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Original Article

GC-MS Analysis and Thrombolytic Property of Methanolic Leaf Extracts of *Terminalia pallida* Brandis against Carrageenan Instigated Tail Thrombosis Model in Mice

Sarvan Kumar Guguloth[1,2], Narender Malothu[1,*], Prasanth DSNBK[1]

ABSTRACT

Background: Several plants of *Terminalia* species are being reported as medicinally useful. *Terminalia pallida* Brandis is one of the plants of the *Combretaceae* family, which constitutes several pharmacologically active substances. Present work explored leaf extract's thrombolytic property, which could serve as the superior choice for currently using drugs. **Objectives:** The present study primarily focused on investigating the phyto-constituents such as phenolic and flavonoid compounds in methanolic leaf extract followed by GC-MS analysis and determining the *in vitro* thrombin inhibition and *in vivo* thrombolysis activity. **Materials and Methods:** Swiss albino mice (total = 36) were randomly distributed into six groups (n=6). Thrombosis was instigated by injecting 40 µL of 1% carrageenan (Type-I) via the subplantar region of the right hind paw of each mouse. The plant extract was screened for its *in vitro* thrombin inhibitory potency. In animal studies, the size of the blood clots in mouse tails was recorded by administering the plant extracts at 100, 200 and 300 mg/kg body weight for every 24, 48 and 72 hr, respectively. **Results:** A dose of 200 and 300 mg/kg of the extract showed significant thrombolytic activity at 24, 48, and 72 hr (p< 0.001) in a concentration-dependent manner when correlated with the control group. A reduction in the length of blood clots (p< 0.01) was observed at 48 and 72 hrs. In acute oral toxicity study, administration of leaf extract showed no mortality and no significant behavioral changes up to 2000 mg/kg dose. The GC-MS analysis explored the occurrence of about 16 eluted compounds; among these, few have been reported as medicinally useful, which accounts for the therapeutic importance of the plant. **Conclusion:** In conclusion, the methanolic extract of *T. pallida* Brandis leaves at 200, and 300 mg/kg showed significant inhibition of the Thrombosis in carrageenan instigated model of mice and *in vitro* thrombin activity.

Key words: Thrombosis, Carrageenan, *Terminallia pallida* Brandis, Heparin, Quercetin.

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INTRODUCTION

Development of clot or Thrombosis is a tricky biochemical and physiological cascade participated by various physiological factors, including rupture of blood vessels, adhesion, and aggregation of platelets.[1,2] Thrombosis is one of the significant causes of morbidity and mortality throughout the world and is responsible for developing various cardiovascular and cerebral diseases with a high impact on the health and socio-economic status of the population.[3] Stroke and heart attacks are mainly triggered by forming a clot in the arteries and atherosclerotic plaques.[4] The haemopoietic system plays a significant role in homeostasis between forming a clot (fibrin) and its lysis (fibrinolysis) to prevent and protect from blood loss and ensure tissue perfusion by forming a platelet plug.[5] To protect against hemorrhage, the physiological interaction between clotting factors and platelets occurs, followed by formation of a hemostatic plug that could stop the free flow of blood at the site of vascular injury. The principal enzyme responsible for the clotting process is thrombin, while plasmin is an enzyme responsible for fibrin and fibrinogen degradation.[6,7] Several synthetic and semi-synthetic drugs such as oral anticoagulants, antiplatelets, or thrombolytics are available for treating various thrombotic disorders (vascular blockage, myocardial or cerebral infarction, venous thromboembolism, and deep vein thrombosis). Most of these drugs exhibit adverse effects like bleeding, severe anaphylactic reactions, overarching safety, and efficacy.[8] Approximately 4% of the world's population has been experiencing excessive bleeding or hemorrhage, 14% of patients worldwide require a blood transfusion. These findings suggest that bleeding is the most alarming side effect of modern synthetic medicines evokes cardiovascular outcomes and carries the risk of death.[9,10]

Despite in cardiovascular diseases the development of coagulopathy is also one of the important signs

