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Original Article

Study on *in vitro* Thrombolytic Efficacy of Polyherbal Formulation to Mitigate Cardiovascular Risks after Recovery from COVID-19

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Abstract

According to WHO reports, after recovering from COVID-19, approximately 10–20% of individuals experience a 1.8-fold higher risk of cardiovascular complications compared to non-infected individuals, even one year after recovery, due to hypercoagulability. This study aimed to develop a herbal remedy to mitigate cardiovascular risks in COVID-19 recovered individuals. An aqueous polyherbal formulation composed of the leaves of *Ocimum sanctum*, *Moringa oleifera*, *Cardiospermum halicacabum*, and the rhizome of *Curcuma amada* was prepared and subjected to phytochemical and spectral analysis. Blood samples from thirty COVID-19 recovered individuals were analysed for the in vitro thrombolytic efficacy and in vitro anti-platelet aggregation efficacy of the polyherbal formulation at different concentrations (8.2, 16.5, 33.0, and 66.0 mg/100 µl). Phytochemical screening revealed the presence of polyphenols, alkaloids, flavonoids, and saponins. Spectral analysis indicated the presence of phenolic O-H groups, conjugated C=O, and C=C bonds as functional groups. The polyherbal formulation demonstrated the highest thrombolytic activity of 79 ± 1.8% at 66 mg/100 µl compared to the standard streptokinase, and an anti-platelet aggregation activity of 83 ± 3.4% (IC_{50} = 17.3 mg/100 µl) compared to standard aspirin. Hence, treatment with this polyherbal formulation may serve as a preventive therapy to mitigate cardiovascular risks following recovery from COVID-19.

Keywords: *Antiplatelet Efficacy; COVID-19-Post Complications; Polyherbal Remedy; Thrombolytic Activity*

Introduction

Cardiovascular disease encompasses a broad spectrum of conditions affecting the heart, blood vessels, and overall blood circulation throughout the body (Curry *et al*., 2018). While the cardiovascular complications associated with acute COVID-19 are well-documented, the long-term cardiovascular effects following recovery from the disease remain less thoroughly explored. According to experts from Johns Hopkins and the American Heart Association (Merschel M. 2022), individuals who have recovered from COVID-19 continue to face a sustained risk of cardiovascular events, with an elevated likelihood of venous clot formation at 0.25% and arterial clot formation at 0.5% even one-year post-infection (Knight *et al.,* 2022).

COVID-19 has been associated with an increased risk of cardiovascular complications, particularly a higher likelihood of thrombotic events (Merschel, 2022). Thrombus formation is a major underlying cause of cardiovascular diseases. In the body, thrombus dissolution occurs naturally through the

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process of fibrinolysis. When natural thrombolysis is inadequate, synthetic thrombolytic agents such as streptokinase and urokinase are employed to activate plasminogen and promote the breakdown of blood clots. However, these agents are often linked to serious side effects, underscoring the need for safer and more cost-effective thrombolytic alternatives (Kim, 2020).

Long-term studies on the cardiovascular outcomes of COVID-19 have provided significant evidence of increased cardiovascular risk, which escalates progressively depending on the severity of the acute infection, ranging from non-hospitalised individuals to those hospitalised and, ultimately, those admitted to intensive care (Xie *et al*., 2022). The consumption of easily digestible, high-fibre, plant-based foods is recommended during the acute phases of heart disease, as they may reduce stress on the heart (Jain *et al*., 2023). When the body's natural thrombolytic processes are compromised or inhibited, the consequences can be severe. COVID-19 is one such condition, where the pathophysiology is largely driven by a hyper-inflammatory response, often referred to as a cytokine storm. This response enhances the activity of tenase and prothrombinase, resulting in increased thrombin-mediated fibrin production and clot formation. Consequently, COVID-19-induced inflammation plays a significant role in the development of thrombosis. Therefore, a combined approach involving anti-inflammatory and thrombolytic therapies is recommended for the management of COVID-19-related complications (Jing *et al*., 2022). The risk may be due to the persistence of the primary COVID-19 infection for a prolonged duration or complications arising from a compromised immune system. After recovery, many individuals present with a variety of long-term complications affecting different organs, collectively known as post-recovery syndrome or post-COVID complications. These include post-COVID lung disease, coagulation disorders, and liver and renal failure (Seyed Alinaghi *et al*., 2021).

