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Influence of Ethanolic Plant Extracts on Covid-19 Sufferer's Platelet Performance

Mukta Sharma[1], Anuradha Rohinkar[2], Krishan Raj Singh[3]*

[1]*Department of Life Sciences, IIMT University Meerut, Uttar Pradesh, India.*
[2]*Department of Biochemistry, Parul University, PO Limda, Vadodara, Gujarat, India.*
[3]*Department of Chemistry, Sanskriti University, Mathura, Uttar Pradesh, India.*

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused coronavirus disease-2019 (COVID-19) is a persistent viral pandemic characterized by abnormal platelet responses that enhance the risk of thrombotic events. Likewise, it has been shown that COVID-19 patients often have platelet hyper reactivity. The antiviral action has been supported by several studies of a few natural compounds, but not only have specifically examined their impact on platelet activity in people with COVID-19. The impact of several medicinal plants in vitro on COVID-19 patients' platelet activation and aggregation, as well as any possible underlying processes, were investigated in this research. Measurements of platelet aggregation and PKC delta translocation and phosphorylation on Tyr311 after stimulation by collagen were used to determine platelet hyper-reactivity; however, Curcuma longa ethanolic extract(EtoHE) was unable to reduce the level of hyper-reactivity shown in COVID-19 patients. According to the current investigation, the EtoHE of Lavandulaangustifolia (L.A) and Rosmarinus officinalis (R.O)may dramatically lessen platelet reaction to low collagen concentrations. These results supported the relationship between the conventionally used medicinal herbs and the outcomes of the study. More research employing randomized controlled trials is advised to fully investigate the advantages of the present study.

Keywords:COVID-19, Platelets, ethanolic extract (EtoHE), PKC delta

Full length article **CorrespondingAuthor*,e-mail:*muktasharma@iimtindia.net*

1. Introduction

Infections carried on by viruses provide a significant challenge owing to the diverse variety of medical manifestations they might take. COVID-19, which is acquired by the SARS-CoV-2, has been estimated to afflict over 8 million individuals throughout the globe and has exhibited very peakmisery and fatality rates within a relatively less amount of time. Coronaviruses have been related to severe acute respiratory infections (SARI), abnormal immunological responses, and thrombosis in the most extreme situations [1]. Gravely unwell patients, on the other hand, exhibit alarmingly high challenges of thrombotic difficulties, such asvenous thromboembolic disease, stroke, and microvascular thrombosis. The increased rate of death in COVID-19 is a result of these issues, which may result in multi-organ dysfunction. It is important to remember that thrombosis-related the top causes of mortality globally are heart disorders. As a direct consequence of this, a significant amount of focus in clinical research has been placed on the investigation of potential methods for reducing the risk of thrombotic consequences in COVID-19 and cardiovascular diseases in general. Following a vascular injury in mammals, the closed circulatory system that functions at high pressure is maintained by a highly regulated physiological process known as hemostatic coordination. This process involves the aggregation of platelets, the clotting of blood, and the subsequent breakdown of fibrin by fibrinolysis. Figure 1 demonstrates that the regulatory system maintains a high level of control over the creation of thrombi, also known as blood clots, within the blood arteries [2]. Thrombi are only formed temporarily and in specific locations under normal physiological circumstances. The formation of a thrombus (also known as a clot) in the veins and arteries is the consequence of hemostasis being overridden by runaway processes, such as those that were realized in COVID-19, or of a swing in the hemostatic equilibrium occurring in the direction of pro-coagulation. Both of these scenarios can occur simultaneously [3]. When it comes to circulation in the microvascular system, the end consequence is a clinical disease that is recognized as thrombosis. Since the mechanisms that underlie increased thrombotic events are not completely understood, there is mounting evidence to suggest that endothelial and platelet activation play a significant role.