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of poor prognosis in patients affected by recent pandemic Covid-19. Elevation of marked D-dimer is a predictor for mortality and hospitalization of Covid-19 patients. The severity of COVID-19 is being associated with coagulation, use of anticoagulants potentially improve prognosis according to various reports from a retrospective study.[11] In the connection of development of new effective anticoagulants the plant extracts have been found as most effective and preventable alternative medicines for thrombus-related diseases.[12] As per the World Health Organization (WHO) reports majority of the populations (over 80%) in developing countries are being using herbal drugs in their health care.[13] As per the recent reports the variety of phytochemical constituents such as coumarins, flavonoids, tannins, and phenols were isolated and identified their significance in the hemostasis.[14] A naturally occurring plant agent rutin, also called rutoside, is chemically flavonoid glycoside abundantly present in *Fagopyrum esculentum* (buckwheat), showed effective thrombolytic activity in mice and humans (IC_{50} value is 6.1 µM). The plant constituent rutin has been proved that it is reversibly inhibiting the protein disulfide isomerase in the process of anticoagulation.[15] In addition, various potential plant extracts and constituents like borneol,[16] sulfated (1-3)-β-L-arabinan of *Codium vermilara,*[17] crude extract of *Erigeron canadensis* L,[18] 2,3,5,4-tetrahydroxy stilbene-2-O-β-D-glucoside of *Polygonum multiforum,*[19] salvianolic acid B-*Salvia miltiorrhiza,*[20] pomolic acid- *Licania pittier,*[21] rhynchophylline,[22] with probable mechanism of action have been reported in various literature sources. Though, no new herbal agent has been established for complete anticoagulant therapy**.** Hence, there is an urge for the development and exploration of potential medicine to treat Thrombosis with safe and effective therapy with no side effects.

Terminalia pallida Brandis is a plant of the *Combretaceae* family, commonly found in tropical and subtropical countries [Figure 1]. It is called with different local names in the southern region of India (i.e., Tellakaraka and Velmakaraka in Telugu, Vallaikkadukkay in Tamil, and white gallnut tree in English.[23]

The plant part constitutes plenty of medicinally useful phytoconstituents. Fruits possess anti-diabetic properties,[24] and the leaves are being used in various herbal formulations, pharmaceuticals, and animal husbandry.[25] The powdered form of fruit concoction is being used to treat the hemorrhoids.[26] In the connection of our investigations on the assessment of thrombolytic properties of *T. pallida* Brandis,[27] the leaf extract was tested to know the thrombin inhibition property as the enzyme is crucial in clot formation. Further, it's *in vivo* thrombolytic activity was determined on animal studies using carrageenan instigated tail thrombosis model of mice. The carrageenan induced tail thrombosis model chosen as it is non-invasive type and simple to perform on small laboratory animals with stress free conditions.[28,29] The present communication described the findings of phytochemical investigation, GC-MS analysis and *in vivo* thrombolytic activity of METP.

Figure 1: Plant material of *T. pallida* Brandis a) Plant leaf b) Leaf powder.

MATERIALS AND METHODS

Chemicals

Carrageenan type-I, low molecular weight heparin, methanol, DMSO, quercetin etc., utilized in this work were procured from SD Fine Chemicals, Mumbai, India.

Collection and authentication of plant

As this plant is widely distributed in the southern region of India, the leaves were collected from Tirumala hills, Chittoor, Andhra Pradesh and authentication of the plant was done by Dr. K. Madhava Shetty, Taxonomist, Sri Venkateswara University, Tirupati, and Andhra Pradesh. A copy of the sample (0821) was referenced for the future.

Procedure for extraction

The leaves were desiccated at ambient temperature (in the air). Dried leaves were powdered and weighed about 250 gm for solvent extraction. The powder was macerated for 24-72 hr and subsequently extracted with methanol (1 lit). Resulted leaf extract was concentrated using a rotary evaporator (*in vacuo*) and was preserved in cold conditions.[27,30]

Estimation of total flavonoid content

Flavonoids are the clusters of polyphenolic admixture, which manifest diverse pharmacological outcomes. It was performed by treating about 2.5 mL of leaf extract with $AlCl_3$ (2.5 mL) in 90% ethanol and kept at ambient temperature for 40 min.[31] The absorbance of resulted mixture was recorded at 415 nm by using a UV-visible spectrophotometer (Shimadzu 1501 model). The experiments were conducted in triplicate by taking ethanol as a blank and quercetin as a reference standard. The total flavonoid content was estimated and indicated in µg/QE/mg of dry extract.

