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Evaluation of the Thrombolytic and Antioxidant Activity of Leaf Extracts of *Plumbago zeylanica* L.

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ABSTRACT

Background: *Plumbago zeylanica* L. is one of the extremely accessible conventionally used herbal plants with various biological activities. However, actions of *P. zeylanica* L on blood clotting and other complications of blood were indisposed therapeutically studied. Therefore, the scope of the current exploration is to screen the thrombolytic, antioxidant, and cytotoxic effects of leaf extracts. **Materials and Methods:** Thrombolytic activity (*in vitro*) was assessed with clot lysis and thrombin inhibitory ability. Further, thrombolytic activity (*in vivo*) was evaluated by a thrombotic tail (carrageenan-induced) animal model. DPPH and nitric oxide (free radical) scavenging methods were employed to check the *in vitro* antioxidant property. Further cytotoxicity and acute oral toxicity were assessed for plant extract. **Results:** The quantitative analysis elicits the presence of the magnificent amount of the total phenolic content (96.8±7.92 mg GAE/g) and total flavonoid content (63.52±4.54 mg QE/g) on the dry weight basis. The maximum clot lysis (96.83%±0.657) of methanolic leaf extract was detected in *in vitro* model at 800 μg/mL in 72 hr. A strong thrombin inhibition (94.63±2.12%) effect was observed for methanol leaf extract at 2 mg/mL. In *in vivo* studies a significant ($p< 0.001$) clot lysis was achieved at the tested dose (100, 200 and 300 mg/kg). DPPH radical and nitric oxide scavenging activity showed the IC_{50} value of 25.47±0.51 and 56.32±0.85, respectively. The methanolic extract was found safer up to the highest lethal dose of 2000 mg/kg. **Conclusion:** These findings suggested that the plant leaves are comprised of significant thrombolytic properties. It could be a promising source for the existence of antioxidant and thrombolytic agents.

Keywords: *Plumbago zeylanica* L., Thrombolytic activity, Anti-oxidant activity, Cytotoxicity, Streptokinase, DPPH radical etc.

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INTRODUCTION

Plants are used in divergent approaches in managing various infirmities. These herbal agents are accomplished to amalgamate dissimilar secondary active metabolites; those possess the potential for remarkable biological activity. Biological activities present in these medicinal plants and their derivatives are of great importance in health care history. *Plumbago zeylanica* L. (Chromosome 2n=24) is also known as "Chitrak", a medicinal herb that belongs to the family of *Plumbaginaceae* (Figure 1).[1] It is the most commonly used medicinal plant, scattered all over the tropical and subtropical regions of the World.[2] The vernacular names of *P. zeylanica* L include, in Kannada- chitramula, in Malyalam-chitrakmula/ bilichitramula, in Tamil-Chita, and in Telugu kodiveli/chitramoolam.[3] It possesses various pharmacological activities like the flowers are used as digestant,[4] roots possess expectorant, abortifacient, laxative, astringent and anti-diarrhea activities.[5] Tincture of bark is used as anti-menstruation and leaves used in remedies for scabies,[6] antimicrobial, anti-inflammatory,[7] androgenic alopecia,[8] anti-fertility,[9] and anti-diabetic.[10] Plumbagin is the secondary

Figure 1: *P. zeylanica* L plant.

metabolite of the plant that possesses antibacterial activity against both Gram (+) and (-) bacteria.[11-12] As per the WHO reports, over 80% of the world population have been using various herbal agents in their primary health care. In general practice, herbal agents are commonly used to treat various kinds of diseases like asthma, different types of skin diseases, premenstrual disorder, rheumatoid joint pain, headache, menopausal manifestations, etc.[13]

