Original Research Article

Evaluation of Phytochemicals and Histochemicals of *Cyperus rotandus* and Its Thrombolytic Activity

ABSTRACT

The main purpose of the research article is to evaluate the thrombolytic activity of the herbal source of *Cyperus rotandus* rhizome. Extracts from various sections of the plant (aerial component, tuber, rhizomes, etc.) produce significant amounts of medicinal active compounds, as well as the chemical structures of phytochemical constituents. The aim of this analysis was to look into the thrombolytic action of *Cyperus rotandus* methanolic extracts. Standard phytochemical methods were used for identify which compounds present in the herbal plant. The fraction's thrombolytic effect was studied in clot lysis experiment. In a thrombolytic activity test, the extract caused 60 % lysis of the blood clot, compared to 70.10 % and 4.70 % lysis for the positive control (streptokinase) and negative control (saline water), respectively. As a result, the extract possessed significant thrombolytic activity. The presence of these phytochemicals was found to be responsible for the plants *in-vitro* thrombolytic action. The methanol extract of *Cyperus rotandus* is a possible candidate for future thrombolytic agents.

Keywords: Cyperus rotandus, Thrombolytic activity, Streptokinase, Phytochemical constituents.

1. INTRODUCTION

Therapeutic plants are important for the modern composite material, which serves as a new enemy of irresistible experts and can be used to validate pharmacological exercises (Tanvir Ahmad Chowdhury *et al*., 2015). Thrombolysis, as well known as thrombolytic healing, is a procedure that dissolves dangerous blood clots, improves blood stream, and prevents tissue and organ damage (Sudipta Roy *et al*., 2015). Thrombolysis, also known as cluster busting, is the pharmacological degradation (lysis) of blood clumps (Bajpay A *et al*., 2018).

Cyprus rotandus is also known as Koraikkizhangu in Siddha medicine and Naagarmothaa in Unani medicine. *Cyperus rotundus* L., as well famous as purple nut sedge or nut grass, is a perennial weed with slender, textured crawling rhizomes that are globular on the bottom and emerge individually from tubers that are 1-3 cm tall (Bhaskar Das *et al*., 2015). The tubers have a distinctive fragrance and are almost blackish in shading resting on the external and ruddy white on the in the interior (Binkowski TA *et al*., 2013). The leaves are straight, dull green, and scored on the superior surface, and the stems cultivate to be about 25 cm long (Edeoga H.O *et al*., 2005). This herb has been exposed to be 7-10 different pharmacological and organic exercises, including anti-candida, anti-diabetic, anti-diarrheal, cytoprotective, anti-mutagenic, anti-microbial, anti-bacterial, cell reinforcement, cytotoxic and apoptotic, hostile to pyretic, and pain-relieving exercises. Nagarmotha (*Cyperus rotundus*) is a plant that can be found all over India (Elumalai A *et al*., 2014).

The aim of our present study was to investigate the thrombolytic activity of methanolic extracts of *Cyperus rotandus* by using an in vitro procedure.

MATERIALS AND METHODS

Plant materials

The rhizome powder of *Cyperus rotundus* were purchased in March 2021 from Country Medicinal Shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Physicochemical analysis

Physicochemical parameters of the powdered sample leaves extractive value content were performed according to the method described in WHO guidelines.

Determination of extractive alcohol soluble

The powdered substance (4 g) was correctly measured and put in a glass stoppered circular bottom flask (RBF). Ethanol (100 mL) was applied to the RBF, which was then thoroughly shook and set aside for 1 hour. A reflux condenser was attached, and the mixture was gently boiled for 1 hour before being cooled and purified. The flask was vigorously shaken before being circulated into a dry filter paper (Al Amin Sikder *et al*., 2011). The filtrate was then moved to a tarred flat bottomed dish and evaporated to dryness over a water bath. The dish was then dried for 6 hours at 105°C, cooled in a desiccator, and measured (Midori A. Yenari *et al*., 1995). The extractable matter content of air dried material (in percent w/w) was calculated as follows:

$$\text{Percentage of alcohol soluble extractive} = \frac{\text{Weight of alcohol soluble residue}}{\text{Weight of sample}} \times 100$$

Plant extracts preparation

In a 250 mL conical flask, 1 gram of *Cyperus rotundus* rhizome powder was kept, and 50 mL of solvent, such as water or ethanol, was applied separately. Cotton was placed over the mouth of the conical flask for 30 minutes of free hand shaking. After finishing the shaking process, the sample was held for 24 hours to allow all active materials to dissolve in the necessary solvent. The extract was then filtered into Whatman no. 1 filter paper. This is the filtrate that was used in the procedure (Collen D *et al*., 2004).