Estimation of total phenolic content

Secondary metabolites of plants consist of the rich source of phenols which usually possess a wide range of pharmacological properties. The test was performed with FC reagent using pyrocatechol as the standard.[31,32] The plant extract (about 0.2 mL) was treated with 10 % w/v of FC (1 mL) reagent in 7.5 % w/v sodium carbonate (0.8 mL). Resulted mixture was kept in incubation for 1 hr and the phenolic content was calculated based on absorbance recorded at 760 nm. The total phenolic content indicated in mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

Experimental animals

The Swiss albino mice weighing between 30-35 grams were employed in the present work. The experimental mice were procured from registered breeders (Venkateswara enterprises, Hyderabad) and were maintained under standard conditions (temperature-22 ±2°C; relative humidity-30-70 %) with a 12:12 light-dark cycle. Also, the animals were fed with a standard pellet diet and water *ad libitum*. The IAEC was approved (1292/ac/09/CPCSEA/47/A) the proposed experiment protocols at Vijaya College of Pharmacy, affiliated to JNTUH, Munaganoor, Hayathnagar, and Ranga Reddy.

Thrombin inhibition assay

Thrombin inhibition assay was performed by employing standard protocol's described by Batra *et al.*[33] Initially, the METP (as thrombin inhibitor) was incubated with Tris-buffer at pH 7.5 in a 96 well plate.

Then, thrombin substrate III (0.2 mM, prepared from Kit) followed by thrombin (1U/mL) was added to incubate. After incubation for 1 hr the 96-well plate was read for fluorescence intensity by taking 450 nm as emission wavelength and 390 nm as excitation wavelength on fluorimeter (Spectra Max M5e, Molecular Devices). The reduction in flouroscence intensity to that of concentration of METP was measured and calculated the percentage (%) inhibition of thrombin activity.

Experimental study design

Swiss albino mice (Total-36 nos) were randomly divided into 6 groups (n=6). The leaf extracts were disintegrated in 20% v/v DMSO in normal saline (diluent). The control group was injected with DMSO (20% v/v) and the experimental treated groups were injected through the intraperitoneal route with 100, 200, and 300 mg/kg of METPs. The standard group animals were injected with 10 IU and 100 IU of low molecular weight heparin. To instigate the blood clot in the tail the animals were injected with 40 µL of 1% carrageenan (type-I) in the subplantar region of the right hind paw of each mouse after one hour of every dose of leaf extract.[34] The size (mm) of blood clots in the tail was recorded every 24, 48, and 72 hrs after the injections of carrageenan, respectively.

Determination of acute oral toxicity (LD50): Acute oral toxicity studies of METP of *T. pallida* Brandis performed according to OECD guideline 425.[35] A total of 28 Swiss albino mice (25-30 gm) in seven groups were employed for toxicity studies. Group I served as control and to the other experimental groups 50, 100, 200, 400, 600 and 2000 mg/kg of the METP was administered using oral feed, respectively. All the mice were noticed for general behavioral changes; signs of toxicity and mortality after treatment for the first 6, 14, and 24 hr, after that daily for 14 days.

GC-MS analysis: GC-MS is one the effective chemical analytical method for confirming phytoconstituents. It will provide an emissary spectral output of all the probable components that get separated from the sample. The procedure involves by injection of the sample to the port of the GC-MS device where vaporization takes place followed by separation was achieved with an analyzer. Each component was producing an ideal specific peak, which is recorded on a paper chart electronically. GC-MS analysis of METP was performed at CSIR- Indian Institute of Chemical Technology (IICT), Habsiguda, Hyderabad. The data was recorded on combined gas chromatogram system (Agilent GC-MS5977B) and mass spectrometer, fitted with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µM, film thickness 0.25 µM), interfaced with 5675C Inert MSD with Triple-Axis detector.[36]

Statistical Analysis

The results of the entire study were statistically presented by using software (Graph pad prism, Version 5.0). The statistical divergence was explored by one-way analysis of variance by Tukey's multiple comparison tests. The obtained data were notified as mean ± standard error mean and the level of significance was contemplated as $p \leq 0.05$.

RESULTS

Total phenolic and flavonoid content in METP

The macerated solvent extract of leaves was concentrated which gave about 2.3 % w/w crude methanolic extract. In which the total phenolic content was found as 16.97 mg GAE/g. While the total flavonoid content in obtained was 7.42 mg/QE/mg.