These herbal agents could be a powerful source for curing thrombus in blood vessels with high safety and efficacy.[14] Coagulation or clotting of blood means cessation of the free flow of blood. The blood clot may form intravascularly and may associate with arterial diseases like myocardial infarction, venous thrombosis, and cerebral infarction diseases which are being major causes of mortality and morbidity in the World.[15] The starting stage which involves the formation of thrombosis because of damage or injury to blood vessels, adhesiveness, and agglomerating the platelets is the major jeopardize factor for the progression of vascular disorders.[16] For treating thrombosis-related diseases, many drugs like anti-platelets, anti-coagulants were applied to lyse the clot in blood vessels. However, among these majority of synthetic drugs are alike with the risk of excessive hemorrhage, severe anaphylactic reactions, and overarching safety and efficacy.[17-18] Continued investigation and research in the hemostasis area will furnish wisdom and promote development towards the formulation of the ideal clot lytic treatment with very less or minimal bleeding and other complications.[19] Besides these several cardiovascular complications there is an urgency of appropriate anticoagulant therapy in emerging infectious diseases like COVID-19. Therefore, it is essential to develop a more effective treatment with considerable safety. A recent study reported the anticoagulant property of the active principle present in the ethanolic root extract of *P. zeylanica* L which resembles the chemical structure of vitamin K.[20] In addition the few primitive communities in the southern region of India practice the use of *P. zeylanica* L leaves for anti-coagulation of blood. These few implications suggested us to search for active principles of plant leaves that could possess anticoagulant properties. The present communication was explored the thrombolytic potentiality of methanolic leaf extracts in *in vitro* and animal models. The plant extract was further tested for its antioxidant and toxicity effects.

MATERIALS AND METHODS

Procurement and authentication: Leaves of *P. zeylanica* L were gathered from Talakona forest (Chittoor, Andhra Pradesh, India) and certified by a botanist (Dr. K. Madhava Shetty) of Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A copy of the sample (0548) was referenced for the future.

Chemicals: DPPH and ascorbic acid were procured from Himedia Labs., Pvt. Ltd., Mumbai. Streptokinase was obtained from SRL Pvt. Ltd., Mumbai. Trichloroacetic acid and thiobarbituric acid were acquired from SD Fine Chemicals Ltd., Mumbai.

Extraction: Freshly dried and powdered leaf material (250 gm) was macerated in methanol for 24-72 hr and successively fractionated with different solvents to get petroleum ether extract (PEPZ), chloroform extract (CEPZ), ethyl acetate extract (EAPZ), methanol extract (MEPZ), and hydro alcohol extract (HAEPZ) etc. The resulted solvent extracts were concentrated and were stored in cold conditions for further investigations.[21]

Phytochemical analysis: Phytochemical analysis was investigated to assess the occurrence of various phytochemicals. Various qualitative tests were employed to find out the presence of glycosides, carbohydrates, tannins, saponins, alkaloids, flavonoids, steroids etc.[22] Occurence of the total soluble flavonoid and phenolic content of dried extract was specified in μg/QE/mg and mg GAE/g, respectively.[23-24]

In vitro thrombolytic activity

Preparation of standard streptokinase: Lyophilized streptokinase (marketed sample) vial of 1500000 IUs was mixed with 5 mL of aseptic pure water. From the resulted solution100 μL (30,000 IU) was withdrawn for *in vitro* thrombolytic assay as reference standard.[25-26]

Collection of blood sample: Approximately 4 mL of whole blood was withdrawn from the experimental animals, and the animals freed from the diseased conditions. The study was conducted by standard

protocols as per Institutional Animal Ethical Committee Certificate (IAEC) (1292/ac/09/CPCSEA/47/A, Vijaya College of Pharmacy, JNTUH, Munaganoor, Hyathnagar, Telangana). About 500 µL of blood was poured into all the 10 pre-measured weights of alpine tubes to form blood clots.

Clot lysis method: Initially the blood sample (500 µL) was poured in previously measured microcentrifuge tubes (sterile) and incubated (at 37°C for 90 min) to acquire blood clot formation. After centrifugation, the serum was collected without distributing the blood clot and measured the weight of tubes to find out the accurate weight of the blood clot.[27] The resulted blood clot was subjected for *in vitro* study to assess the clot lysis ability of leaf extract (at 200, 400, 600, and 800 µg/mL).[28] It was assessed for different incubation time intervals (at 24, 48, and 72 hr) at 37°C to find out maximum efficacy.

In vitro thrombin inhibition assay

The *in vitro* inhibitory effect on thrombin was assayed as per standard protocols where the decrease in fluorescence intensity could be observed on application of different concentrations of MEPZ.[29] Based on the measured intensity calculated the percentage (%) inhibition of thrombin activity.