PHYTOCHEMICAL SCREENING

Test for Tannins

About 1ml of sample is boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride is added and observed for brownish green or a blue-black colouration.

Test for Saponin

About 2 ml of sample is boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate is mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing is mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Steroids

2 ml of acetic anhydride is added to 1ml of extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

5 ml of each extract is mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive results for the presence of terpenoids.

Test for triterpenoids

1ml of the extract is added in 1 ml of chloroform, 1 ml of acetic anhydride is added following the addition of 2 ml of concentrated $H_2 SO_4$. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for alkaloids

Mayer's test: To a few (one) ml of the extract, a drop of Mayer's reagent is added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

Test for anthraquinones

Five ml of the extract solution is hydrolyzed with diluted concentrated H_2SO_4 extracted with benzene. 1 ml of dilute ammonia is added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Polyphenols

Ethanol (4 ml) is added to each extracts (1ml) and the resulting solution is transferred in test tubes and warmed in a water bath (15 minutes). Three drops of freshly prepared ferric cyanide solution were added to the extract solution. Formation of a blue green colour indicated the presence of polyphenols.

Test for Cardiac glycosides

5 ml of each extracts is treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This is underlayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Coumarins

2 ml of each extracts is treated with 3 ml of 10% NaOH. A yellow colouration observed in each extract indicated the presence of Coumarins.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Determination of total phenols by spectrophotometric method

Plant powder (2g) was boiled in 50 mL ether for 15 minutes to remove the phenolic portion. 5 mL extract was pipetted into a 50 mL flask, followed by 10 mL distilled water. There was also 2 mL ammonium hydroxide solution and 5 mL concentrated amylalcohol added. The samples were prepared to the specifications and left to react for 30 minutes to allow for colour growth. This was measured at a wavelength of 505 nm (Swaroop S. Kumar *et al*., 2015).

Determination of Flavonoids

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Prashanthi Gandhamalla *et al*., 2018).

Estimation of total terpenoid content

1 g of plant powder was taken separately and soaked in alcohol (50ml) for 24 hrs. Then filtered, the filtrate was extracted with petroleum ether (40ml) for 2 hours. The dried ether extract was evaporated by complete elimination of petroleum ether under reduced pressure. The dried ether extract was treated as total terpenoid (Barton G. M *et al*., 1952).

HISTOCHEMICAL TESTS

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemical was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes (Sikandar Khan Sherwani *et al*., 2013).

UV-VISIBLE AND FTIR SPECTROSCOPIC ANALYSIS

The methanol extract were examined under UV-visible and FTIR spectrophotometer analysis were scanned in the wavelength ranging, characteristic peaks were detected (Sai Sandeep.Y *et al*., 2012).

COLUMN CHROMATOGRAPHY ANALYSIS

Extraction of polyphenolic compounds

Extraction of phenolic compounds along with sugars was carried out in the following manner. Twenty five grams of sample (Powder) were introduced into a 100 ml dark glass bottle and suspended in 200 ml of methanol-water or acetone-water (80:20, v/v). Tightly capped bottles placed in water bath at 80^0C. After 15 min during which the content was shaken twice, the extract was cooled and filtered under partial vacuum. The material left on the filter paper was transferred back to dark glass bottles for further extraction with 200 ml of the same extraction solution (Azad A.K *et al*., 2015). This procedure was repeated two times over 30 and 60 min of extraction, each time collecting the solution for analysis. Supernatants were combined and evaporated using rotary vacuum evaporator to remove any remaining solvent; the water was then removed by lyophilization (Kamrul Islam Siddique *et al*., 2013).