A 2024 UK Biobank analysis found that the risk of heart attack, stroke, and death remained elevated for up to three years post-COVID-19 infection, comparable to other established cardiovascular risk factors. This risk was particularly higher in hospitalised individuals with blood types A, B, and AB (Williamson, 2024). Plant-based herbs are among nature's most remarkable gifts to humanity. Studies by Jain *et al*. (2023) demonstrated that plant-derived compounds such as quercetin, piperine, curcumin, apigenin, kaempferol, luteolin, thymol, rutin, eugenol, and oleanolic acid exhibit antiviral activity against SARS-CoV-2 and possess anticoagulant functions. The presence of biologically active compounds and therapeutic properties, including antioxidant effects, inhibition of platelet aggregation, and anti-inflammatory activity, provides strong evidence supporting the preference for plant-based traditional remedies for thrombolysis over conventional medicines. Literature surveys indicate that the leaves of *Ocimum sanctum* have significant thrombolytic efficacy (Khan *et al*., 2011) as well as antiplatelet aggregation activity (Tohti *et al*., 2006; Amrani *et al*., 2009). Studies by Helmy *et al*. (2017) and Kunwar *et al*. (2022) showed that the leaves of *Moringa oleifera* possess antihyperlipidaemic and clot-lysis effects, respectively. The leaves of *Cardiospermum halicacabum* demonstrate both antioxidant and anti-inflammatory activity (Aishwarya *et al*., 2014; Cheng *et al*., 2013). The rhizome of *Curcuma amada* has been reported to exert thrombolytic action (S. *et al*., 2017). More recently, research by Srilatha *et al*. (2025) investigated the thrombolytic potential of *C. amada* using both in vitro and in silico approaches, with findings that highlight its promising efficacy in clot dissolution. The present study investigates the in vitro thrombolytic efficacy of a polyherbal formulation, with the aim of reducing cardiovascular risks in individuals who have recovered from COVID-19.

Materials and Methods

Collection of plant specimens

The leaves of *Ocimum sanctum*, *Moringa oleifera*, *Cardiospermum halicacabum*, and the rhizome of *Curcuma amada* were collected locally in Tiruchirappalli and authenticated at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. All the collected materials were shade-dried and ground into a fine powder. Aqueous extracts of each individual plant material were prepared, and a polyherbal formulation (1:1:1:1 w/w) comprising all the selected plant materials was also prepared using a Soxhlet apparatus.

Phytochemical analysis of aqueous extracts

All five aqueous extracts were subjected to preliminary phytochemical analysis using the standard protocol (Harborne, 1998) to identify the presence of secondary metabolites such as alkaloids, anthraquinones, coumarins, flavonoids, phenols, proteins, quinones, saponins, sugars, steroids, terpenoids, and tannins.

GC-MS and FT-IR Analysis polyherbal formulation

GC-MS and FT-IR analysis were conducted solely on the polyherbal formulation. The GC-MS analysis was performed using a Perkinelmer claire 500 Gas Chromatograph, coupled with a mass detector. The spectral scan range was set between 40 and 450 (mhz). The functional group of polyherbal formulation was detected by FTIR analysis and the absorption band was set between 4000- 400 cm**.**

Analysis of clotting time

With prior consent, venous blood samples were collected from thirty (n = 30) COVID-19 recovered individuals who visited SMS Hospital, Uraiyur, Tiruchirappalli. The clotting time was determined using the capillary tube method, described briefly as follows: a finger was pricked with a sterile lancet, and blood was drawn into two non-heparinised capillary tubes and left for 4 minutes. The tubes were then broken at 30-second intervals, and the time was recorded when a fibrin thread formed between the two broken ends (Tabassum *et al*., 2017). The observed clotting time was compared with the normal clotting time of 8–12 minutes.