In vitro antioxidant activity

As the methanolic leaf extract of *P. zeylanica* L was expressed potential *in vitro* thrombolytic activity it was further evaluated for its *in vitro* antioxidant potentiality with the aid of DDPH and nitric oxide (NO) scavenging ability method.

DPPH assay: The free radical (DPPH) scavenging ability of MEPZ was estimated employing standard protocols.[30] MEPZ and the reference standard (ascorbic acid) solutions were prepared separately. The plant extract (0.1 mL) was mixed in 3 mL of methanolic solution of DPPH (0.004%). The mixture was oscillated and allowed to stand for 30 min and the optical density was measured at 517 nm by using a UV-visible spectrophotometer. The IC_{50} value was determined by plotting a calibration graph of concentration (µg/mL) *versus* % radical scavenging $[\{(A_0-A_1)/A_0\} \times 100]$ [A_0 = absorbance with the control; A_1 = absorbance with the MEPZ].

NO scavenging assay: The test works on the basis of scavenging ability of plant extract to that of curcumin standard. Aqueous sodium nitroprusside solution is releases NO at physiological conditions; it can be generate nitrite ions by reacting with O_2, which can be assessed by the use of the Griess Illosvoy reaction.[31]

In vitro cytotoxicity

Cytotoxicity was assessed with the help of brine shrimp nauplii method.[32] It was carried out by using saline arrangement shrimp eggs (*Artemia salina* Leach). The shrimp eggs are produced for 48 hr for maturity in the 3.8% NaCl solution which is artificial seawater and matured shrimp called nauplii. The requisite quantity of MEPZ was dissolved in DMSO (50 µL in 5 mL solution) with artificial seawater to obtain the sterility of diluted concentrations of 12.5, 25, 50, 100, 200, 400, and 800 µg/mL of plant extract. Vincristine sulfate (VC) had taken as a positive control in serial dilutions (0.06, 0.125, 0.25, 0.5, 1, 5 and 10 µg/mL). After 24 hr all the vials were examined by amplifying the glass and the number of survived nauplii in each vial was detected and recorded.

$$\text{Mortality (\%)} = N_0\text{-}N_1/N_0{*}100$$

[Where, N_0= Number of nauplii taken, N_1 = Number of nauplii alive].

In vivo Experimental Design

The study animals were purchased from local breeders (Venkateswara Enterprises, Hyderabad) and were maintained as per CPCSEA norms. The animals were marked with a solution of picric acid to easily recognize the different experimental groups. The experimental protocol was approved by IAEC (1292/ac/09/CPCSEA/47/A). A total number of 30 Swiss albino mice were selected and arbitrarily divided into five (*n*=6). The test samples of extracts were prepared in 20% v/v of DMSO. Group I served as normal and administered with DMSO (20% v/v) solution. Group II served as standard which was administered with aspirin (20 mg/kg). Group III, IV, and V were treated with MEPZ at various doses (100, 200, and 300 mg/kg) of extract orally. After seven days of treatment, Groups II-V were injected intraperitoneally with carrageenan (30 mg/kg) to induce tail thrombosis in mice.[33] The blood clot size (mm) in was measured every 24 and 48 hr in clot induced tail.

Acute oral toxicity

The OECD guidelines 425 were adapted for acute oral toxicity of MEPZ.[34] Swiss albino mice (28 nos.) weighing 25-30 gm were divided into seven groups, with four in each group. Group I considered as control and to the Group II- VII the MEPZ extract was administered at 50, 100, 200, 400, 600 and 2000 mg/kg using oral gavage, respectively. The behavioral changes; toxicity indications and mortality were detected after treatment

in predetermined time slots (initially for the first 6, 14, and 24 hr followed by daily for 14 days).

Statistical analysis

The experiment results were statistically indicated with the help of Tukey's multiple comparison tests using Graph pad prism software (Version 5.0). The obtained data were expressed with SEM (±) and the level of significance was expressed as $p \leq 0.05$.

RESULTS

Extraction: On successive fractionation, the percentage yield of crude solvent extracts was found as 0.94% w/w of PEPZ, 3.8% w/w of EAPZ, 4.9% w/w of CEPZ, 3.9% w/w of MEPZ and 2.28% w/w HAEPZ.