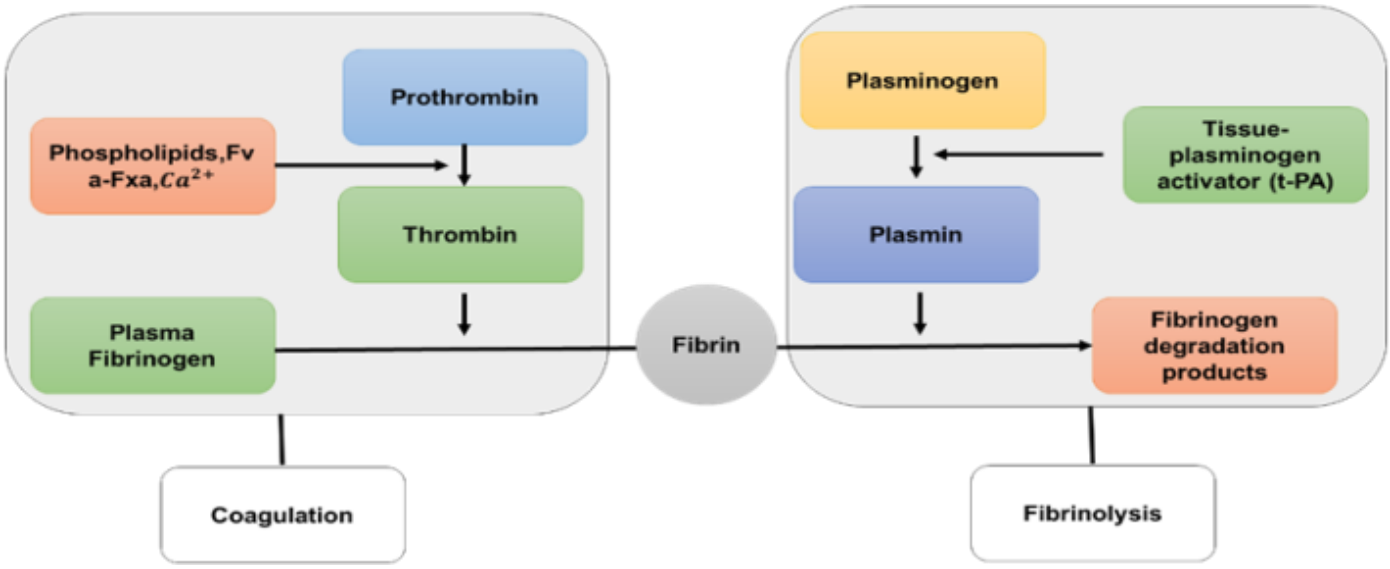

Figure 1. Fibrin clot formation and Fibrinolysis

As infectious aggregates in endothelial cells were discovered, it was hypothesized that endothelial cell stimulation and damage may be the driving force behind platelet activation and the resultant coagulopathy [4]. Consequently, analyzing the involvement of platelets in COVID-19 severe sickness is essential to both comprehending the biology of SARS-CoV-2 infection and locating potential therapy methods. Platelet P-selectin is an important thrombo-inflammatory molecule that has a role in both platelet stimulation and function. It has been proven that it plays an important part in primary hemostasis by normalizing platelet–leukocyte communication, fibrin, and tissue factor enrollment into platelet aggregates, and thrombus formation [5].The research was carried out by the principles listed in the Declaration of Helsinki, and at the time of enrollment, every one of the patients who took part in it or a member of their close family was required to sign a written permission form.