In vitro thrombolytic activity of aqueous extracts

The in vitro thrombolytic efficacy of all five extracts was analysed using standard protocols (Hussain *et al*., 2014). Each 500 µl venous blood sample from COVID-19 recovered individuals was transferred into a pre-weighed, sterilised microfuge tube and incubated at 37 °C for 10 minutes to promote clot formation. Serum was then removed by centrifugation, and the clot weight in each tube was recorded. To assess the clot lysis efficacy of the five extracts, 500 µl of each extract at different concentrations (8.2, 16.5, 33.0, and 66.0 mg/100 µl) was added to the clots. Clots treated with streptokinase (30,000 I.U) served as the positive control, while clots treated with 500 µl of distilled water served as the negative control. All tubes were incubated at 37 °C for 1 hour 30 minutes and then centrifuged to remove the supernatant. The weight of the unlysed clot in each tube was determined, and the percentage of clot lysis was calculated as follows:

$$\text{Percentage clot lysis} = \frac{\text{Weight of clot before lysis} - \text{Weight of the after-lysis clot}}{\text{Weight of clot before lysis}} \times 100$$

In vitro anti platelet aggregation efficacy of aqueous extracts - Microtitre plate method

The in vitro platelet aggregation inhibition efficacy of all five extracts was determined using standard protocols (Wu *et al*., 2020). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation at 100 g and 2300 g for 10 minutes, respectively. Different concentrations of each extract (8.2, 16.5, 33.0, and 66.0 mg/100 µl) were added to 100 µl of PRP and 100 µl of Tyrode buffer (pH 7.4) in a microtiter plate, and absorbance was measured at 570 nm. After the addition of a stirrer, the PRP was incubated at 37 °C for 5 minutes and vibrated at 1000 rpm. The platelet aggregation rate was calculated by comparing the absorbance before and after stimulation using the formula below. Aspirin was used as the reference standard, and the absorbance of PPP was taken as the background value, as follows:

$$\%\ \text{of Platelet aggregation} = \frac{\text{Absorbance after stimulation} - \text{Absorbance (PPP)}}{\text{Absorbance before stimulation} - \text{Absorbance (PPP)}} \times 100$$

Statistical Analysis

Data are expressed as the mean ± standard deviation of the mean. The significance between percentage of clot lysis and anti-platelet aggregation by the test samples and controls were tested by the one-way ANOVA by SPSS package at $p < 0.05$.

Results

Phytochemical analysis of extracts

All extracts demonstrated the presence of secondary metabolites such as flavonoids, phenolic compounds, steroids, terpenoids, and tannins. The polyherbal formulation contained a greater number of secondary metabolites compared with the individual plant extracts, as shown in Table 1.

Table 1: Phytochemical Qualitative Analysis of Aqueous Extracts

S.No	Phyto chemicals	*O.s*	*M.o*	*C.h*	*C.a*	Polyherbal formulation
1	Alkaloids	++	++	+++	+	+++
2	Anthraquinone	A	++	A	A	+++
3.	Coumarin	++	A	A	++	+
4	Flavonoids	+++	+++	+++	+++	+++
5	Phenol	+	+++	+++	+	+++
6	Protein	+	++	++	+	++
7	Quinone	A	+++	++	++	+++
8.	Saponins	+++	A	+	+++	+++
9	Sugar	+++	++	+++	+	++
10	Steroids	+++	+++	+++	++	+++
11	Terpenoids	+++	+++	+++	+++	+++
12	Tannins	+++	+++	+++	+++	+++

* + Trace ++ Moderate +++ strong A- Absence

GC - MS Analysis of polyherbal formulation

The results of GC-MS analysis of polyherbal formulation were given in Table 2 and Figure 1. The GC-MS analysis of the polyherbal formulation showed a total of 25 peaks and the highest peak was observed at 18 with 1-Hexyl-2- nitrocyclohexane 34.83% and the second highest peaks were found at 2 with heptasiloxane, hexadecamethyl at 19.12%.