Phytochemical analysis: As per the preliminary phytochemical investigations the *P. zeylanica* L. leaves manifested the residence of alkaloids, flavonoids, saponins, tannins, steroids, and glycosides (Table 1). It was well known fact that the secondary metabolites could possess a diverse range of pharmacological activities. The literature reports suggests that the flavonoids could serve in curing cardiovascular diseases, cancer, and other diseases.[35-36] An ample interest has been focused on the occurrence of antioxidant properties of flavonoids as they reduce the free radical formation.[37-38] Therefore, there is a necessity to quantify the phenolic and flavonoid content. As per the results, the total phenolic and flavonoid content of the MEPZ was found as 96.8±7.92 (mg GAE/g) and 63.52±4.54 (mg QE/g), respectively.

In vitro thrombolytic activity

In an *in vitro* thrombolytic model, about 96.83%±0.657 of clot lysis was achieved at the concentration of 800 µg/mL for MEPZ in 72 hr. On other hand, the HAEPZ also possess 92.73%±0.768 of clot lysis. The other solvent extracts PEPZ, EAPZ and CEPZ also found with moderate clot lysis effect i.e., 68.67%±0.974, 71.16%±0.235, and 73.68%±0.975, respectively (Table 2). The streptokinase standard and control displayed 97.26%±0.974 and 41.33%±0.843 of clot lysis, respectively (Table 3). The *in vitro* studies indicated that the phytoconstituents comprised in methanol and hydroalcoholic extracts served prominent thrombolytic properties (Figure 2 and 3).

Table 1: Phytochemical profile of *P. zeylanica* L leaf extract.

Test	EAPZ	EEPZ	CEPZ	MEPZ	HEPZ
Alkaloids	+	+	+	-	+
Flavonoids	-	-	-	+	+
Saponins	-	+	-	+	+
Tannins	-	+	-	+	+
Steroids	+	+	-	-	-
Glycosides	+	-	+	-	-
Carbohydrates	-	-	-	-	-
Proteins	-	+	+	+	+
Amino acids	-	+	+	+	+
Mucilage	-	-	-	-	-

Note: + indicates presence of compounds, - indicates absence of compounds.

Table 2: *In vitro* thrombolytic activity of solvent extracts of *P. zeylanica* L leaves.

Conc. of leaf extract (µg/mL)	Incubation time (hr)	Clot lysis in percentage (%) Mean±SEM				
		PEPZ	EEPZ	CEPZ	MEPZ	HAEPZ
200	24	8.63%±0.681	8.36%±0.574	10.66%±0.785	11.68%±0.884	9.98%±0.887
	48	23.54%±0.324	25.45%±0.568	27.84%±0.741	31.83%±0.978	26.34%±0.884
	72	39.64%±0.543	39.87%±0.427	44.78%±0.357	47.85%±0.768	46.93%±0.987
400	24	9.89%±0.684	11.23%±0.781	13.38%±0.765	19.85%±0.769	15.75%±0.858
	48	25.98%±0.981	30.54%±0.256	31.24%±0.236	38.28%±0.258	30.28%±0.258
	72	43.87%±0.688	44.89%±0.284	48.68%±0.314	63.76%±0.858	49.65%±0.665
600	24	13.23%±0.657	13.53%±0.581	13.83%±0.854	19.54%±0.657	17.65%±0.988
	48	28.98%±0.596	33.97%±0.541	39.92%±0.689	59.88%±0.894	47.78%±0.987
	72	52.21%±0.859	48.88%±0.234	51.98%±0.877	82.27%±0.858	69.67%±0.754
800	24	16.36%±0.945	16.86%±0.254	16.88%±0.778	23.75%±0.678	21.87%±0.857
	48	33.85%±0.648	40.81%±0.288	43.68%±0.882	68.86%±0.997	61.76%±0.567
	72	68.67%±0.974	71.16%±0.235*	73.68%±0.975**	96.83%±0.657***	92.73%±0.768**

Note: Sample volume (n) =3, SEM= Standard Error Mean; PEPZ= Petroleum ether extract; EEPZ= Ethyl acetate extract; CEPZ= Chloroform extract; MEPZ= Methanol extract and HAEPZ= Hydro alcoholic extract.