Authors of [6] demonstrate that A. auriculiformis is a prized source of compounds with therapeutic value and offers a reliable foundation for their potential application in modern medicine. To understand the issues with traditional usage, it is necessary to isolate novel phytochemicals and examine their mechanisms of action, which must exhibit pharmacological effects. This is because research areas are restricted. Clinical investigations on isolated bioactive compounds from this plant, which are necessary to explain pharmacological effects, also have drawbacks [7].Commercially available influenza-specific antivirals may exhibit synergistic activity when tested in combination with betel leaf components, resulting in a reduction in the sufficient dose of antivirals, lowering drug pressure, and protecting the emergence of resistant viral strains in treated individuals. This is because betel leaf extracts can treat influenza-like illnesses [8].Rats exposed to ovalbumin were tested to see if the plant affected their tracheal responsiveness and lung pathological characteristics. While the sensitization phase, animals received a daily dose of dexamethasone and a C. Longa (C.L) extract in their water. As a comparison to group S, all C.L concentrations markedly reduced interstitial fibrosis. The essential role of platelets in primary hemostasis is compromised in severely wounded individuals due to an unidentified mechanism, which contributes to an acute coagulopathy that worsens bleeding and raises fatality [9]. Infusing healthy platelets with histone H4 caused ballooning platelets, which mimicked the alterations in platelet shape and function seen in trauma patients. Histone H4 is a damage-associated molecular pattern that is produced in large numbers after heavy abuse. It is thought that using herbal medicines and chemicals, which mostly have antiviral properties with specific effectiveness against RNA viruses and plants in general, has fewer adverse effects, is relatively inexpensive, and has the added benefit of being readily available. The highlighted compounds and medicinal plants were found in the category of plants that were chosen for screening [10]. These compounds and medicinal plants could aid in the development of an innovative drug for the urgently needed treatment of respiratory syncytial virus and COVID-19.FDA-approved medicine of choice for RSV, however, it has drawbacks due to toxicity and administration challenges, among other things [11].The encouraging results against several viral diseases that many herbs and mushrooms have. It has brought attention to Inonotus obliquus's medicinal potential as a safe and effective natural antiviral therapy for SARS-COV-2. The potential of natural agents against COVID-19 has not received much attention up to this point, therefore expanding the study of this area may reveal the potential that including extracts may have against SARS-CoV-2. Despite the lack of knowledge on the innate immune response in COVID-19 patients, the majority of research has demonstrated that lymphopenia often develops in these individuals [12].Identifying the indications and symptoms that may result from ACE2's failure to properly metabolize several significant substances in humans is crucial. Given our affordability, many of us may already be using the therapeutic plants we've described [13]. Using these herbs over time will unquestionably boost immunity and aid in the prevention of several diseases. Even though they are a traditional source of medicine, improper use of them does not always result in increased resistance to disease. For their medicine for

numerous infectious illnesses [14]. As the main issue in contemporary medicine, today is antimicrobial resistance, researchers are persuaded to choose and standardize plant remedies because they cure or prevent infectious illnesses. To pinpoint the antibacterial simulation of plants, precise in vitro and in vivo research is required. Just a small portion of the world's plant species are of high grade [15].

The goal of this research was to identify the impact of several medicinal plants on the aggregation and activation of platelets in COVID-19 patients, according to the most probable underlying mechanisms. To the best of our knowledge, no research has looked at how medicinal plant extracts affect patients with COVID-19 patients' platelet function. Employing ethanol as an industrial solvent is an economical technique to get high-quality plant extract from lots of plants. Cold and warm extraction techniques are used in ethanol extraction systems to make the distillation process easier.

The remainder portion of the study is structured as follows: The issue statement and earlier research are presented in Section 2. Part 3 presents the materials and methods of the study. In Section 4, the performance study is presented. The conclusion and recommendations for further study are included in Section 5.

2. Materials and Methods

2.1. Plant materials

In this study, three food and medicine herbs were chosen because of their increased phenolics, antioxidant activities, and low or nonexistent toxicity. Table 1 displays the findings of a spectrophotometric study of various plant extracts. RAB112424: L.A; RAB112425: R.O; and RAB112426: C.L is the voucher numbers for the plants.

2.2. Patients

- Inclusion criteria: hospitalized or not, Patients, who introduced a confirmed diagnosis of COVID-19 through the use of an RT-PCRquantitation on nasopharyngeal swabs were collected and who were acknowledged to A between 7th December 2020, and 8th march 2021.
- Exclusion criteria: absence of medical records.

The enrollment was given the go-ahead by the Ethics Committee of B, and it is in line with the principles outlined in the Helsinki Declaration. Everyone who participated agreed to participate after providing their informed consent permission. The clinical features of individuals diagnosed with COVID-19 are outlined in Table 2.

2.3. Process of making plant extracts

Once the materials were finely slender and placed onto a Soxhlet column, extraction was performed using 70% v/v ethanol by volume in water at a temperature between 75 and 79 degrees Celsius for 15 hours. The resulting extract was heated to 45 degrees Celsius and then drained. Following that, the EtoHE was sequentially split by a succession of strengthening polar solvents such asbutanol, distilled water, andethyl acetate. Ultimately, the EtoHEwas distilled water.

2.4. Evaluation of platelet aggregation

An eight-channel optical aggrego-meter was used to monitor the aggregation process at 37 degrees Celsius while the mixture was agitated. In a nutshell, collagen was used to activate the extracts from Chrono-Log, which was present in quantities of 0.5 and 5 g/mL. After incorporating a sufficient amount of collagen, platelet aggregation was documented and observed. This continued tillindication stability and light transmission were maximum aggregation point measurements.

