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ISOLATION OF THROMBOLYTIC PRINCIPLE FROM LEAF EXTRACT OF *AMARANTHUS TRICOLOR*

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ABSTRACT

Background and objectives: The study is regarded for standardization of leaf extract of *Amaranthus tricolor,* study focus on phytochemical investigations, isolation of flavonoids and evaluation for thrombolytic activity from various solvents. **Methods:** Pharmacognostical, phytochemical studies and *in-vitro* thrombolytic activity of leaves extract of *Amaranthus tricolor* was carried out by using *in-vitro* model. The extracts were subjected to qualitative chemical analysis, chromatographic studies (TLC and 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^{o}C for 45mins, the fluid obtained after clot lysis removed and tube again weighed. The clot lysis = (Wt before clot lysis – Wt after clot lysis) x 100. **Results:** An alcoholic extract (20mg/100µl and 10mg/100µl) was evaluated in incubated blood. An attempt made to standardize leaf extract of *Amaranthus tricolor*, was successful and showed significant thrombolytic effect in comparison with thrombosis control in comparison with standard thrombolytic agent streptokinase (30000unit/100µl). **Interpretation and conclusion:** 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 and 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 efficacy for thrombolytic activity.

KEYWORDS

Thrombolytic activity, *Amaranthus tricolor* and Streptokinase.

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INTRODUCTION

Thrombosis is the formation of a 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[3]. There 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 *Amaranthus tricolor* 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.

MATERIAL AND METHODS

Collection of plant material

The plant leaves of *Amaranthus tricolor* 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[5]

Different parameters viz; macroscopy, microscopy and proximate values are investigated. The macroscopical features and microscopical features were investigated as per standard protocols[4].

Phytochemical studies[6]

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 *Amaranthus tricolor*. The extracts are dissolved in small amount of mobile phase. Benzene and Acetic acid in the ratio 4.6:0.4 were 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[4].

HPTLC Studies

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

The solution of alcohol extract of 2µl was applied as 10mm bands on a precoated silica gel G 60 F 254 for HPTLC with Linomat V applicator using a Camag 100µl 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 8.0mm. The plates were kept for development, to a migration distance of 75mm. 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 0.30mm, Micro, scanning speed 20mm/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[7].

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 100µ l of streptokinase (standard drug). 10mg/100µl and 20mg/100µl of isolated constituent of *Amaranthus tricolor* 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[8,9].

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 and petiolate, redish pink / Purplish pink colour taste slightly bitter, having mild flavor, Size of leaf are variables 5-12cm long and 2.5-5cm wide.

Powder Microscopical characters leaf

Amaranthus tricolor is a light green shows lignified vessels with spiral thickening, micro sphenoidal crystals of calcium oxalate, fragments of irregular sinuous, poly-hedral, thin-walled, parenchymatous epidermal cells and palisade cells.

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.

Preliminary photochemical screening

The results are presented in Table No.1.

TLC studies

TLC study of ethanolic extract for flavonoid has given in Table No.2.

HPTLC studies

HPTLC finger printing of the alcoholic extract of *Amaranthus tricolor* was performed. In this study the alcoholic extract revealed the present of one phyto-constituents i.e, is o-quercetin and quercetin with Rf-value 0.59 and 0.67 respectively. Plate was viewed under UV light 254nm and 366nm under normal white light.

The photographs are shown in Figure No.3 and Figure No.4 below:

Thrombolytic activity

The percentage of clot lysis for standard streptokinase (30000 unit/100µl) 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 Figure No.7, Table No.3 and Table No.4.