In vitro Thrombin Inhibitory Activity

MEPZ in seven concentrations ranging from 50 µg/mL-2 mg/mL were incubated in buffer solution with thrombin substrate for 5 min followed by with thrombin (1U/mL). Based upon intensity of fluorescence (after 60 min incubation) it was displayed thrombin inhibition with increasing dose of MEPZ extract. The leaf extract at 2 mg/mL inhibited thrombin activity with highest potency (94.63±2.12%). The test concentrations ranging from 50-200 µg/mL of leaf extract moderately inhibited. Furthermore, the results indicated the considerable thrombin inhibitory activity i.e., 76.82±2.18%, 84.91±2.41% and 92.32±1.28% at 500, 800 and 1000 µg/mL of MEPZ, respectively (Figure 4).

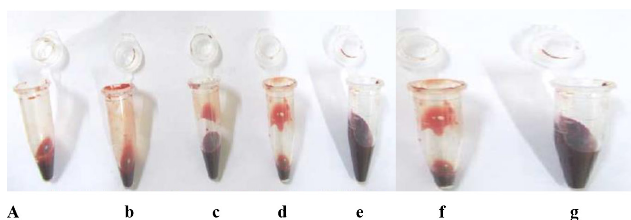

Figure 2: Clot lysis images of different extracts of *P. zeylanica* L leaves. a) PEPZ b) EEPZ c) CEPZ d) MEPZ e) HAEPZ f) Water and g) Streptokinase.

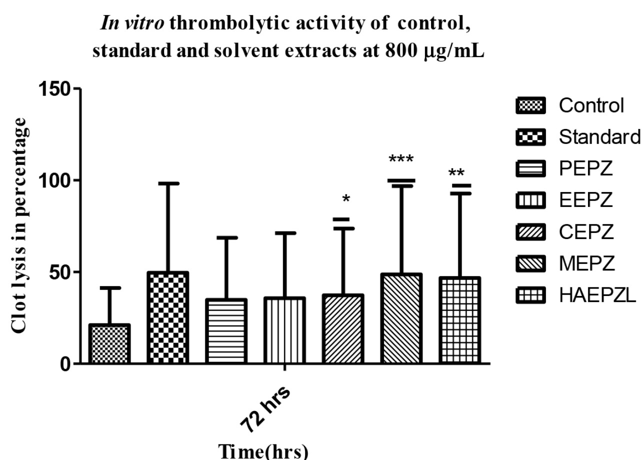

Figure 3: Graphical report of clot lysis effect of control, standard and solvent extracts of *P. zeylanica* L leaves at 800 µg/mL concentration.

In vitro antioxidant activity

DPPH assay: Antioxidant activity of MEPZ was quantitatively determined by DPPH assay.[39] It was found that the percentage inhibition at a dose of 40 µg/mL of the MEPZ was almost equivalent to 10 µg/mL of standard. The IC_{50} value of MEPZ was indicated the reduction in DPPH activity and the results found statistically significant (Table 4).

NO assay: NO scavenging effect of extract proportionally increased at tested concentrations. The MEPZ effectively reduced the generation of NO radicals.[40] The IC_{50} value of extract was found at 56.32 µg/mL whereas curcumin standard was showed at 25.20 µg/mL (Table 5).

In vitro cytotoxicity

The brine shrimp lethality bioassay was accomplished as it is a simple and effective method to assess the toxic effect of plant extract on biological systems. The lethality study of a MEPZ with the aid of simple organism like shrimp (*Artemia salina*) was achieved by cytotoxic evaluation. As per the bioassay, the LC_{50} value is lower than 1000 µg/mL was considered as bioactive. In our study, lethality shown by MEPZ was found to be commensurate to the congregation of the extract concentration ranging from 12.5 µg/mL to 800 µg/mL (Table 6). The concentration-dependent mortality (%) of brine shrimp nauplii obtained by the MEPZ designated the occurrence of the cytotoxic constituents. Perhaps the observed cytotoxic action could be due to the effect of some kinds of alkaloid and steroidal

Table 4: DPPH radical scavenging activity of MEPZ.