In vitro thrombin inhibition activity

The crude METP was incubated in buffer solution with thrombin substrate (50 µg/mL-2 mg/mL) for 5 min, then with thrombin (1U/mL). The fluorescence was read out was recorded by fluorescence reader after 60 min. Treatment of methanolic leaf extract showed thrombin inhibition in dose dependent manner. The maximum thrombin inhibition (93.76±2.98%) was achieved at 2 mg/mL. Moderate to remarkable inhibition was observed for test concentration 50-200 µg/mL. Moreover, there was substantial increment in percent inhibition was found for 500 µg/mL, 800 µg/mL and 1 mg/mL of METP (Table 1, Figure 2).

In vivo thrombolytic activity

Test groups-III and IV treated with plant extract (at 200 and 300 mg/kg) were showed significant activity at 24 hr ($p< 0.001$) to that of control group [Figure 3]. Also it showed a reduction in length of blood clots ($p< 0.01$) at 48 and 72 hr at 200 and 300 mg/kg. The standard group (Group-V) treated with 10IU of low molecular weight heparin was not shown significance at 24, 48 and 72 hr when correlated with the control group [Figure 4]. The thrombolytic activity of METPs against carrageenan instigated tail thrombosis model in mice at 24, 48 and 72 hr was summarized in Table 2.

Acute toxicity profile

In oral toxicity study, administrations of 50, 100, 200, 400, 600 and 2000 mg/kg of METP were not produced any clinical sign of toxicity, as well as no animal was died. The behavioral patterns of animals were recorded for the first 6, 14, and 24 hr after the administration of plant extract. The test animals were found normal and did not showed any notable changes in behavior, skin reactions. Further no animals were noticed the disability in food and water intake, breathing as well as postural abnormalities and signs of alopecia.

Table 1: Thrombin inhibitory activity data of METP.

S. No	Concentration of METP	Percentage inhibition (% Mean±SEM)
1	50 µg/mL	42.10±2.75
2	100 µg/mL	55.64±2.60
3	200 µg/mL	76.46±2.65
4	500 µg/mL	82.85±1.18
5	800 µg/mL	88.90±2.10
6	1 mg/mL	91.85±1.58
7	2 mg/mL	93.76±2.98

Note: SEM-Standard Error Mean.

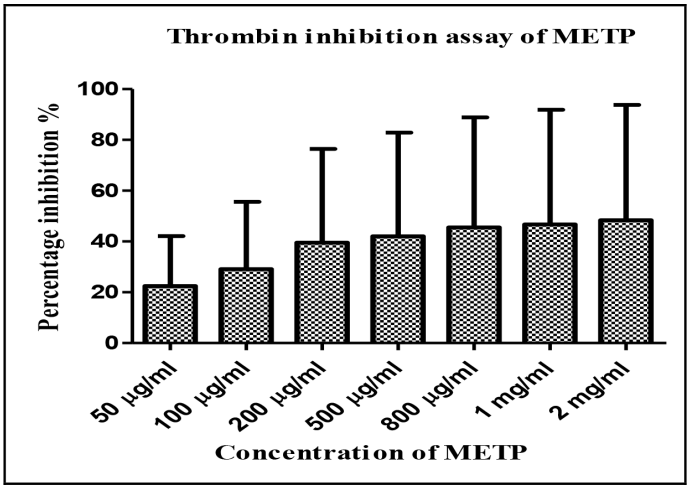


Figure 2: Graphical report of thrombin inhibitory activity of METP.

