Invitro Thrombolytic Activity on Leaf Extract of Vitex Leucoxylon

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ABSTRACT

The study is regarded for standardization of leaf extract of Vitex leucoxylon, study focus on phytochemical investigations, isolation of flavonoids and evaluation for thrombolytic activity from various solvents. Pharmacognostical, phytochemical studies and in-vitro thrombolytic activity of leaves extract of Vitex leucoxylon was carried out by using in-Vitro model. The extracts were subjected to qualitative chemical analysis; chromatographic studies (TLC & HPTLC) were performed for flavonoids detection. The samples blood transferred to sterile microcentrifuge tubes incubated at 300C for 90mins. The serum removed completely and each tube having clot weighed to get clot weight. The serum with the clot added standard drug or plant extract. The tubes again incubated at 370 C for 45mins, the fluid obtained after clot lysis removed and tube again weighed. An alcoholic extract (20mg/100µl & 10mg/100µl) was evaluated in incubated blood. An attempt made to standardize leaf extract of Vitex leucoxylon, was successful and showed significant thrombolytic effect in comparison with thrombosis control in comparison with standard thrombolytic agent streptokinase (30000unit/100µl). Morphological study has provided a characteristic identity of leaf which have purplish pink/reddish pink colour, variable in-shape such as ovate, lanceolate, obtuse apex petiolate, and was found to be bitter in taste & mild flavour odour. And also determined the present of alkaloids, flavonoids, phenols, tannins, carbohydrates, saponins, glycosides. From the above-mentioned studies, it can be concluded that the Pharmacognostical standards generated will be useful for the proper identification of plant and also to differentiate it from its closely related species and adulterants. With the support of in vitro studies and phytochemical screening, the ethanolic extracts were showed more efficacies for thrombolytic activity.

Keywords: Thrombolytic activity, Vitex leucoxylon, Streptokinase.

INTRODUCTION:

Thrombosis is the formation of blood clot internal blood vessels, obstructing the glide of blood thru the circulating gadget. When a blood vessel is injured, the body makes use of platelets (thrombocytes) and fibrin to shape a blood clot to prevent blood loss. Even while a blood vessel isn't injured, blood clots may shape in the body below sure conditions. A clot that breaks unfastened and starts off evolved to travel round the frame is called an embolus. The clot itself is termed as thrombus.1 Thrombo embolism is both thrombosis and its most important complication, that is embolisation. Thrombosis, thrombus and the prefix thrombo all come from the Greek thrombos meaning a lump or clump or a curd or clot of milk. When a thrombus occupie more than 75% of surface vicinity of the lumen of an artery, blood float to the tissue furnished is reduced enough to cause systems because of decreased oxygen and amassed of metabolic merchandise like lactic acid, more than 90% obstruction can bring about anoxia, the entire

deprivation of oxygen and infraction a mode of cellular death.2 Thrombolytic drugs hastily lyse thrombi by way of catalyzing the formation of plasmin from plasmino- gen. These pills create a generalized lytic state whilst administered intravenously. Thus, each defensive hemostatic thrombi and target throm- boemboli are broken down. Thrombolytics or fibrinolytics can remove installed thrombi and emboli.3There are many traditional systems of medicine in the world, every with extraordinary associated philosophies and cultural origins. Some of these, along with Tibetan conventional medicine, remain surprisingly localised in their united states of origin; at the same time as others which includes Ayurvedic and Chinese conventional drug treatments are more and more utilized in many exclusive regions of the world. Ayurveda is the maximum broadly practised of the Indian traditional remedy systems, but there are others such as Siddha and Unani which might be additionally used within the Indian subcontinent.4 There is limited source of drugs for treating thrombosis. The above some plants Vitex leucoxylon reported for having thrombolytic activity from the literature, the flavonoids have major role in thrombolytic activity. As the plant reported for the presence of flavonoids and also reported for treating wide range of ailments, the plant selected for present investigation to establish scientific evidence for having thrombolytic activity.