2.5. PKC delta Phosphorylation

Collagen was used to activate platelets for five minutes while an EtoHE was also present or its carrier at a temperature of 37 degrees Celsius and under shear circumstances. The process was terminated by the addition of the required amount of 4 Laemmli buffer. After being heated for 5 minutes at 95 degrees Celsius, the platelet lysates were chilled to a temperature of -20 degrees Celsius before being subjected to further SDS-PAGE analysis. The proteins were separated using eight percent SDS-PAGE lotions, transmitted onto cellulose acetate epithelial cells, ceased with 5% non-fat dry milk for just a 60 minutes, three times cleaned with Tablespoons (150 molar ratio NaCl, 20 molar ratio Tris, pH 7.4, 0.1percent) of the respondents Product description), and afterwards underwent a 24-hour incubation at 4 degree C with antibody levels against phospho-PKC delta Tyr311. After the steps of rinsing, the membranes were tagged with a detection antibody that was conjugated with horseradish peroxidase for an hour. After this, the membranes were rinsed again, and enhanced chemiluminescence was used to examine the associated peroxidase activity. To determine whether or not the membranes were loaded with the same quantities of protein, they were stripped and blotted for the total PKC delta.

2.6. A study using Confocal Microscopy

Platelets were immobilized on penta microscope slides for 8 hours at 4 degrees Celsius after being fixed for 30 mins with 2percent (v/v) formalin solution in phosphorus saline. Platelets were stimulated with collagen during 5 minutes at 37 degrees Celsius in the availability of EtoHE or its vehicle. A 20-minute permeabilization procedure using 0.1percentage Triton X-100 to produce 2 percentage albumin from bovine serum was performed on the adhering platelets.

Table 1. Phenolic extracts from food and medicine plants in methanol

MedicinalPlant	**Flavonoids ($mgCATEg^{-1}DW$)**	**Tannins($mgECEg^{-1}DW$)**	**Totalphenols($mgGAEg^{-1}$ DW)**	**Ortho-diphenols($mgGAEg^{-1}DW$)**
L.A	35.72±0.70	23.58±0.44	41.92±0.64	108.84±0.67
C.L	45.39±1.06	95.27±0.16	13.26±1.77	39.16±0.93
R.Officinalis	49.14±0.83	18.10±0.77	48.48±0.13	133.88±1.78

Table 2. COVID-19 patients

Index	**p-value**	**Patients with COVID-19**	**Reliable**
Age, years	0.82	53±16.27	50.32±12.24
Female/Males	-	5/5	5/5
N° of patients		10	10
Platelet number x 10^9/L	0.21	187.5±96.61	238±75.59
ALT,U/L	0.01	34.22±15.48	20.54±5.43
Weight,kg	0.94	82.67±13.94	79.44±26.82
LLDH,U/L	0.01	616.30±192.36	362.89±134.37
AST, U/	0.01	36.17±14.81	19.62±4.40
Lymphocyte number x 10^9/L	0.36	1.08±0.53	1.19±0.87
D-dimers,mg/L	0.01	1.46±0.62	0.56±0.38
C-reactiveprotein,mg/L	0.05	22.64±12.43	8.39±4.11

After that, the platelets were washed and branded with secondary antibodies against rabbit IgG Alexa-555 and mouse IgG Alexa488 for one hour at room temperature. After that, the platelets were allowed to remain in an incubation solution containing Cursor anti-human monoclonal antibody against tubulin and rabbit anti-human polyclonal anti-PKC antibody for three hours. An LSM 510 confocal microscope was used to take a succession of fluorescent confocal images of coverslips placed on microscopic plates.

3. Results and Discussions

According to this study, COVID-19 patients had the greatest levels of platelet activation, aggregation, and platelet-leukocyte aggregates than healthy blood donors reported. Although it has shown the occurrence of SARS-CoV-2 virions in platelets, several studies have confirmed the presence of platelets in thrombi identified in several organs of COVID-19 postmortem patients. These results show that SARS-CoV-2 infection increases platelet reactivity, and they hypothesize that in individuals with severe COVID-19, platelet hyperactivity may be the primary factor causing thrombosis, causing organ failure and death. Our research looked at whether platelet hyper-reactivity might be reversed after treatment with an EtoHE of several medicinal herbs.