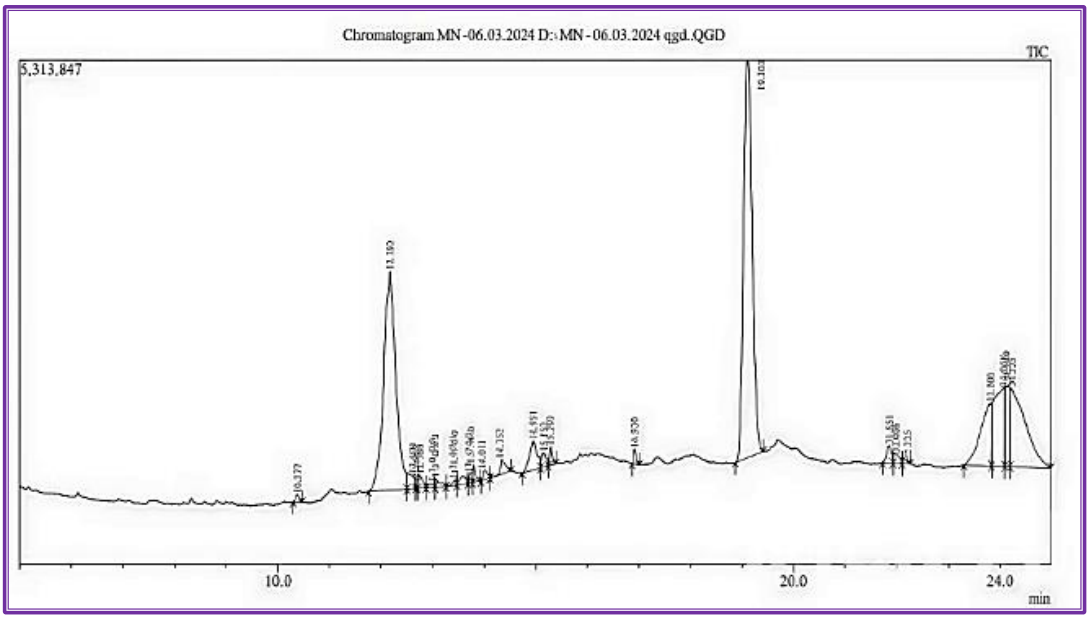

Figure 1: *GC-MS spectrum of polyherbal formulation*

Table 2: GC-MS Analysis of Polyherbal Formulation

Peak Report							
Peak	**R.Time**	**Area**	**Area%**	**Height**	**Height%**	**A/H**	**Name**
1	10.377	349232	0.24	79948	0.66	4.37	Benzeneethanamine,N-[(pentafluorophenyl) methylene]
2	12.182	3741872	25.68	2301007	19.12	16.26	Heptasiloxane, hexadecamethyl
3	12.608	1304863	0.90	131176	1.09	9.95	1H-cycloprop[E]azulen-4ol, DECA
4	12.692	290212	0.20	104597	0.87	2.77	alpha.-L-Galactopyranoside, methyl 6-deoxy
5	12.769	805210	0.55	133159	1.11	6.05	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,
6	13.008	359718	0.25	49331	0.41	7.29	Benzeneethanamine, N-[(pentafluorophenyl)
7	13.083	505064	0.35	82899	0.69	6.09	Glycine, N-[N-(2-Hydroxybenzoyl)
8	13.433	354608	0.24	56642	0.47	6.26	3-Hydroxy-6-(N,N-dimethylamino)methylpyr
9	13.500	956150	0.66	78252	0.65	12.22	Pentadecanoic acid methyl ester
10	13.742	453982	0.31	102416	0.85	4.43	2,4a,8,8-Tetramethyldecahydrocycloprop a[d]n
11	13.804	583480	0.40	184236	1.53	3.17	:3,4-Dihydroxymandelic acid – tetratms
12	14.011	675593	0.46	101399	0.84	6.66	n-Hexadecanoic acid
13	14.352	1069618	0.73	148372	1.23	7.21	Pseduosarsasapogenin-5,20-dien methyl ether
14	14.951	3176570	2.18	305138	2.54	10.41	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4
15	15.153	972310	0.67	154587	1.28	6.29	n-Nonadecanol-1
16	15.292	659655	0.45	193490	1.61	3.41	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,
17	16.920	504694	0.35	159961	1.33	3.16	Cyclononasiloxane, octadecamethyl-
18	19.103	49547531	34.01	4192884	34.83	11.82	1-Hexyl-2- nitrocyclohexane
19	21.851	1162575	0.80	180670	1.50	6.43	1H-purin-6-amine, [(2-fluoropheny)
20	22.008	884962	0.61	99657	0.83	8.88	1H-Benzocyclohepten-7-ol, 2,3,4,4A
21	22.225	311392	0.21	24098	0.20	12.92	1,4-methanoazulen-7(1H)-one,
22	23.808	9876039	6.78	658765	5.47	14.99	Methyl commated D
23	24.058	1181550	8.11	829697	6.89	14.24	4,4,6a,6b,8a,11,11,14b-Octamethyl-
24	24.144	4600849	3.16	857672	7.13	5.36	D-Norandrostan-16-ol, acetate
25	24.225	1705983	11.71	826927	6.87	20.63	Benzoic acid, 2,6- Bis (Trimethylsil)