MATERIALS AND METHODS:

Collection of plant material:

The plant leaves of *Vitex leucoxylon* was collected from Chittur district of Andhra Pardesh. It is dried under shade and made coarse powder. The plant material collected was identified and authenticated by Assistant Prof (Dr) K.Madhava Chetty, Department of Botany, Shree Venkateswara University Tirupati Chittur district, Andhra Pradesh.

Pharmracognostical studies:

Different parameters viz; macroscopy, microscopy and proximate values are investigated. The macroscopical features and microscopical features were investigated as per standard protocols.

Phytochemical studies:

Phytochemical screening carried out by the methods referred from text book authored Pulok Mukherjee and Kokate. Chromatographic studies were carried out by referring text book by E. Stall and Wagner et al.

Preparation of extract:

The previously powdered drug was used for preparing extract. Different extracts are prepared by extracting the plant material with different solvents with increasing polarity.

Preparation of alcoholic and aqueous extract:

About 300g of powdered drug is taken into a maceration chamber and made wet with 95% ethanol; the solvent level is maintained above the bed of powdered drug material. Maceration is carried out for 14 days with intermediate shaking. Another 300gm of powdered drug is macerated with chloroform – water following the above procedure. Both the extracts obtained were filtered carefully and the solvent was evaporated at room temperature. The extractive value (%) was calculated with reference to air dried drug. Detection of chemical constituents: Different chemical tests were performed for detecting various chemical constituents.

Chromatographic Studies:

TLC:

Thin layer chromatography is a technique used for separation and identification of different components in an extract. In the current study TLC of flavonoid is performed for separation and identification of different flavonoids present in total alcoholic extract of *Vitex leucoxylon.* The extracts are dissolved in small amount of mobile phase. Benzene and Acetic acid in the ratio 4.6:0.4were used as mobile phase. The mobile phase is kept for saturation (for about 1 hr) in development chamber.Detection was done using UV light and ammonia.[7]

HPTLC Studies:

In the present work Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Camag Reprostar3, with 12bit CCD camera for photo documentation, controlled by WinCATS software was used. All the solvents used were of HPLC grade obtained from MERCK.

The solution of alcohol extract of 2 µl was applied as 10 mm bands on a precoated silica gel G 60 F 254 for HPTLC with Linomat V applicator using a Camag 100 µl syringe. The mobile phase used for alcohol extract was

benzene: acetic acid (4.5:0.4). No pre-washing of the plates was done. Chamber saturation time was 1 hr. The sample application position on HPTLC plates was 8.0 mm. The plates were kept for development, to a migration distance of 75 mm. The developed plates were dried with hot air and scanned using Camag TLC Scanner 3 at wavelength 366 nm, 254nm; slit dimension 4.00 x 0.30 mm, Micro, scanning speed 20 mm/sec. No post derivatisation was done prior to scanning. The Rf and peak area were interpreted by using the software. The developed plates were photo documented under 254nm, 366nm and visible light, using Camag Reprostar-3.

Pharmacological studies:
The commercially available lyophilized streptokinase vial (15, 00,000 i.u.) 5ml Phosphate buffered saline (PBS) was added and mixed properly. This suspension was used as a standard drug.[8]
The dried extract was dissolved in distilled water and this extract was taken as sample. The thrombolytic activity was performed by in-vitro model. The blood samples (collected from slaughter house) were transferred in different pre-weighed sterile micro centrifuge tube (500µl/tube) and incubated at 37°c for 45 min and allowed to stand. The serum was completely removed (aspirated out without disturbing the clot formed) after clot formation. Each tube having clot was again weighed to determine the clot weight -Clot weight = weight of clot containing tube — weight of tube alone. Each micro centrifuge tube containing clot was properly labelled and 100 µ l of streptokinase (standard drug). 10mg/100µl and 20mg/100µl of isolated constituent of *Vitex leucoxylon* and distilled water (as control) was added. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The test was repeated six times with all different dilution of the extract, standard drug and Control.