Column Chromatography

The column of about 15cm long and 4cm in width was completely washed with cleanser, flushed with refined water and afterward permitted to dry. At the point when the column has been completely dried, little piece of glass fleece was embedded into the lower part of the column and the column was upheld using a clap and report stand (Saddam Hussain Md *et al*., 2016). A channel is connected to the open end and minimal clean white sand was poured on top of the glass fleece previously embedded, after this some amount of the dissolvable was poured down the column. The silica gel (40micron) utilized was initiated in the broiler at 120 ^{o}C f or 2hours. An all around mixed suspension of silica gel (100–150 g in oil ether at 60°C–80°C was filled a column (150 cm long and 50 mm in width).At the point when the spongy was very much settled, the abundance of petroleum ether was permitted to go through the column (Rajeswari S *et al*., 2017). The slurry was gone through the silica gel in petroleum ether and was processed to very much blended column. Slow setting was orchestrated by keeping a delicate disturbance while there was dissolvable move through the column to acquire a homogenous pressing. A 1.0 g part of the concentrate was broken down in 5 m1 of methanol and applied to a chromatographic column (3.4 x 50 cm) loaded with Sephadex LH-20 and eluted with methanol. Portions (6 ml) were

gathered utilizing a division authority and their subjective examination of polyphenol test (Hossan sakib Md *et al*., 2015).

Polyphenol test

Ethanol (4 ml) is added to each fraction (1ml) and the ensuing plan is moved in test tubes and warmed in a water shower (15 minutes). Three drops of recently organized ferric cyanide game plan were added to the concentrate course of action. Improvement of a blue green concealing showed the presence of polyphenol (Arifur Rahman Chowdhury Md *et al*., 2017).

THIN LAYER CHROMATOGRAPHY

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces (Rashaduz Zaman *et al*., 2015).

Rf value

It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$Rf = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

IN VITRO THROMBOLYTIC ACTIVITY

3ml venous blood drawn from own blood was distributed in four different pre- weighted eppendorf tubes and incubated at 370 c for 45minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). To each eppendorf tube containing pre-weighted clot, 100µl (100µg/ml) of sample was added and another eppendorf tube containing pre-weighted clot, 100µl (200µg/ml) of sample was added. As a negative control, 100µl of distilled water was added to the control tube (Anand Prakash *et al*., 2019). For positive control, 100µl of streptokinase (SK) was added. All the tubes were then incubated at 370C for 90minutes and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighted 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 (Pushpendra kumar Jain *et al*., 2016). The equation for calculating weight of clot is given below.

Clot weight = Weight of clot filled tube –Weight of empty tube

% of clot lysis = (Weight of lysis/weight of clot before lysis) × 100

RESULTS

Extractive value of analysis

Table 1 represents the extractive value of *Cyperus rotandus* rhizome extract. Alcohol and water soluble extract value of *Cyperus rotandus* rhizome investigated (Vikas Kumar, 2016). Among the two extract value, alcohol extract (17%) value has higher than water extract (4%) value.

Table 1. Extractive value of *Cyperus rotundus* rhizome

S. No	Extractive solvent	Result (%)
1	Alcohol soluble extractive	17.00
2	Water soluble extractive	4.00

QUALITATIVE AND QUANTITATIVE ANALYSIS

Qualitative and quantitative analysis *Cyperus rotandus* rhizome studied. Significant amount of flavonoids (110.00±7.70), terpenoids (10.00±0.70), and phenol (150.47±10.53) were present in *Cyperus rotandus* rhizome. The phytoconstituents were tested as per the standard methods (Sri Ranjani Sivapalan, 2013).

Table 2. Quantitative phytochemical analysis of *Cyperus rotundus* rhizome powder

S. No	Phytochemicals	Results (mg/gm)
1	Flavonoids	110.00±7.70

2	Terpenoids	10.00±0.70
3	Phenol	150.47±10.53

Values are expressed as mean ± SD for triplicates.