FT-IR analysis of polyherbal formulation

The probable functional groups responsible for the biological activity of the polyherbal formulation were determined by FT–IR analysis and are presented in Figure 2 and Table 3. The FT–IR spectrum displayed distinct absorption bands confirming the presence of diverse functional groups. A broad peak

at 3447 cm^{-1} corresponded to O–H stretching vibrations of alcohols, phenols, or carboxylic acids, suggesting strong hydrogen bonding, whereas characteristic C–H stretching of methylene and methyl groups was observed at 2924 and 2854 cm^{-1}, confirming the presence of aliphatic groups. A sharp band at 2296 cm^{-1} indicated the presence of nitrile (C≡N) groups, while the strong absorption at 1642 cm^{-1} corresponded to carbonyl (C=O) stretching, possibly from ketones, carboxyl, or amide linkages. Peaks at 1384, 1336, and 1054 cm^{-1} reflected C–H bending and C–N stretching, supporting the presence of amine or amide functionalities. Additionally, a peak at 1109 cm^{-1} corresponded to aliphatic C–C stretching, and absorptions at 778 and 625 cm^{-1} suggested the presence of chlorinated compounds and aliphatic C–H bending, respectively. Collectively, these results indicate that the material contains hydroxyl, carbonyl, amine/amide, and hydrocarbon groups, with additional contributions from nitrile and halogenated moieties, highlighting a complex organic structure that may be derived from proteinaceous or polymeric modifications.

Table 3: FTIR Analysis of Polyherbal Formulation

Sl.No	Frequency	Type of Band	Type of Functional Group
1.	3447.73cm-1	OH Stretching	Alcohol, phenols, carboxylic acid
2.	2924.48 cm-1	CH2 Stretching	Methylene group
3.	2854.74 cm-1	(-CH3) Stretching	Methyl, methylene
4.	2296.99 cm-1	CN Stretching	Cyanides
5.	1642.08 cm-1	(C=O) Stretching	Carbonyl group
6.	1384.44 cm-1	CH Stretching	Methyl, methylene, methylidyne
7.	1336.49 cm-1	C-N Stretching	Amines, amides
8.	1109.88 cm-1	C-C Stretching	Aliphatic or saturated hydrocarbon
9.	1054.65 cm-1	C-N Stretching	Amines, amides
10.	778.12 cm-1	C-CL Stretching	Chlorinated compound
11.	625.01 cm-1	C-H Stretching	Aliphatic hydrocarbon