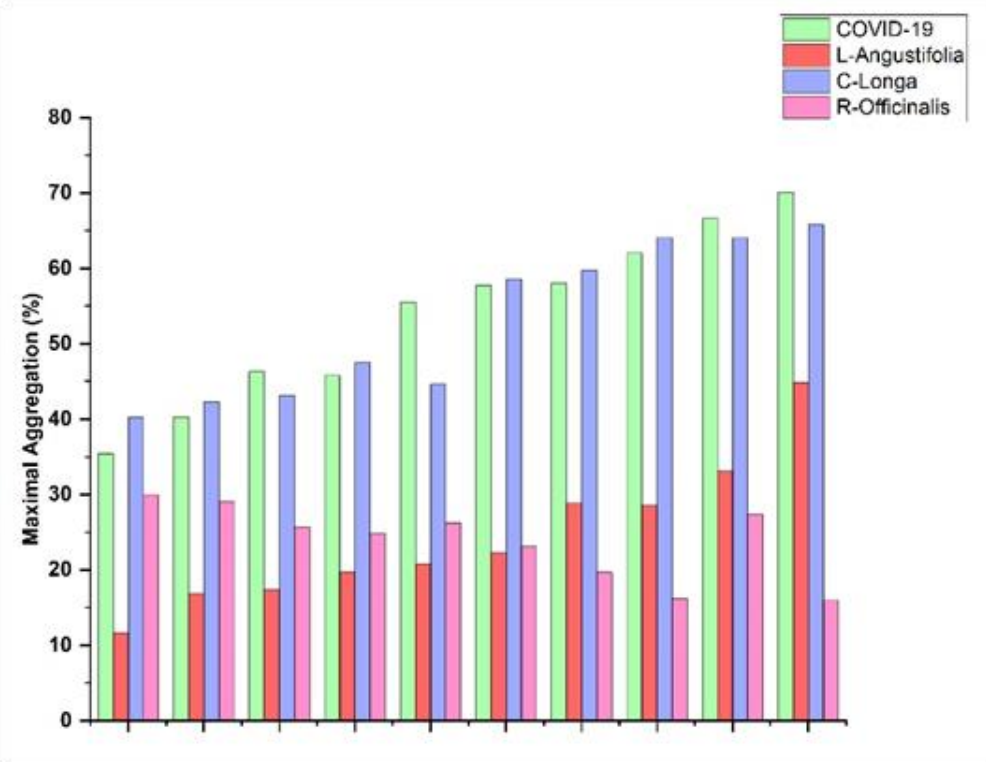

Figure 2. Maximum aggregation of collagen ($0.5\mu g/mL$)

