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In Vitro thrombolytic and antibacterial properties of *Typha angustifolia* Fibre extracts

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Abstract
Objective: The aim of this study was to investigate the antibacterial activity and *in vitro* anti-thrombolytic activity of *Typha angustifolia* fibre extracts.
Methods: The thrombolytic activity was screened by *in-vitro* clot lysis model. In this model the blood sample (500µl/tube) from human volunteer has been collected in micro centrifuge tubes. After incubation the standard drug and different dilutions of test drug were added whereas water is taken as control. Percentage of Clot lysis was calculated on the basis of the weight difference of micro centrifuge tubes obtained before and after clot lysis. The antibacterial properties were determined by agar well diffusion method against *S. aureus, B. subtilis, K. pneumoniae, E. coli, P. aerugenosa.*
Result: Solvent extracts of *T. angustifolia* significance clot lysis activity with reference of Streptokinase as standard. Ethanol and Acetone showed strong antibacterial activity against *S. aureus, E. coli* and *P. aerugenosa* (18mm), Methanol showed moderate antibacterial activity against *S. aureus, B. subtilis* and *P. aerugenosa* (17mm). Aqueous solution showed antibacterial activity against *B. subtilis, P. aerugenosa, S. aureous, E. coli* (16mm). Acetone showed moderate antibacterial activity against *K.pneumoniae*. Ethanol and Methanol showed mild antibacterial activity against *K. pneumonia* and *E. coli (15mm).* Aqueous solution showed mild antibacterial activity against *K. pneumonia.*
Conclusion: The present investigation revealed that *Typha angustifolia* fibre extracts Possesses positive thrombolytic properties that could lysis blood clots. Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Typha angustifolia* fibre extracts as a thrombolytic drug ethanol and acetone extract of *T. angustifolia* possess strong anti-bacterial activity against bacterial pathogen.

Keywords: thrombolytic, clot lysis, *Typha angustifolia*, antibacterial activity

Introduction
Nature had been known as stockyard of medicinal agents since the time immemorial. Herbal products are extensively perceived as safe because they are "natural" having less or no side effects. Medicinal plants contain large number of secondary metabolites which have potential therapeutic properties that can be utilized in the treatment of human diseases [1].
Thrombus (blood clot) developed in the circulatory system due to failure of hemostasis causes Vascular blockage and leads to serious consequences in thrombolytic diseases such as acute Myocardial or cerebral infarction which may cause death [20]. Thrombolytic drugs are used to Dissolve blood clots in a procedure termed thrombolysis [3]. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) are commonly used thrombolytic agents to dissolve clots [4]. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Continued investigation in this area will provide new insights and promote progress towards the development of the ideal thrombolytic activity which are characterized by maximal coronary arteral thrombolysis with minimal bleeding [5]. Selective third generation thrombolytic activity such as monoteplase, tenecteplase, reteplase etc. result in a greater angiographic potency in patients with acute myocardial infarction, although so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials [3]. In recent years, it is observed that the heart diseases are increasing to a great extent and side effects of synthetic drugs are becoming an everincreasing therapeutic problem. Almost all the available thrombolytic agents still have significant shortcomings [6]. According to one of the reports, approximately, 30% of the pharmaceuticals are prepared from plants worldwide [7] and are considered to be less toxic and freer from side effects than the synthetic one [8]. Hence, it is needed to find out the safe, less or no side effective herbal drugs, because natural products of higher plants may give a new source of thrombolytic agents, as well as antimitotic agents [9]. Primary bioassay screens are most important for the initial screening of plants for bioactive principles and are often the first step in drug development.