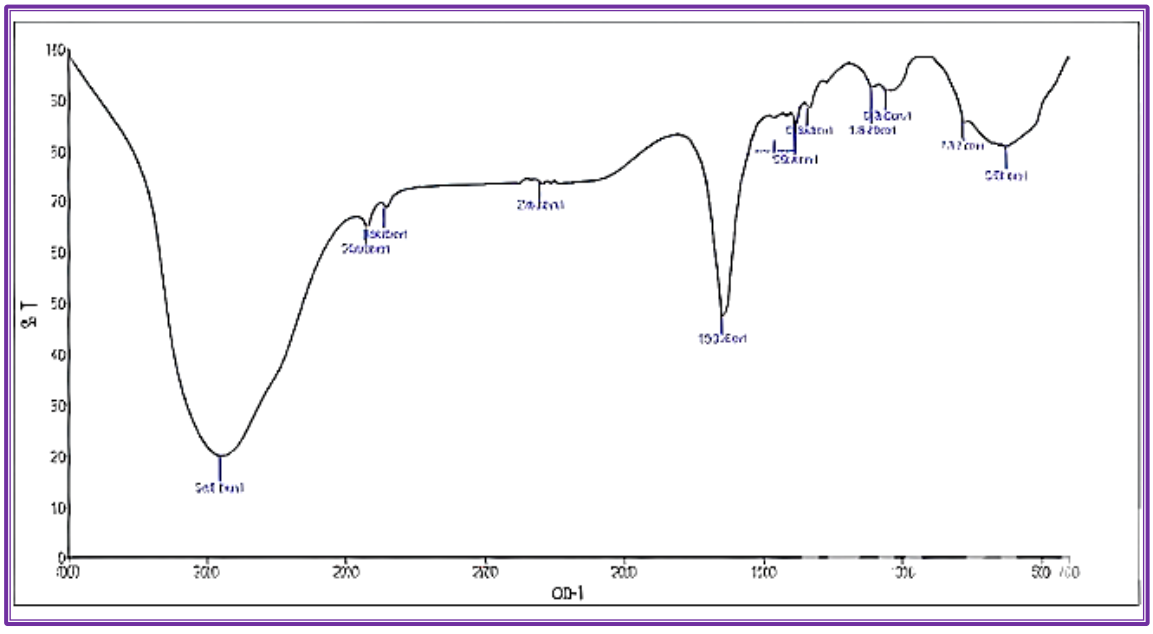

Figure 2: *FTIR Spectrum of Polyherbal Formulation*

Analysis of blood clotting time

The blood clotting time analysis of 30 individuals recovered from COVID-19 showed a mean clotting time of 6.5 ± 0.5 minutes, compared with 8 ± 0.5 minutes in normal individuals (Figure 3). A significant difference ($p < 0.05$) in clotting time between COVID-19 recovered individuals and normal controls indicates the presence of hypercoagulability in the blood of those who have recovered from COVID-19.