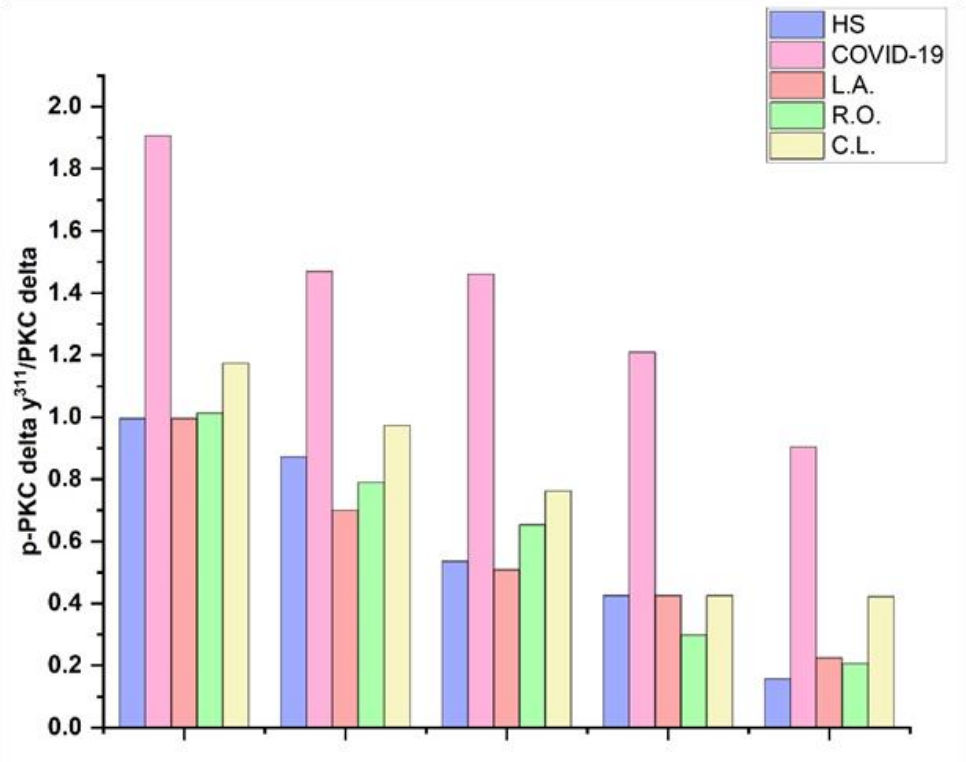

Figure 3. Aggregation of collagen ($0.5\frac{\mu g}{mL}$)

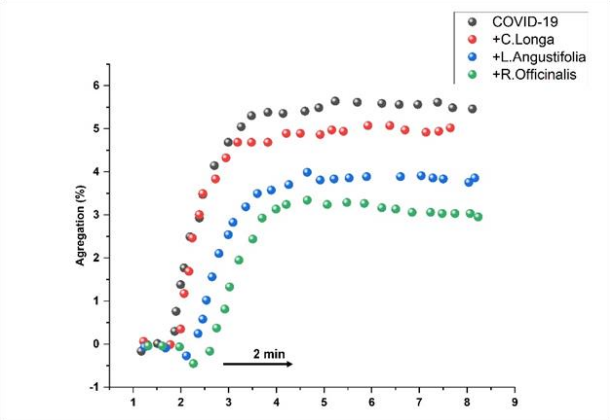

Figure 4.PKC delta

The example aggregation traces are shown in figure 3 under constant stirring at 37 °C.It was shown that subjecting acid-soluble human collagen to heat treatment at a pH of 2.5 resulted in a significant decrease in the relative viscosity of the collagen as well as platelet aggregating activity. This discovery was found when researchers were looking at various collagen characteristics, including those that are essential for collagen's capacity to aggregate platelets. When collagen is exposed to circulating blood during the development of a thrombus after arterial wall damage, platelet attachment, and subsequent aggregation are started. Since it functions as both an adhesive surface and a strong activator of platelets, collagen is a special kind of agonist. Figure 4 demonstrates that in individuals who had elevated COVID-19 and platelet pretreatment with EtoHE of LA, R, and C for 5 minutes at 37°C, the following results were observed. PKC delta phosphorylation on Tyr311 residue is elevated in response to collagen. SDS-PAGE was used to examine platelet lysates for P-PKC Tyr311. All circumstances resulted in a total PKC evaluation. Immunoblot (top) shows 5 donors as a whole. P-PKC Tyr311 was subjected to a densitometric (lower) examination and its values were represented as comparative optical densities (n=5). Measurement of the maximum amount of platelet aggregation in pre-incubated NaCl (n=5).

The current investigation showed that C.L, L.A, and R.O were effective in vitro at preventing platelet hyper-reactivity when SARS-CoV-2 was infected. These results provide further evidence that platelets taken from COVID-19 patients are high, as seen in figure 2, in response to a suboptimal concentration of collagen (0.5 g/mL).Platelet hyperreactivity to collagen may be reduced using a therapy consisting of L. A and R.O. Platelets were extracted from a total of ten reliable donors and ten patients with heavy COVID-19. Whether on purpose or not, platelet from COVID-19 patients received therapy with an EtoHE containing L.A, R.O, and C.Lfor (n=10) and 5 minutes at 37 degrees Celsius in acknowledgment to less. Quantification of maximal platelet aggregation was performed.

4. Conclusions

The study demonstrates that an extract of medicinal plants made with ethanol may diminish the platelet hyper-reactivity that is seen in individuals with COVID-19. According to the findings of the current research, EtoHE of L. A and R.O. have the potential to dramatically inhibit platelet activation in reactions to low concentrations of collagen. Based on these findings, there is a desirable and potential therapeutic candidate for anti-SARSCoV-2 treatment. Ethnomedicinal suppliers may complete the difficult tasks if they respect industry standards for manufacturing and quality control, but these medications shouldn't be marketed without Quality assurance and Quality control certification. In the future, wound treatment has great potential thanks to scientific research and clinical trials utilizing conventional and alternative medicine.

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