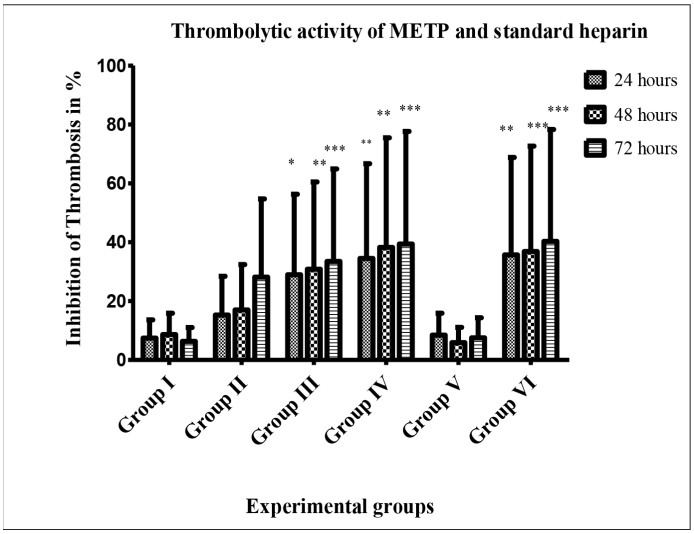


Figure 3: Thrombolytic activity of Group-I (Normal Control). Group-II-IV (METP of *T. pallida* Brandis at 100, 200 and 300 mg/kg), and Group-V and VI (Standard Heparin) on carrageenan instigated tail thrombosis model in mice.

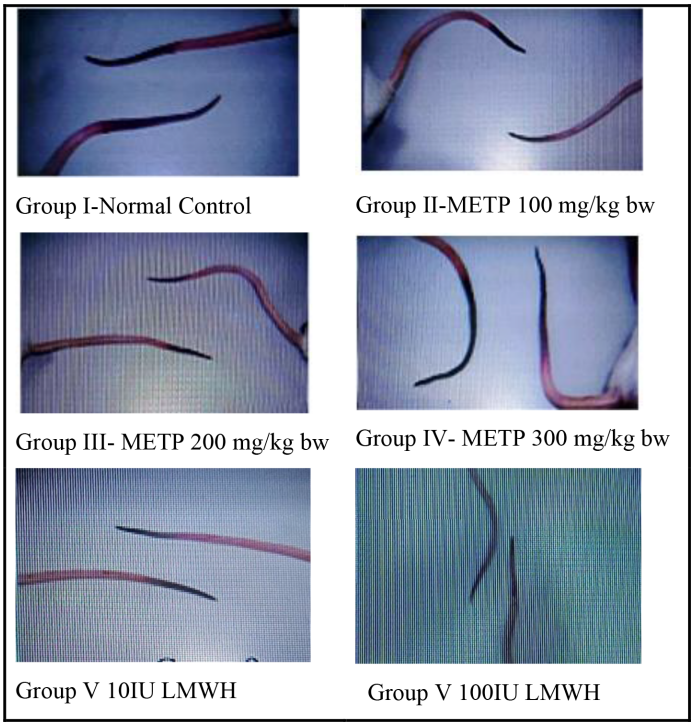


Figure 4: Photographs of tail thrombosis of mice tail at 48 hr, showing thrombolytic effect of Normal Control (Group I), METP of *T. pallida* Brandis (Group-II-IV) and standard (Group-V and VI) in carrageenan instigated thrombosis mice model (after 48 hr carrageenan injection).

GC-MS analysis of METP

In the GC-MS chromatogram of METP, a total number of 16 compounds were identified [Figure 5]. The compounds were eluted with different retention times and peak area which designates the presence of chemically diverse plant constituents. The retention time (Rt), peak area (A) and percentage (A %) of the eluted components were depicted in Table 3.

Table 2: Thrombolytic activity of METP in carrageenan instigated tail thrombosis model in mice.

Experimental group	Dose	Inhibition of blood clot in the tail (%Mean±SEM, *n*=6)		
		24 hr	48 hr	72 hr
I	20% DMSO	13.63±1.21	15.88±0.38	11.04±1.63
II	100 mg/kg	28.43±1.52	32.43±2.12	54.73±1.58
III	200 mg/kg	56.30±1.57*	60.46±1.15**	64.89±2.01**
IV	300 mg/kg	66.68±2.32**	74.45±1.02**	77.65±1.09***
V	10 IU	15.89±0.99	11.08±0.65	14.36±0.69
VI	100 IU	68.79±2.61**	72.62±1.08***	78.31±2.27***

Note: Group I = Normal control; Group II, III and IV = METP of *T. pallid* brandis (100, 200 and 300 mg/kg) Group V and VI = Low molecular weight heparin; Data were represented as Mean±SEM (n=6).*p<0.05, **p<0.01, *** p<0.001 significant when correlated control group.