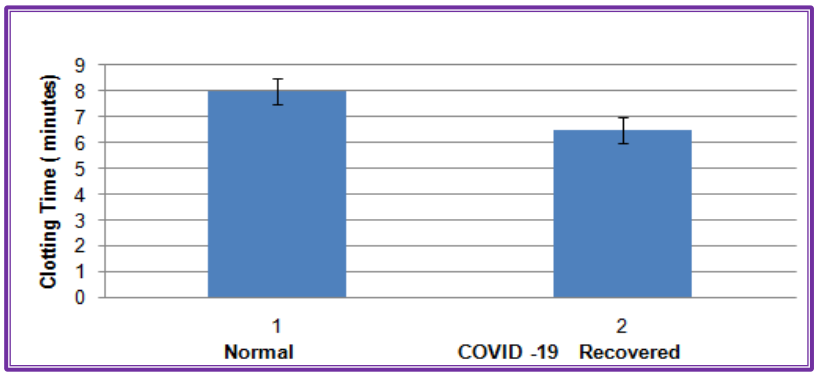

***Figure 3**: Analysis of Clotting Time*

In vitro thrombolytic activity of aqueous extracts

The results of thrombolytic efficacy of extracts at 8.2, 16.5, 33.0, 66.0 mg /100µl concentrations for *O. sanctum* (23.6 ± 1.7, 33.5 ± 1.0, 52 ±0.9, 70 ± 1.5%) *M. oleifera* (29.6 ± 1.6, 30 ±1.3, 49 ±1.0, 60 ± 1.5 %), *C. halicacabum* (29.1 ± 1.8, 34.2 ±1.3, 49 ± 1.5, .69 ± 1.1%) *C.amada* (27 ± 1.4, 32 ± 1.3, 50 ± 1.0, 60 ± 1.4%) and polyherbal formulation (34 ± 1.5, 46 ±1.5, 55± 2.0, 79 ± 1.8%) respectively showed a direct relationship between concentration and percentage thrombolytic activity of extracts (Figure 4). The percentage of clot lysis for streptokinase treated samples was found to be 94± 1%. The results showed that all the 5 extracts contained active compounds for thrombolytic activity which is comparable to streptokinase (positive control) activity. Among all the extracts, polyherbal formulation showed the significant ($p < 0.05$) thrombolytic activity of 79 ± 1.8% at 66.0 mg /100µl concentration compared to standard.

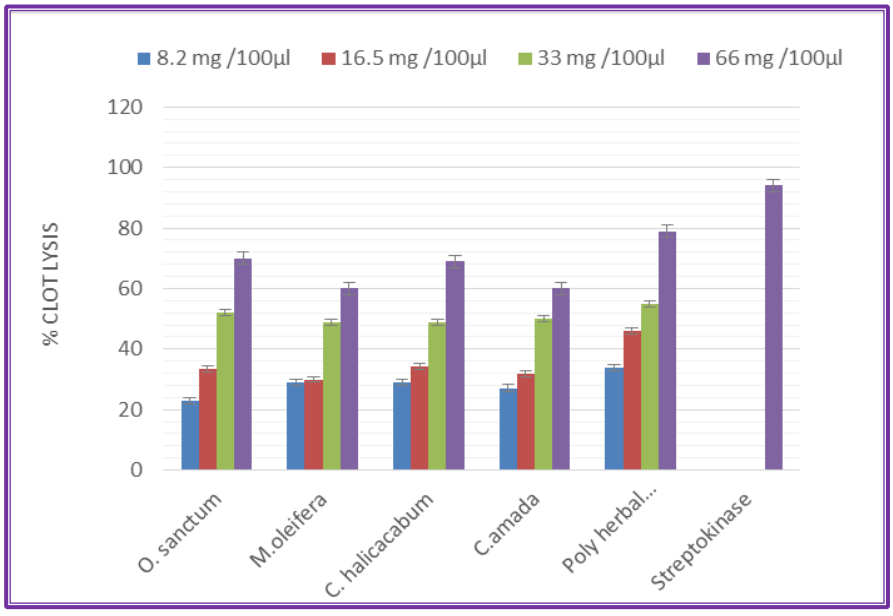

***Figure 4:** in vitro Thrombolytic Activity of Herbal Extracts*

In vitro anti platelet aggregation analysis

The antiplatelet aggregation efficacy of all five extracts at different concentrations is presented in Table 4. At concentrations of 8.2, 16.5, 33.0, and 66.0 mg/100 µl, *O. sanctum* (25.6 ± 2.0, 43 ± 1.2, 51 ± 2.1, 65 ± 1.5%), *M. oleifera* (29 ± 2.2, 35.3 ± 1.8, 48 ± 1.9, 61 ± 2.5%), *C. halicacabum* (32.1 ± 2.9, 46.2 ± 1.9, 59 ± 2.5, 73 ± 3.2%), *C. amada* (26 ± 2.4, 40 ± 2.8, 53 ± 1.0, 62 ± 1.4%), and the polyherbal formulation (36.5 ± 2.8, 52.2 ± 2.5, 69 ± 2.3, 83 ± 3.4%) each demonstrated a clear dose-dependent relationship between concentration and percentage inhibition of platelet aggregation. The IC_{50} value obtained for the polyherbal formulation was 17.3 mg/100 µl. The percentage inhibition of platelet aggregation in aspirin-treated samples (positive control) was 92 ± 2.2%. The findings confirmed that all

five extracts contained active compounds with antiplatelet aggregation activity comparable to aspirin. Among all the extracts, the polyherbal formulation showed significant ($p < 0.05$) inhibition of platelet aggregation (79 ± 1.8% at 66.0 mg/100 µl) relative to the standard. Overall, the results demonstrated that the polyherbal formulation possesses superior thrombolytic and antiplatelet aggregation efficacy compared with the individual extracts.