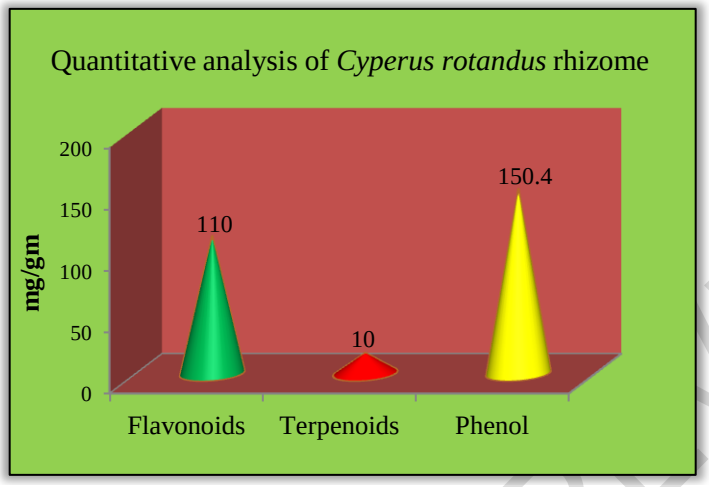


Figure 1. Quantitative phytochemical analysis of *Cyperus rotundus* rhizome

HISTOCHEMICAL ANALYSIS

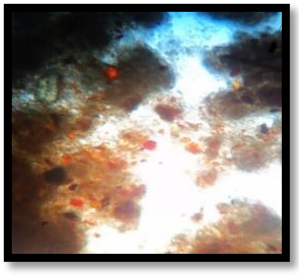
Histochemical analysis of *Cyperus rotundus* rhizome powder was investigated. *Cyperus rotandus* rhizome powder treatment with different reagents and observation under microscope (Ashokkumar R *et al*., 2014). The results revealed the presence of Tannin, Flavonoids, Polyphenol and Terpenoids. In this results further confirmed the presence of phytochemicals (table 3 and figure 2).

Table 3: Histochemical analysis of *Cyperus rotundus rhizome* powder

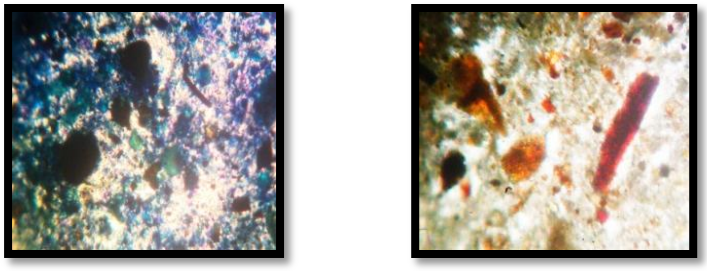
S.No	Phytochemicals	Results	Colour observation
1	Tannin	++	Black
2	Flavonoids	++	Yellow
3	Polyphenol	++	Blue, Green and Red
4	Terpenoids	++	Dark orange

Note: (+) Presence; (++) present with high intensity of the colour

a)Tannin



b) Flavanoids



c) Polyphenol d) Terpenoids

Figure 2. Histochemical analysis of *Cyperus rotundus* rhizome powder

UV-VISIBLE ANALYSIS

The UV-VIS analysis preformed for identification of phytoconstituents present in *Cyperus rotundus* rhizome ethanolic extract. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings (Dibyajyoti S *et al*., 2014).