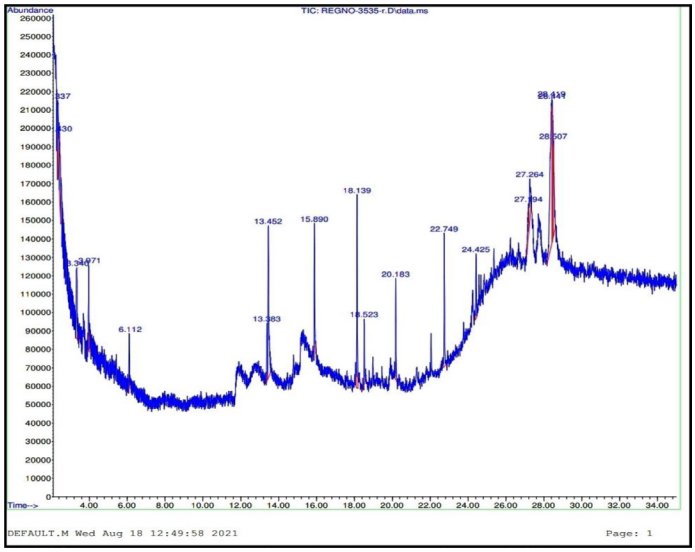


Figure 5: GC-MS chromatogram of METP of *T. pallida* Brandis.

In GC-MS analysis, the phyto-constituents were characterized by considering the percent elution followed by interpretation of mass spectrum with appropriate hits (NIST library etc). The molecular weight (MW), molecular formula (MF) and structure of characterized compounds are summarized in Table 4. Majority of the identified compounds were found biological active and they are reported as anticonvulsant, muscle relaxant (Propyl carbamate),[37] anti-cancer, anti-diabetic (Cyclotrisilioxane hexamethyl),[38] estrogenic (Decamethyltetrasiloxane),[39] and anti-oxidant (Adrenaline),[40] agents [Table 4].

Analysis of chromatogram and mass spectrum data of hit compounds revealed the presence of variety of plant components. Among these pyridine, 3-[2, 2-bis (trimethylsilyloxy) vinyl] - (20.88%), 3,7-ditert-butylnaphthalen-1-ol (10.252 %) were comprised majorly. The mass spectrums of the eluted compounds were shown in Figure 6.

DISCUSSION

Polyphenols and flavonoids are secondary plant metabolites naturally present in plants. As per earlier reports, most flavonoids possess antithrombotic properties.[41-44] the preliminary phyotchemical study

Table 3: Chromatographic parameters of GC-MS report.

S.No.	Chemical Name	Retention time (min)	Peak area	Percentage (%)
1	*N,N*-Dimethylformamide dipropyl acetal	2.33	1014006	3.98
2	Propyl carbamate	2.43	1043856	4.10
3	Methyl carbamate	3.34	1245544	4.88
4	1,2,4 Butanetriol	3.97	862219	3.38
5	Ethyl(dimethyl)isopropoxysilane	6.11	502932	1.97
6	3,7-Ditert-butylnaphthalen-1-ol	13.45	2643455	10.37
7	Octadecane	18.13	1839919	7.22
8	Adrenaline	18.52	518930	2.04
9	Eicosane	20.18	879341	3.45
10	Benzo[b]thiophen-2-amine, 3-phenyl-*N*-(phenylmethylene)	22.74	1420282	5.57
11	Octadecanoic acid, tert-butyldimethylsilyl ester	24.42	786513	3.09
12	Decamethyltetrasiloxane	27.19	585772	2.30
13	Benzo[h]quinoline, 2,4-dimethyl	27.26	951148	3.73
14	Benzoic acid, 2,4-dimethyl-, (2,4-dimethylphenyl) methyl ester	28.41	5321081	20.88
15	Pyridine, 3-[2,2-bis(trimethylsilyloxy) vinyl]-	28.43	951148	10.25
16	Cyclotrisilioxane hexamethyl	28.50	5321081	6.29

Table 4: Chemical composition identified in METP extract by GC-MS.