Table 4: In Vitro Antiplatelet Aggregation Analysis of Herbal Extracts

Extract	Concentration			
	8.2 mg/100µl	16.5 mg/100µl	33.0 mg/100µl	66.0 mg/100µl
O. sanctum	25.6 ± 2.0	43 ±1.2	51 ±2.1	65 ± 1.5
M. oleifera	29 ± 2.2	35.3 ± 1.8	48 ±1.9	61 ± 2.5
C. halicacabum	32.1 ± 2.9	46.2 ±1.9	59 ± 2.5	73 ± 3.2
C. amada	26 ± 2.4	40 ± 2.8	53 ±1.0	62 ± 1.4
Polyherbal formulation	36.5 ± 2.8	52.2 ±2.5	69 ± 2.3	83 ± 3.4

Discussion

The hypercoagulable state of COVID-19 patients has been demonstrated even four months after recovery (von Meijenfeldt *et al*., 2021). A survey by the American Heart Association, Inc. (2022) reported that in the first week following COVID-19 infection, the risk of venous thrombosis was 33 times higher. By the third and fourth weeks, the risk decreased to eight times higher, yet even between 27 and 49 weeks post-infection, it remained 1.8 times greater compared with individuals who had never contracted COVID-19 (Lin *et al*., 2021). Cytokine storm and IL-6–mediated platelet activation further exacerbate the hypercoagulable condition in COVID-19 (Barrett *et al*., 2021; Hottz *et al*., 2020).

These findings highlight the importance of implementing targeted therapeutic interventions to optimise patient outcomes and improve the long-term prognosis of COVID-19 survivors. Plant-derived thrombolytic compounds are considered attractive and safe options for the prevention and management of thromboembolism. In this study, the polyherbal formulation and other extracts were found to contain phytochemicals such as flavonoids, phenolic compounds, steroids, terpenoids, and tannins. Previous studies, including the thrombolytic and anti-inflammatory efficacy of *Achyranthes aspera* aerial part extracts (Ha *et al*., 2024), the inhibition of platelet activation and thrombus formation by tannic acid (Zhu, 2018), the antithrombotic efficacy of the alkaloid ambinine and antiplatelet aggregation by tannins in the tuber of *Corydalis ambigua var. amurensis* (Chang *et al*., 2018), and the prevention of thrombus formation through inhibition of platelet aggregation by certain flavonoids (Bojić *et al*., 2019), further support the clot-lytic and antiplatelet effects of plant-based drugs. Our results demonstrated that the polyherbal formulation exhibited superior thrombolytic and antiplatelet aggregation efficacy compared with the individual extracts. This enhanced activity may be attributed to the synergistic action of the active principles present in the polyherbal formulation. Hence, this extract has the potential to mitigate the cytokine storm-induced hypercoagulable state observed in COVID-19 recovered individuals. Therefore, the consumption of a decoction prepared from the four selected edible plant materials may help optimise the clotting mechanism.

Conclusion

This study demonstrated the potent thrombolytic efficacy of a nutraceutical formulation comprising the leaves of *Ocimum sanctum*, *Moringa oleifera*, *Cardiospermum halicacabum*, and the rhizome of *Curcuma amada*. Hence, it may serve as a promising source for further investigation to identify lead compounds with thrombolytic and antiplatelet activity, highlighting its potential application in mitigating cardiovascular risks among COVID-19 recovered individuals.

Conflict of Interest

The authors report no conflicts of interest in this work.

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