Table 4. UV-Visible analysis of *Cyperus rotundus* rhizome ethanolic extract

S. No	Wavelength(nm)	Absorbance (OD)
1	400	2.000
2	640	0.371
3	670	0.298

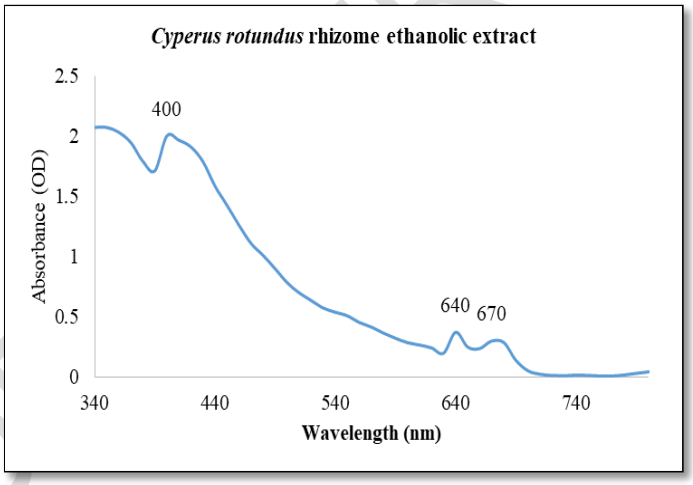


Figure 3. UV-Visible analysis of *Cyperus rotundus* rhizome ethanolic extract

FTIR ANALYSIS

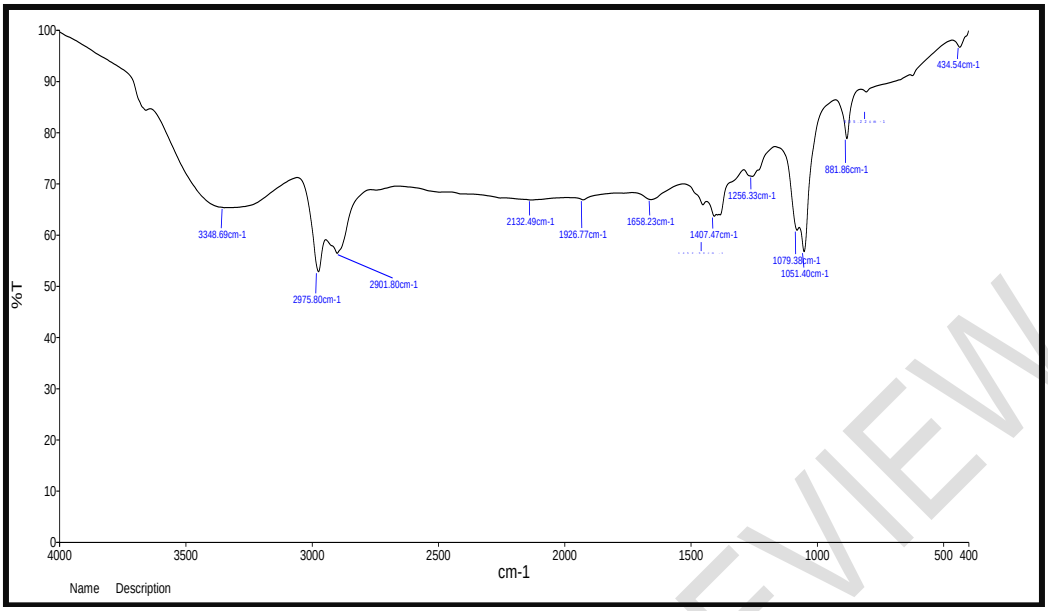


Figure 4. FTIR peak of *Cyperus rotandus*

Table 5. FTIR analysis of *Cyperus rotundus* rhizome extract

Peak	Bond	Functional group
3348.69	N-H stretch	1°, 2° amines, amides
2975.80	C-H stretch	Alkanes
2901.80	C-H stretch	Alkanes
2132.49	-C≡C- stretch	Alkyne
1658.23	-C=C- stretch	Alkenes
1452.50	C-H bend	Alkanes
1407.47	C-C stretch (in ring)	Aromatics
1256.33	C-N stretch	Aromatic amines
1079.38	C-N stretch	Aliphatic amines
1051.40	C-N stretch	Aliphatic amines
881.86	C-H “oop”	Aromatics
805.22	C-Cl stretch	Alkyl halides

COLUMN CHROMOTOGRAPHY

To separation and identification of the phenolic compound using Column chromatography and TLC from *Cyperus rotundus* rhizome. The *Cyperus rotundus* rhizome extracted with methanol-water (80:20, v/v). This procedure was repeated two times over 30 and 60 min of extraction. The highest extraction of phenolic compounds from plant material with methanol-water (80:20, v/v) was achieved during the extraction (Figure 5).The collected fractions further tested in phenol (Table 6) by qualitatively (Sikandar Khan Sherwani *et al*., 2013). All the fractions showed the strong positive reaction in phenol and concentrated the fractions for further TLC analysis. TLC plate showed the presence of Gallic acid and quercetin as compared with the standard Gallic acid and quercetin (Figure 5). The results of the study concluded that *Cyperus rotundus* rhizome extract contain Gallic acid and quercetin (Barton G. M *et al*., 1952).

Figure 5. Column chromotography

THIN LAYER CHROMOTOGRAPHY

Thin layer Chromatography is based upon the principles of column and partition Chromatography.