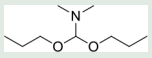
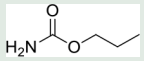
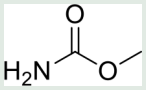
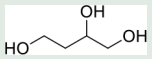
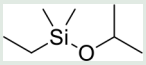
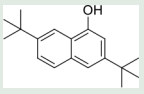
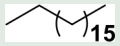
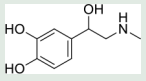
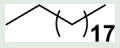
S. No.	Chemical Name	IUPAC Name	Mass (g/mol)	Molecular formula	Structure
1	*N,N*-Dimethylforma-mide dipropyl acetal	*N,N*-Dimethyl-1,1-dipropoxymethanamine	175.27	$C_9H_{21}NO_2$	
2	Propyl carbamate	Propyl carbamate	103.12	$C_4H_9NO_2$	
3	Methyl carbamate	Methyl carbamate	75.03	$C_2H_5NO_2$	
4	1,2,4 Butanetriol	1,2,4-Butanetriol			
5	Ethyl(dimethyl)isopropoxysilane	Ethyl(dimethyl)isopropoxysilane	146.11	$C_7H_{18}OSi$	
6	3,7-Ditert-butyl naphthalen-1-ol	3,7-Ditert-butyl naphthalen-1-ol	256.34	$C_{18}H_{24}O$	
7	Octadecane	Octadecane	254.49	$C_{18}H_{38}$	
8	Adrenaline	4-[1-Hydroxy-2-(methyl amino) ethyl] benzene-1,2-diol	183.2	$C_9H_{13}NO_3$	
9	Eicosane	Eicosane	282.33	$C_{20}H_{42}$	

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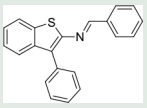
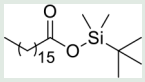
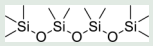
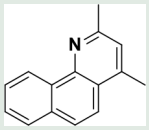
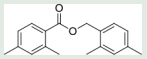
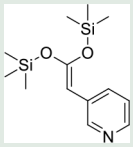
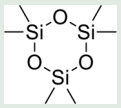
Table 4: Cont'd.

S. No.	Chemical Name	IUPAC Name	Mass (g/mol)	Molecular formula	Structure
10	Benzo[b]thiophen-2-amine, 3-phenyl-*N*-(phenylmethylene)	1-Phenyl-N-(3-phenyl-1-benzo thiophen-2-yl) methanimine	313.4	$C_{21}H_{15}NS$	
11	Octadecanoic acid, tert-butyldimethylsilyl ester	*t*-Butyl(dimethyl) silyl stearate	188.12	$C_9H_{20}O_2Si$	
12	Decamethyltetrasiloxane	[Dimethyl(trimethylsilyloxy)silyl]oxy-dimethyl-trimethylsilyloxysilane	310.68	$C_{10}H_{30}O_3Si_4$	
13	Benzo[h]quinoline, 2,4-dimethyl	2,4-Dimethylbenzo[h] quinoline	207.10	$C_{15}H_{13}N$	
14	Benzoic Acid, 2,4-Dimethyl-, (2,4-Dimethylphenyl) Methyl Ester	(2,4-dimethylphenyl)methyl 2,4-dimethylbenzoate	268.3l	$C_{18}H_{20}O_2$	
15	Pyridine, 3-[2,2-bis(trimethylsilyloxy)vinyl]-	Trimethyl-(2-pyridin-3-yl-1-trimethylsilyl oxyethenoxy)silane	281.50	$C_{13}H_{23}NO_2Si_2$	
16	Cyclotrisilioxane hexamethyl	2,2,4,4,6,6-Hexamethyl-1,3,5,2,4,6-trioxatri silinane	222.46	$C_6H_{18}O_3Si_3$	

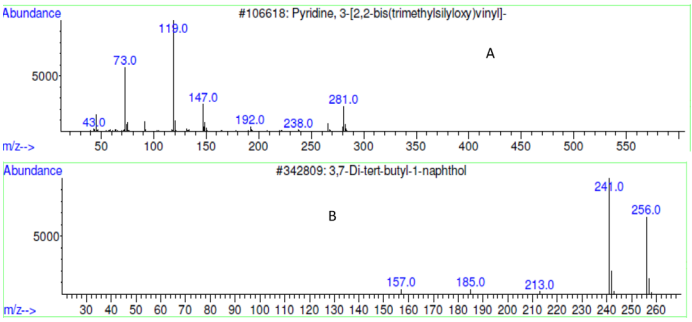


Figure 6: Mass spectrum of eluted compounds pyridine, A. 3-[2,2-bis(trimethylsilyloxy)vinyl]- (20.88%); B. 3,7-Ditert-butylnaphthalen-1-ol (10.252 %).