RESULTS AND DISCUSSION:

Pharmacognostical studies:
Organoleptic and macro – morphological study of the fresh leaf part shown is an annual or perennial, Leaf shaped is simple and asymmetrical, triangular, lanceolate, obtuse apex & petiolate, redish pink / Purplish pink colour taste slightly bitter, having mild flavor, Size of leaf are variables 5-12cm long & 2.5-5 cm wide.

Proximate values:
Various physical constants of aerial part of plant were performed like loss on drying, ash values and extractive values. Loss on drying is 5%, is very less, which indicates lower quantities or absence of volatile constituents. It also shows that the drug is dried enough to control the bacterial growth. Total Ash value and Acid – insoluble Ash value were found to be 10% and 2% respectively. The very low values of Acid – insoluble Ash represents that the drug is less adhered with dirt and sand which in turn represents the purity of the drug.

Preliminaryphotochemical screening:
The results are presented in table No1.

Table 1:Preliminary photochemical screening

Test	Dry powder	PetEther	Chloroform	Ethanol	water
ALKALOIDS	+	-	+	+	+
CARBOHYDRATE	+	+	+	+	+
FLAVONOIDS	+	-	+	+	+
GLYCOSIDES	-	-	-	-	-
PROTEINS	+	-	-	+	+
PHENOLS	+	-	+	+	+
SAPONINS	-	-	-	-	-
STEROIDS	+	+	+	+	+
TANNINS	+	+	-	-	+

TLC studies:
TLC study of ethanolic extract for flavonoid has given in table 2.

Table 2: TLC study of ethanolic extract for flavonoid

Sl.No	Extractused	Numberof spot	Rfvalueofspot
1.	Ethanolicextract	1	SpotA:0.72

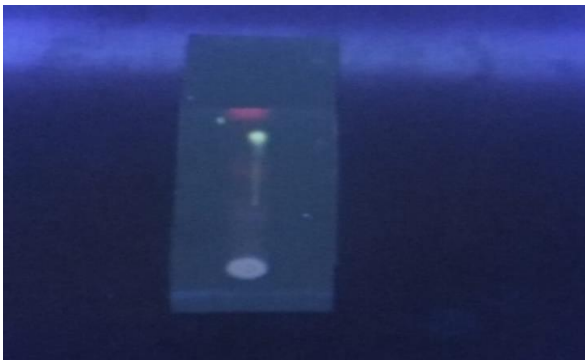

Fig.2:TLC of ethanolic extract.

HPTLC studies:
HPTLC finger printing of the alcoholic extract of *Vitex leucoxylon* was performed. In this study the alcoholic extract revealed the present of one phytoconstituents i.e,iso-quercetin and quercetin with Rf-value0.59and0.67 respectively. Plate was viewed under UV light 254 nm and 366 nm under normal white light.

The photographs are shown in fig 3& 4 below:

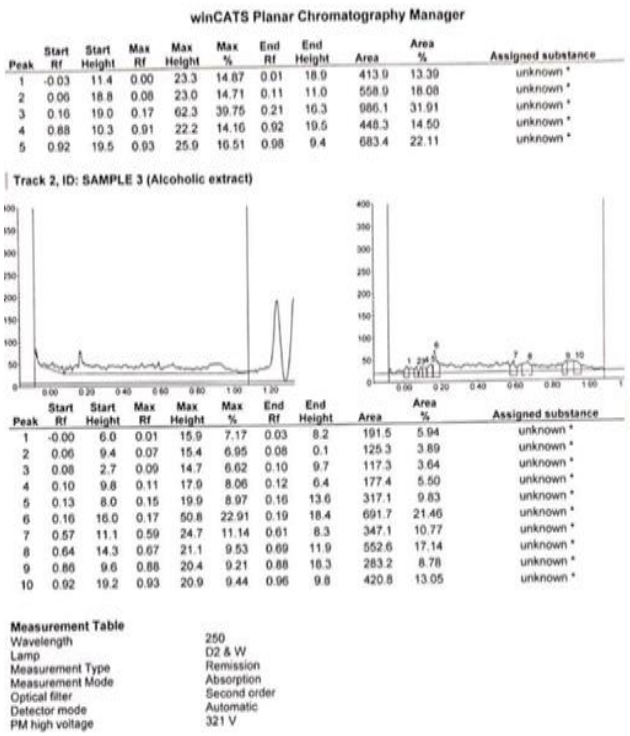

Fig. 3: HPTLC at 254 nm

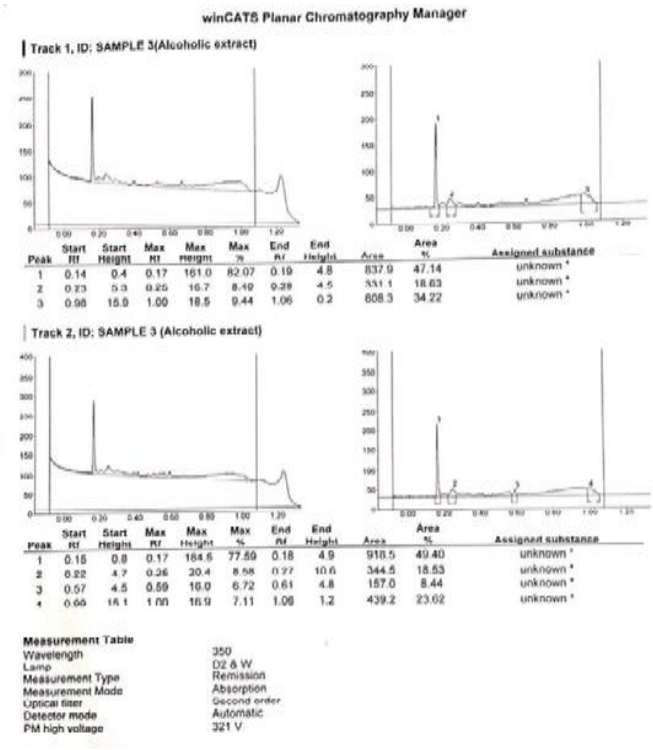

Fig .4: HPTLC graph alcoholic extract

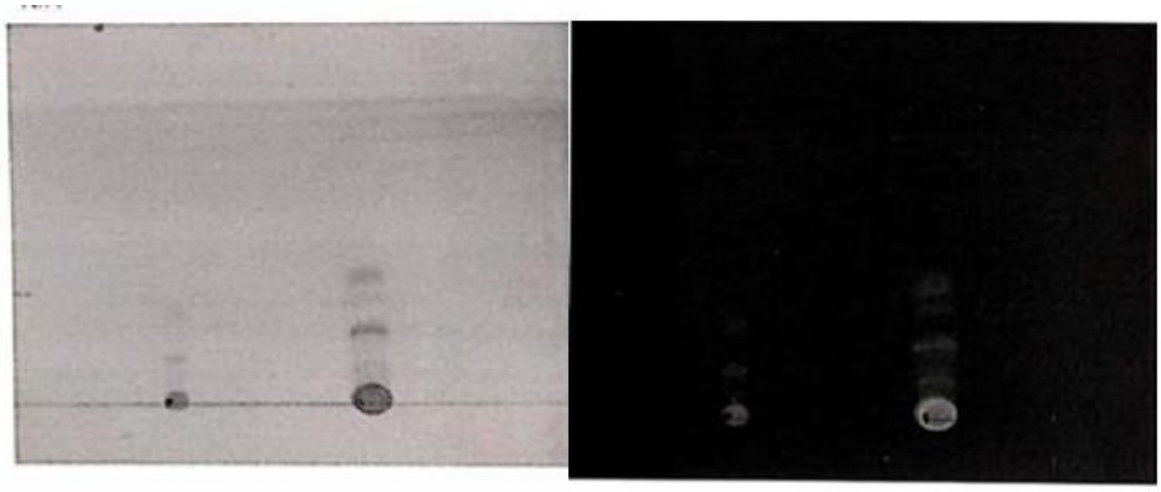

Fig .5: HPTLC of alcoholic extract at 254nm and 366 nm.