Test compound	Percentage (%) DPPH scavenging activity in Mean±SEM					IC_{50} (µg/mL)
	2.5 µg/mL	5 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	
AA	27.28±1.52	41.24±3.38	68.05±8.12	76.74±9.42	93.65 ±8.53	7.9±0.61
MEPZ	8.63±0.82	18.17±1.17	34.33±2.24	48.55±1.38	65.37±3.10	25.47±0.51

Note: Values are in mean ± SEM (n=3); AA: Ascorbic acid.

Table 5: NO scavenging activity of MEPZ.

Test compound	Percentage (%) NO scavenging activity in Mean±SEM					IC_{50} µg/mL
	2.5 µg/mL	5 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	
Curcumin	35.18±2.17	45.24±1.65	66.85±8.12	86.74±5.42	91.65 ± 8.63	25.20±0.53
MEPZ	10.11±0.82	19.47±0.87	37.33±2.34	56.55±3.38	78.37±4.16	56.32±0.85

Note: Values are in mean ± SEM (*n*=3).

Table 6: Brine shrimp lethality bioassay (LC_{50}) MEPZ.

Test sample	Concentrations (µg/mL)	Percentage (%) mortality	LC_{50} (µg/mL)
MEPZ	12.5	40	45.42
	25	40	
	50	50	
	100	60	
	200	80	
	400	100	
	800	100	
VC	0.06	10	0.699
	0.125	20	
	0.25	30	
	0.5	40	
	1	50	
	5	90	
	10	100	

Note: VC; Vincristine.

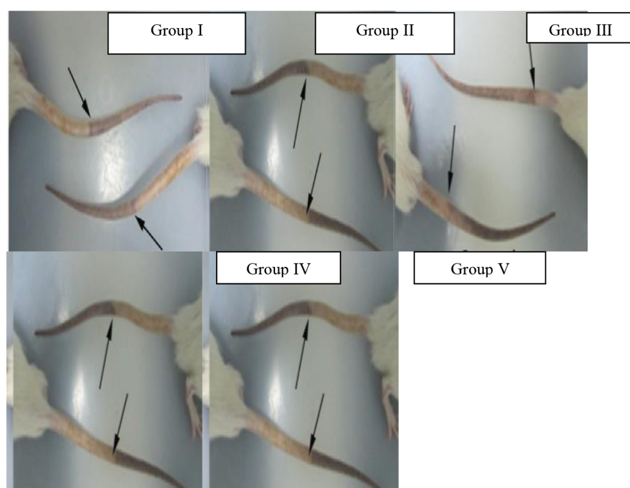

Figure 5: Photographs of tail thrombosis of mice tail in 48 hr showed the effects of MEPZ in animal model (Mice).

constituents. Several reports have been explored the role of alkaloids and steroids in the cytotoxic activity in plants. However, phenolics and flavonoids have been also known to show cytotoxicity as per Hoechst 33258 fluorescence assay.

In vivo thrombolytic activity

The full length of the mice's tail was measured in this experiment and obtained data were analyzed statistically. The length of tail thrombosis was recorded at 24 and 48 hr were shown in (Figure 5). The average length of the tail thrombosis in the normal group was 12.40±1.36 at 24 hr and 12.36±1.12 at 48 hr. The length of the tail thrombosis was reduced to 9.64±1.45, 8.63±1.61 and 7.96±1.67 at 24 hr and 9.36± 1.97, 7.69±0.39 and 7.03±1.07 at 48 hr after treatment of 100, 200 and 300 mg/kg of MEPZ, respectively (Table 7). When the results were compared with Group II (standard), at tested doses of MEPZ (100, 200 and 300 mg/kg) remarkably ($p< 0.001$) inhibited the thrombus formation with every increment of dose. The results of *in vivo* study suggested that MEPZ could stop the development of tail thrombosis in mice by carrageenan. Group I Normal -20% DMSO, Group II-20 mg/kg of Aspirin, Group III, IV and V = MEPZ (100, 200 and 300 mg/kg).

Table 7: *In vivo* thrombolytic activity of MEPZ in animal model.