confirmed the occurrence of both flavonoid and phenolic compounds in methanolic extract. The *in vitro* thrombolytic screening (clot lysis: 95.43%±0.697) results showed potent activity at the test concentration (800 µg/mL) in our earlier studies.[27] Furthermore, the plant extract was tested with thrombin inhibition assay. In these studies, METP showed inhibition of thrombin activity (93.76±2.98%) at 2 mg/mL. The thrombin inhibition property indicated us to screen the effect of plant extract in Thrombosis induced animal model. In this connection the thrombolytic property of leaf extract was assessed in animal studies. The formation of a blood clot in vascular-related diseases seems to be one of the important risk factors which are responsible for mortality and morbidity in the world.[45] By considering the suitability of the study the carrageenan-instigated tail thrombosis model was employed to know the thrombolytic behavior. In the carrageenan-induced tail thrombosis model, a red color appears in the tip of the tail of the experimental animal and the length of the blood clot in the tail (thrombosis) increased with the time transpired followed by development of necrosis.[34] Results obtained suggested that *in vivo* thrombolytic effect of METP at 200 and 300 mg/kg at 24, 48 and 72 hr ($p<0.001$) significant thrombolytic activity in concentration dependent manner. In general, oral acute toxicity studies are being conducted to acquire suitable dose for chronic toxicity tests and find out the pretentious organs at the end of the treatment.[46,47] In oral acute toxicity, at the preferred higher dose (2000 mg/kg) the plant extract was not showed any significant toxicological signs, and mortality throughout the 14 days of treatment.

The preliminary GC-MS investigations confirmed the constitution of phenolic compounds in the plant extracts, which was correlating with the phytochemical tests. Probably, occurrence of these constituents primarily indicated the thrombolytic properties of plant leaves. Further studies such as isolation and structure elucidation of the components of the methanolic extract could explore the active principle there by possible mechanism of action.

CONCLUSION

In the present study, the preliminary phytochemical investigations were indicated the presence of phenolic and polyphenolic compounds in the methanolic extracts. In thrombin inhibition assay**,** the plant extract showed 93.76±2.98% of inhibition at 2 mg/mL which indicated its thrombin lysis property. In the assessment of thrombolytic property against carrageenan instigated tail thrombosis in mice model showed significant inhibition (clot lysis-67.45±1.02**) of Thrombosis at 200 and 300 mg/kg of plant extract in concentration dependent manner. However, the exact mechanism of the blood clot dissolving property of these extracts was needed to be established. The GC-MS studies confirmed the presence of biologically active constituents. As per the analysis the methanolic extract was majorly comprised with phenolic compounds. This study could serve as a new insight for future investigation for herbal thrombolytic agent's which can lead the identification and characterization.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of statement.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **OECD:** Organization for Economic and Cooperation Development; **IAEC:** Institutional Animal Ethical Committee; **METP:** Methanolic extract of *Terminalia pallida* Brandis; **FC:** Folin Ciocalteu's; ***T. pallida:*** *Terminalia pallida* Brandis; **DMSO:** Dimethyl Sulfoxide.

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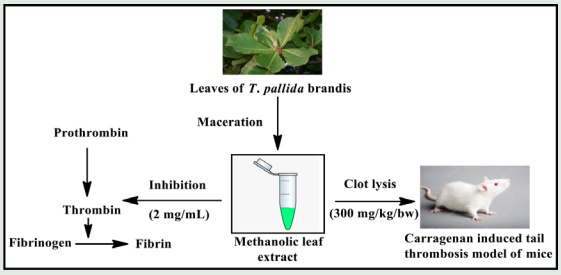
GRAPHICAL ABSTRACT



SUMMARY

The methanolic extract obtained from the dried leaves of *T. Pallida* Brandis was screened for flavonoid and phenolic content and evaluated the GC-MS data. *In vitro* thrombin inhibition assay was tested for extracts. Further the thrombolytic activity was tested on carrageenan instigated tail thrombosis animal model in mice. In these studies GC-MS reports suggests the occurrence of 16 compounds. The thrombolytic activity was found significant at test doses in *in vivo* animal model.

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