Table No.1: Preliminary photochemical screening

S.No	Test	Dry powder	Pet Ether	Chloroform	Ethanol	water
1	Alkaloids	+	-	+	+	+
2	Carbohydrate	+	+	+	+	+
3	Flavonoids	+	-	+	+	+
4	Glycosides	-	-	-	-	-
5	Proteins	+	-	-	+	+
6	Phenols	+	-	+	+	+
7	Saponins	-	-	-	-	-
8	Steroids	+	+	+	+	+
9	Tannins	+	+	-	-	+

Table No.2: TLC study of ethanolic extract for flavonoid

S.No	Extract used	Number of spot	Rf value of spot
1	Ethanolic extract	1	Spot A:0.72

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

Group	Treatment	Concentration	Weight of clot (before treatment) (ing)	Weight of clot after treatment (ing)	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 No.4: Percentage of clot lysis in different concentration of plants extracts

Group	Treatment	concentration	%of clot lysis
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%

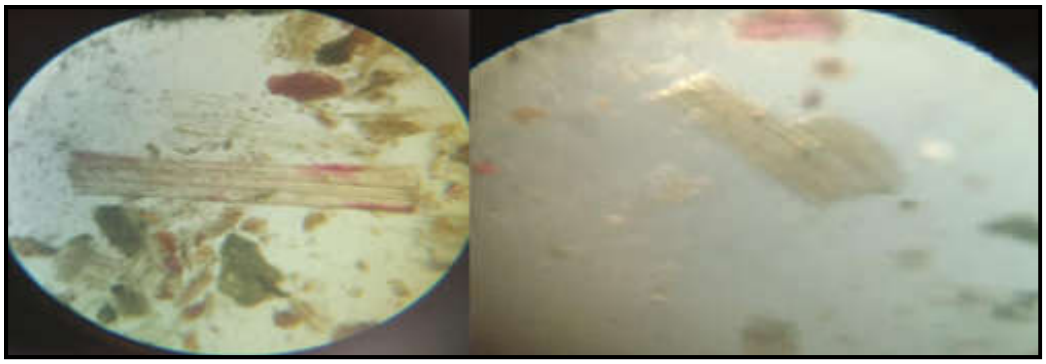

Figure No.1: Powder Microscopy of *Amaranthus tricolor*

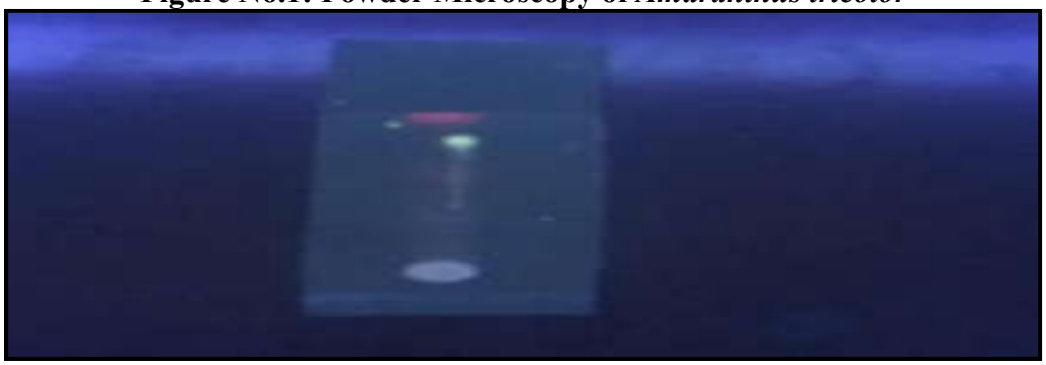

Figure No.2: TLC of ethanolic extract

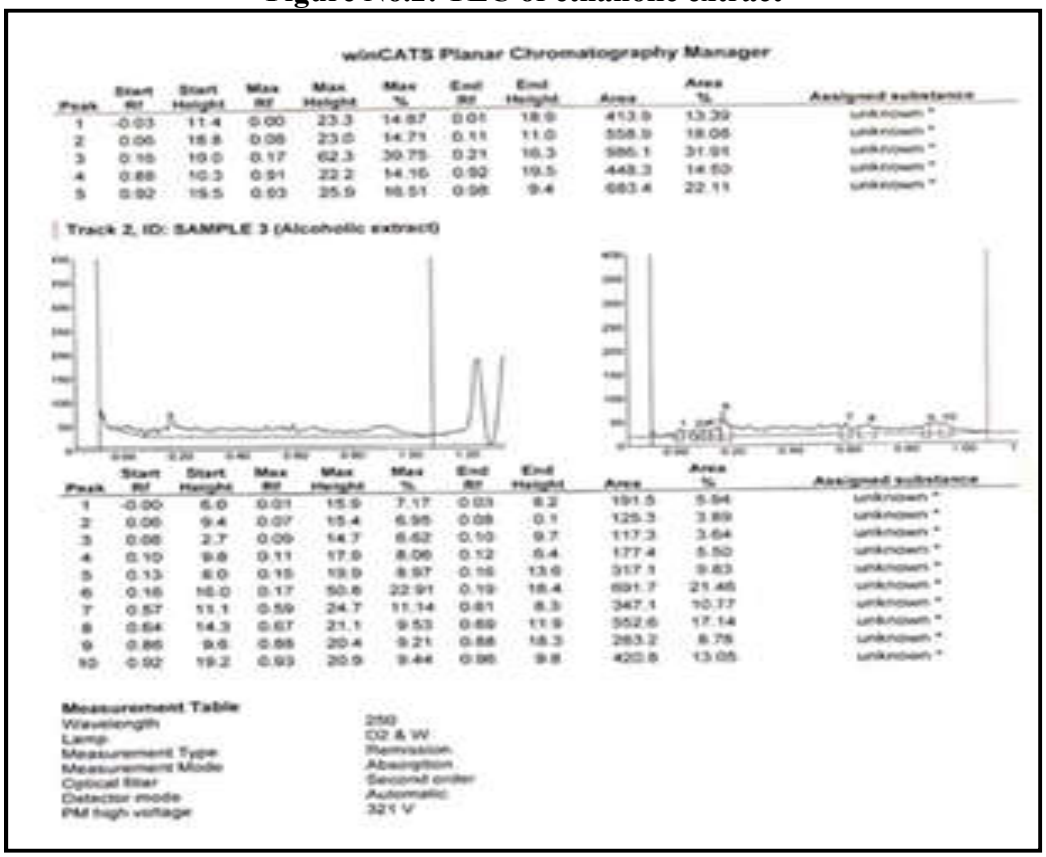

Figure No.3: HPTLC at 254nm

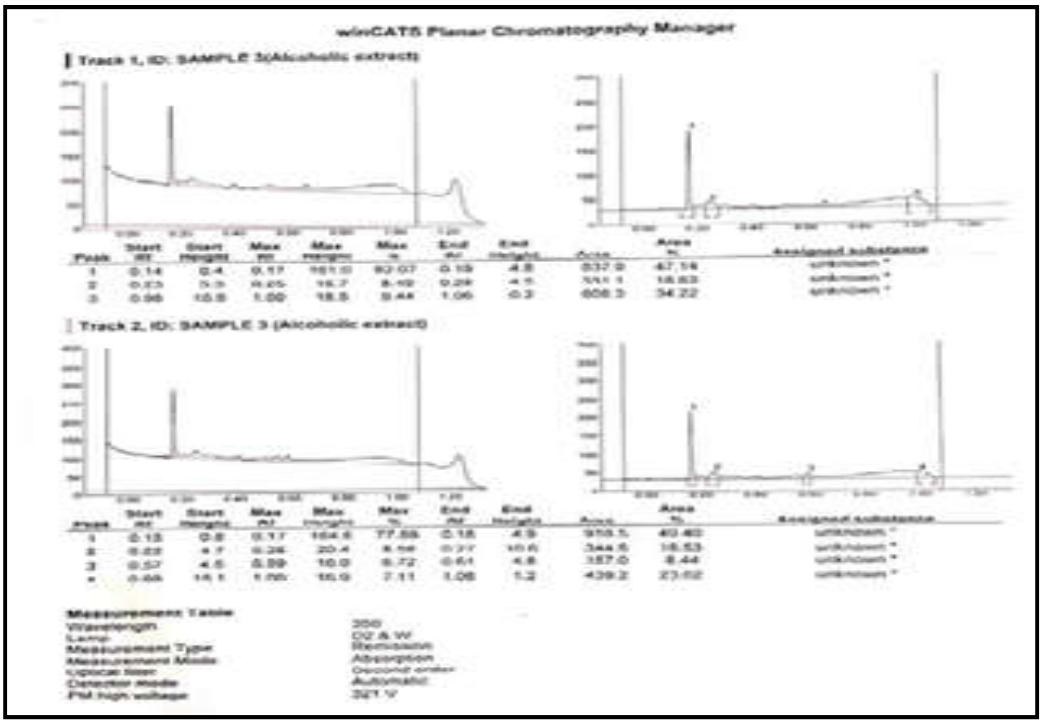

Figure No.4: HPTLC graph alcoholic extract