Typha is a monocot genus of the monotypic family Typhaceae with about 12 species distributed in the tropical and temperate regions of the world in marshes and wetlands of varied depth. It a common plant of wetlands, is an unexploited taxon which can be used as a good source of food, medicines, and, fibres. *Typha angustifolia* are herbaceous, colonial, rhizomatous, perennial plant with long, slender, green stalks topped with brown, fluffy, sausage-shaped flowering heads. It is a perennial growing up to 3 m (9ft) often forming extensive colonies along shores of shallow ponds, lakes and marshes. The results of Varpe SS reveal that the aqueous and 70% methanol extracts of *T. angustifolia* pollen grains exhibits anti-inflammatory activity [10]. In the present situation it has been proposed that *Typha* could be utilized as a biomass crop for renewable energy [11].The present study aimed to evaluate antibacterial activity and thrombolytic properties of fibre extracts of *T. angustifolia*.

Materials and Methods

Collection and extraction of plant material

The plant fibre of *T. angustifolia* was collected from the Godavari river area of Kopargaon. The plant fibres were dried in shade 1-2 weeks. Precaution was to avoid direct sunlight contact of fibres otherwise it will destroyed the active compounds of plant fibre. After drying the different extracts were prepared using different solvents including Acetone, Methanol, Ethanol and Aqueous extracts.

Preparation of plant extract

The air dried and powdered plant material 100 gm was extracted successfully with 1 liter of ethanol, methanol, acetone and water by using a soxlet extractor until a complete extract were effected (10-12h) at a temperature not exceeding the boiling point. The extracts were evaporated to dryness under reduced pressure using a Rota vapor and the resulting extracts were stored in a refrigerator for antibacterial [12] and thrombolytic screening [13]

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Klebsiella pneumoniae	109
Bacillus subtilis	2414

Antibacterial activity using disc diffusion method: The modified paper disc diffusion method was employed to determine the antibacterial activity of diffrenet extracts of resins of *T. angustifolia*. Turbidity of inoculums was matched with McFarland turbidity standard [12]. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C. The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

Determination of thrombolytic activity

The eppendorf tubes were incubated at 37 °C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each eppendorf tube having clot was again weighted to determine the clot weight (clot weight= weight of clot containing tube- weight of tube alone.) To each eppendorf tube containing pre-weighted clot, 100 µ aqueous solutions of different extracts along with the crude extract was added separately. As a positive control, 100µ of streptokinase and a negative non-thrombolytic control, 100µof distilled water were separately added to the control eppendorf tubes. All the eppendorf tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and eppendorf tubes were again weighted to observe the difference weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis is shown below:

% clot lysis= (Weight of the lysis clot/ Weight of clot beforelysis)*100

Result and Dicsussion

Acetone and Methanol plant fibre extracts showed the strongest antibacterial activity against S. aureus (18mm). Acetone, Ethanol and aqueous extracts showed moderate antibacterial activity against *S. aureus*, *B. subtilis*, *P. aerugenosa* (17mm and16mm). Ethanol, Methanol and Aqueous sol. Showed mild antibacterial activity against *K. pneumoniae* and *E. coli* (15mm).

Table 2: Antibacterial activity of *T. angustifolia* fibre extracts against bacterial pathogens (Zone of inhibition of growth in mm, average of 3 readings)

Bacterial Pathogens	Acetone					Ethanol					Methanol					Aqueous					Ampicillin (10mcg)
	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	
B. subtilis	24	23	18	16	14	23	18	16	15	13	23	20	15	14	13	17	15	13	-	-	16
S. aureus	26	24	21	17	16	24	22	20	18	16	20	19	17	15	13	23	20	17	15	13	24
E.coli	22	18	16	13	12	20	17	15	13	12	23	20	17	14	12	25	22	17	14	13	11
P. aeruginosa	24	23	21	18	16	23	20	15	14	12	21	24	21	17	15	24	21	18	16	14	16
K. pneumoniae	24	20	17	15	13	15	21	18	15	13	20	23	17	15	12	23	21	17	16	14	19