Experimental group	Dose (mg/kg)	Blood clot length of the tail (Mean±SEM, *n*=6)		
		Full length of thrombotic tail	After 24 hr treatment	After 48 hr treatment
Group I	-	16.99±0.83	12.40±1.36	12.36±1.12
Group II	20	17.78±0.13	11.57±1.57	11.57±1.45
Group III	100	17.42±0.65	9.64±1.45***	9.36±1.97***
Group IV	200	15.52±0.36	8.63±1.61***	7.69±0.39***
Group V	300	14.25±0.63	7.96±1.67***	7.03±1.07***

Note: Group I = Normal; Group II; Aspirin treated, Group III, IV and V =MEPZ (100, 200 and 300 mg/kg) Data were represented as Mean±SEM (*n*=6).*$p<0.05$, **$p<0.01$, *** $p<0.001$ significant when correlated control group.

Acute Toxicity Study

The acute toxicity profile of MEPZ at different concentrations 50, 100, 200, 400, 600 and 2000 mg/kg not showed the signs of toxicity with no deaths of the animal. For every 6, 14, and 24 hr, the behavioral patterns of animals were recorded in a treatment groups. The animals in both groups showed no remarkable change in behavioral patterns like catatonia, muscle rigidity, watery saliva, diarrhea, tremors and water consumption.

DISCUSSION

The phenolic compounds are present as per phytochemical tests. The pervasive constituents of many herbal plants have tremendously added for research interest, because of their beneficial properties as antioxidants. Thus it is advisable to search plants with polyphenolic, flavonoid content, and antioxidant activity.[41] Awkwardly have been centered on the discovery of plant derivatives as constructive appurtenance or even proxy to those ongoing antithrombotic drugs.[42] Natural antioxidants are an excellent source obtained from plants and their primary function is safety towards

oxidative stress of free radicals.[43] Progress of reactive oxygen species plays a vital role in oxidative stress,[44] and literature review revealed that it can be minimized by few plant agents like quercetin and gallic acid,[45-48] and also they have been effectively works in various free radical induced diseases. In this study, the MEPZ exhibited potential antioxidant activity ($p<0.001$) to the standard used. The experimental findings suggests that occurrence of *in vivo* thrombolytic effect of MEPZ at three test concentrations (100, 200, and 300 mg/kg) at 24 and 48 hr ($p<0.001$). The administered higher dose (2000 mg/kg) not showed any notable oral toxicological signs and mortality in the 14 days of treatment. Further phytochemical isolation and their structure elucidation existing in the leaves could help in exploring the active principle with the mechanistic pathways.

CONCLUSION

In the light of the present findings of the current research explored the adequateness of methanolic leaf extract of *P. zeylanica* L in clot dissolving (thrombolytic) properties with enough oxidative potential. These findings support in identifying the new chemical entities potential pharmacological properties with apposite molecular mechanisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; ***P. zeylanica* L:** *Plumbago zeylanica* L, **DPPH:** 1, 1-Diphenyl-2-picrylhydrazyl; **DMSO:** Dimethyl sulfoxide, **OECD:** Organization for Economic Cooperation and Development; **IC_{50}:** Half-maximal Inhibitory concentration; **g:** Gram; **mg:** Milligram; **IU:** International Units.

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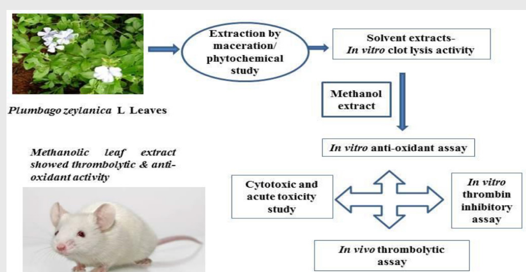
PICTORIAL ABSTRACT



SUMMARY

A maceration process was employed for the extraction of phytoconstituents from leaves of one of the conventionally useful medicinal plants *P. zeylanica* L. The phytochemical study indicated the occurrence of phenolic and flavonoid content in leaves. As per the *in vitro* clot lysis assay, the methanolic leaf extract exhibited its thrombolytic properties. Furthermore, it was confirmed with its thrombin inhibitory effects. Thus, the methanolic extract was subjected to *in vivo* thrombolytic screening on clot induced model of mice. The leaf extract at tested concentration exhibited significant ($p<0.001$) thrombolytic activity to the heparin standard. To predict the antioxidant property, the leaf extract was further subjected to DPPH and nitric oxide assay. These studies elicited the antioxidant properties in addition to clot lysis effects. Further studies such as isolation and characterization of phytoconstituents and the establishment of mechanistic ways may strengthen the current findings.

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