Thrombolytic activity:

The percentage of clot lysis for standard streptokinase (30000 unit/100 µl) was found to be 77.785%. For alcoholic extract (20mg/100µl and 10mg/100µl) was found to be 39.5% and 24.39% respectively. The percentage clot lysis of alcoholic extract in comparison with other extracts alcoholic extract having significant activity as per literature, the flavonoids present in the alcoholic extract may be responsible for thrombolytic activity.

Thrombolytic activity of plants showed in fig no. 7, Table no. 3 and 4.

Fig. 7: Thrombolytic activity of standard drug and Plant extracts

Table No.3: Thrombolytic activity of standard drug and plant extracts

Group	Treatment	Concentration	Weight of clot (before treatment) (in g)	Weight of clot after treatment (in g)	Weight of clot lysis	% of clot lysis
1.	Streptokinase	30000unit/100µl	0.400	0.090	0.310	77.50
			0.400	0.080	0.320	80.00
			0.390	0.100	0.290	74.21
			0.400	0.090	0.310	77.50
			0.390	0.90	0.310	77.50
			0.400	0.080	0.320	80.00
2.	Water extract	10mg/100µl	0.380	0.320	0.060	15.73
			0.390	0.390	0.000	0.000
			0.390	0.340	0.050	12.80
		20mg/100µl	0.400	0.340	0.060	15.00
			0.390	0.320	0.070	17.41
			0.390	0.320	0.070	17.41
3.	Alcoholic extract	10mg/100µl	0.400	0.290	0.110	27.50
			0.390	0.300	0.090	23.70
			0.410	0.320	0.090	21.99
		20mg/100µl	0.410	0.260	0.150	36.50
			0.390	0.230	0.160	41.00
			0.390	0.230	0.160	41.00
4.	Chloroform extract	10mg/100µl	0.410	0.360	0.050	12.19
			0.400	0.340	0.060	15.00
			0.400	0.360	0.040	10.00
		20mg/100µl	0.380	0.320	0.060	15.70
			0.390	0.290	0.010	25.64
			0.390	0.300	0.090	23.07
5.	Pet .ether extract	10mg/100µl	0.390	0.330	0.060	15.3
			0.380	0.350	0.030	7.89
			0.380	0.360	0.040	10.52
		20mg/100µl	0.400	0.320	0.080	20.00
			0.380	0.300	0.080	21.05
			0.400	0.330	0.070	17.50

Table 4: Percentage of clot lysis in different concentration of plants extracts.

Group	Treatment	concentration	%of clotlysis
1.	Streptokinase(std drug)	30000unit/100µl	77.785%
2.	Water extract	10mg/100µl	9.51%
		20mg/100µl	16.6%%
3.	Alcoholic extract	10mg/100µl	24.39%
		20mg/100µl	39.5%
4.	Chloroform extract	10mg/100µl	12.39%
		20mg/100µl	21.47%
5.	Pet.ether extract	10mg/100µl	11.23%

		20mg/100µl	19.51%

CONCLUSION:

An attempt was made to standardize the leaf of Vitex leucoxylon for morphological, microscopical and proximate values. HPTLC studies of alcoholic extract showed the presence of 2 spots which corresponds to different phytoconstituents. Ethanolic extract at dose levels of 10 mg/100µl and 20mg/100µl are tested for thrombolytic activity in animal blood. This extract showed significant activity on comparison with standard drug streptokinase.

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