According to antibacterial profile (Table 2), maximum inhibitory effect of the Methanol extract observed only on *Staphylococcus aureus, K. pneumoniae* and moderate antibacterial against *Escherichia coli, Pseudomonas aeruginosa, ium* but mild inhibitory effect on *B. subtilis.* Acetone extract showed strong antibacterial effect against *Staphylococcus aureus and P. aeruginosa* and moderate antibacterial against *Escherichia coli,* but mild effect on *B. subtilis.*

Table 3: Effect of fibre of *T. angustifolia* extracts on *in-vitro* clot lysis

Extracts	Percentage of Clot Lysis (Mean± S.D)
Acetone	34.48±4.43%
Ethanol	31.71±3.18%
Methanol	38.32±2.49%
Aqueous	28.77±6.14%
Negative Control	2.96±0.63%
Positive Control	75.75±4.16%

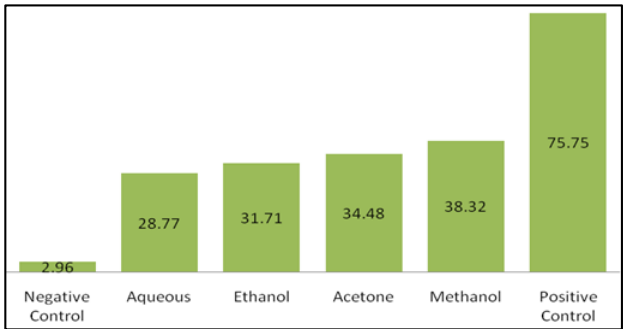

Fig 1: clot Lysis (%) of standard and different Solvent Extract of *T. angustifolia*

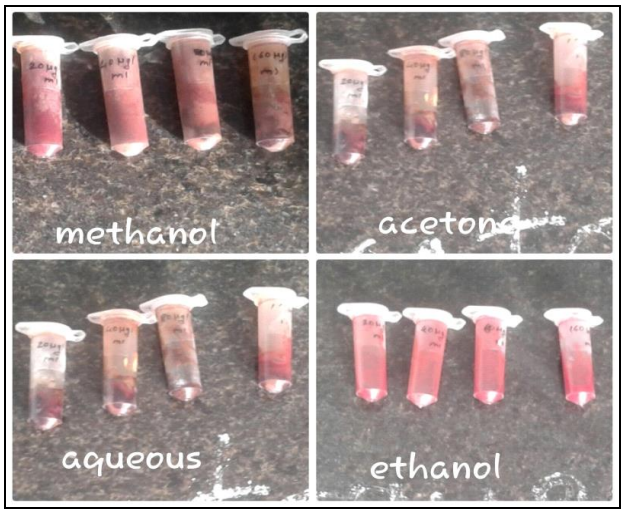

Fig 2: Clot Lysis (%) of different Solvent Extract of *T. angustifolia*

The normal range of blood clotting in humans is 140 sec to 240 sec. All *Typha angustifolia* fibre extracts showed immediate blood clotting at 160µg/ml concentration than other concentration. According to the table it showed that Acetone fibre extract was more active in blood clotting than other extract. Acetone fibre extract showed immediate blood clotting at different time than other plant fibre extract (Methanol, Ethanol, Aqueous extract). In India though Streptokinase [14-15] and urokinase are commonly used as thrombolytic drugs but these are the drugs with some serious adverse effect like they have short half-life, increase the bleeding time, higher dose. So in the current study it was tried to screen the thrombolytic drug from natural origins. Some polyphenolic constituents show the clot lysis activity in dose dependent manner.

Conclusion

Typha angustifolia fibre is also rich source of Phytochemicals. The fibre extract of *Typha angustifolia* fibre show the positive results for thrombolytic activity. So there may be chances that the other plant of the *Typha angustifolia* fibre also shows the positive antibacterial activity. Further we can screen the thrombolytic activity to other plants of same genera by in-vitro and in-vivo methods as well as can try to find out the phytoconstituent which are responsible for the activity.

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