Table 6. Separation and identification of the phenolic compound

Fraction	Fraction colour	Qualitative analysis polyphenol	Rf value
1	**Orange**	**++**	0.85
			0.55
2	**Brown**	**+**	**0.80**
			0.55
3	**Yellow**	**++**	**0.71**
			0.55
Standard (Gallic acid)			**0.74**
Standard (Quercetin)			**0.72**

(+)Presence; (++) present with high intensity of the colour

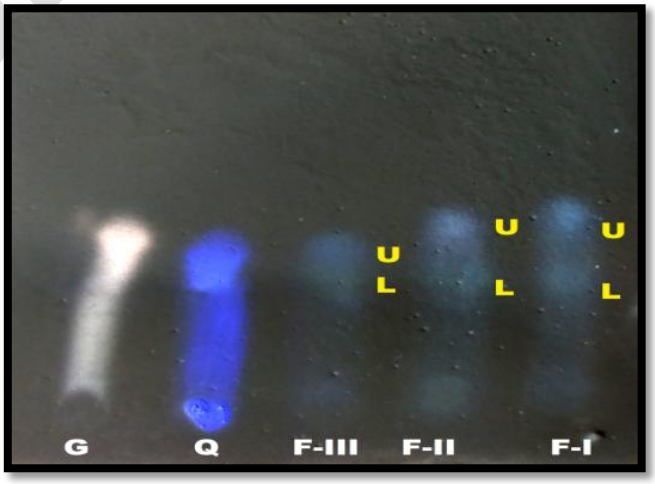


Figure 6. Identification of the phenolic compound using Column Chromatography eluting and TLC

TO FIND THE THROMBOLYTIC ACTIVITY OF *Cyperus rotundus* RHIZOME EXTRACT

Thrombolytic activity of the leaves of the *Cyperus rotundus* rhizome. The leaves contain the maximum thrombolytic activity than the leaves Streptokinase (SK), a known thrombolytic drug is used as a positive control. Water, on the other hand, was selected as a negative control. The present study a significant thrombolytic activity was observed after treating the clots with *Cyperus rotundus* rhizome ethanolic extract shows 60.00±5.18% in 200µg/ml of clot lysis while SK shows the 70±4.46% clot lysis (table 7). So it can be concluded as significant anti-coagulant agent.

Table 7: Thrombolytic activity of *Cyperus rotundus* rhizome extract

Concentrations(µg/ml)	**% of blood lysis**
Control	4.70±0.58
C. rotundus(100µg/ml)	26.30±4.50*
C. rotundus 200µg/ml	60.00±5.18*
Standard	70.10±4.46*

Values are expressed as mean ± SD for triplicates Data were analyzed by one-way ANOVA followed by post-hoc Tukey HSD test. Statistically significant variation was derived by comparing Control versus 100µg/ml *Cyperus rotundus* rhizome extract, 200µg/ml *Cyperus rotundus* rhizome extract and Standard. Significance level α 0.05.*P<0.05 statistically significant and NS= Non significant (P>0.05) compared with Control.

Overall effect on *Cyperus rotundus* rhizome extract were 100µg/ml, 200µg/ml and Standard was statistically significance deference from α 0.05 significant level compared with Control.