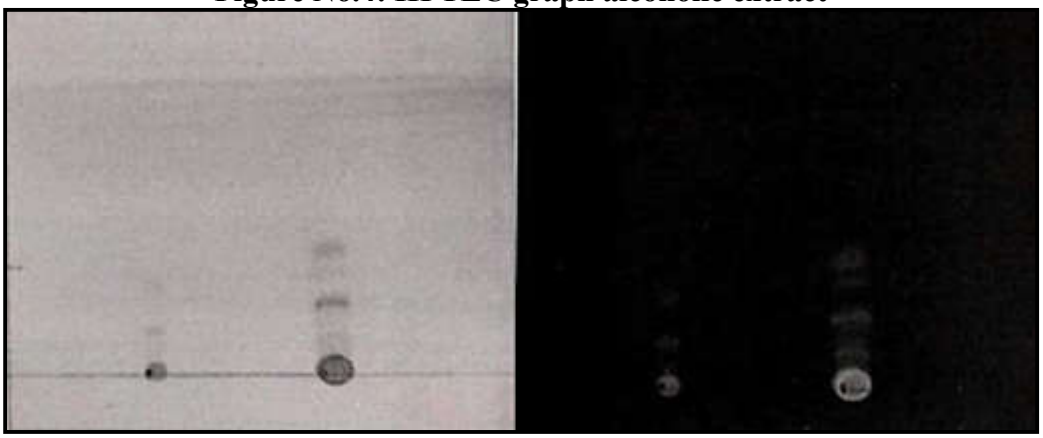

Figure No.5: HPTLC graph alcoholic extract at 254nm and 366nm

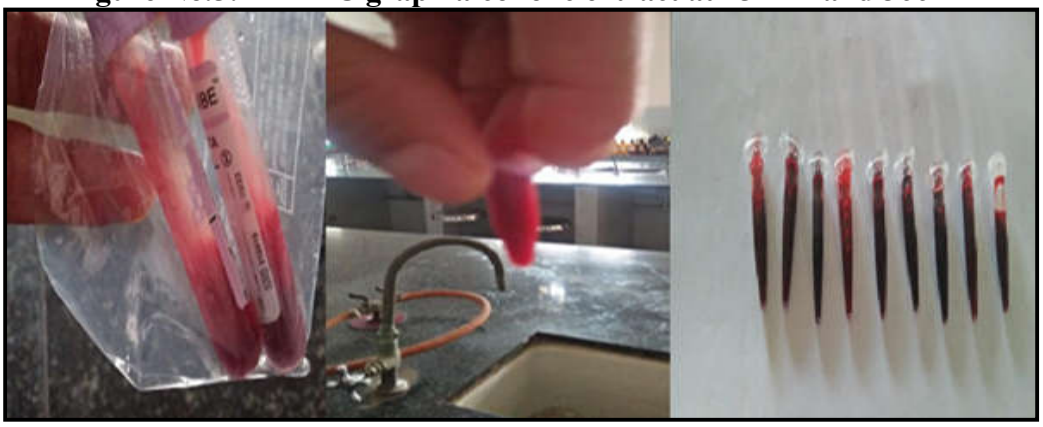

Figure No.6: Thrombolytic activity of standard drug and plant extracts

CONCLUSION

An attempt was made to standardize the leaf of *Amaranthus tricolor* 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 10mg/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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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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