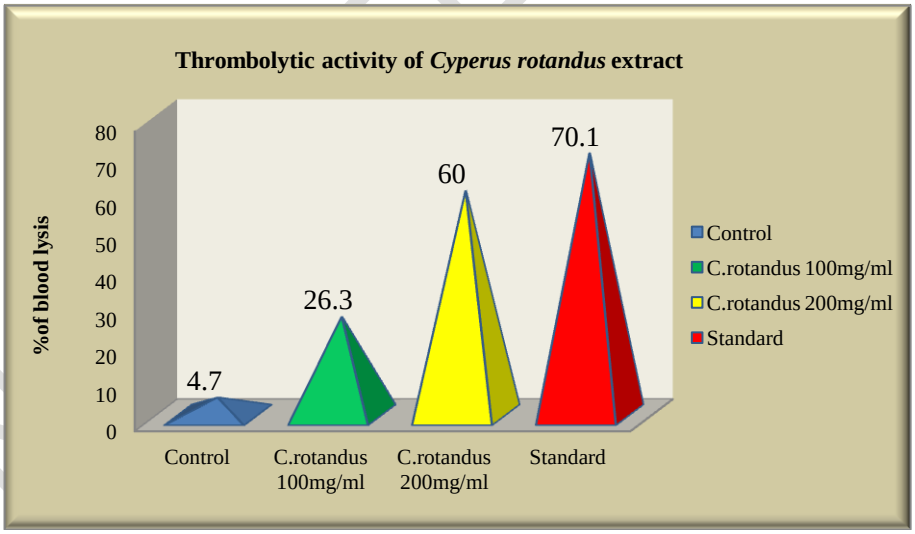


Figure 7: Thrombolytic activity of *Cyperus rotundus* rhizome extract

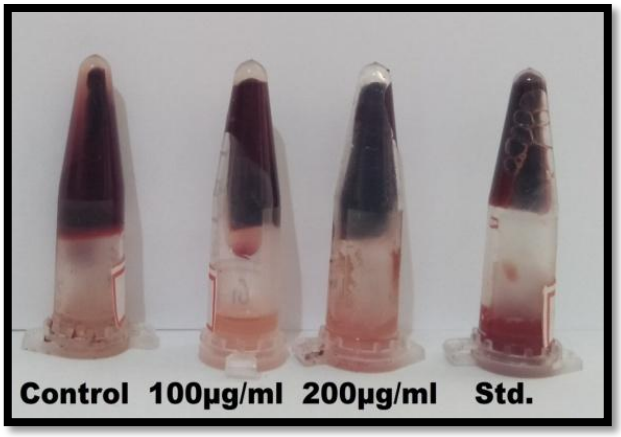


Figure 8: Experiment of thrombolytic activity of *Cyperus rotundus* rhizome extract

The present study a significant thrombolytic activity was observed after treating the clots with *Cyperus rotundus* rhizome ethanolic extract shows 60.00±5.18% in 200µg/ml of clot lysis while Streptokinase shows the 70±4.46% clot lysis (table 7).So it can be concluded as significant anti-coagulant agent compared to the other plants.

DISCUSSION

Many reports such as, the phytochemical study of four different plants of the *Asteraceae* family of different solvent extracts has shown. Saponin and steroids were found in the alcoholic extract of this herb, and steroids were found in the chloroform extract (Fatema Tabassum *et al*., 2017). The *Cyperus rotandus* was found to be high in phenolic compounds such as flavonoids, alkaloids, Saponin, and other secondary metabolites such as terpenoids in the report. Phenolic compounds have been shown to have a variety of pharmacological effects and to play an important part in cancer prevention and treatment (Ghose AK *et al*., 1987). The present study indicates high level phytochemical constituents of Tannin, Flavonoids, Polyphenol and Terpenoids from *Cyperus rotandus* like other plants. Column chromatography is one of the most commonly employed separation methods to classify both organic and inorganic products, implying its possible utility in chemical analysis of complex extract content in this research (Shruthisrivastava, 2012). The effectiveness of column-chromatographic techniques for the separation of biologically active secondary metabolites from plant samples was demonstrated in this study. By this study, it was concluded that methanolic extract of *Cyperus rotandus* analysis such as UV-Visible spectroscopy, FTIR, Column chromotography and TLC were showed high activity compared to another plants (Trott.O *et al*., 2010). The methanol extract of *Cyperus rotandus* may be a possible candidate for future thrombolytic agents, according to the findings of the report. While this is a preliminary review, it is an important addition to the catalogue of natural plant products that have recently been tested for thrombolytic action (Binkowski TA *et al*., 2003). As a result, the whole community is now searching for and developing molecules that may have therapeutic potential in atherothrombotic disorders such as myocardial or cerebral infarction (Sheikh Anwar Md et al., 2011).

CONCLUSION

A medicinal plant contains bioactive compounds that can be used for beneficial purposes and that serve as precursors to the production of effective drugs. The in vitro thrombolytic function of *Cyperus rotandus* was investigated in this research. Along with aim of determining the phytochemical screening and thrombolytic involvement of *Cyperus rotandus* in this research. Overall, it concluded that the above results suggest that the extract of *Cyperus rotandus* has rich source of phytochemicals confirmed by qualitative and quantitative. The present investigation provides. The experimental studies of *Cyperus rotandus* extract exhibited considerable thrombolytic activity.

NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Tanvir Ahmad Chowdhury, Mustafa Kamal A M, Kazi Ashfak Ahmed Chowdhury, Afrina Jahan, Shakhawat Hossain Md, Arafatul Mamur, Mahmudul Hasan and Javed Hossain. Cytotoxic& Thrombolytic activity of methanolic extract of Macaranga denticulata Bark. The Pharma Innovation Journal, 2015; Vol 4(5).
2. Sudipta Roy, Aziz Abdur Rahman, Hazrat Ali,Mohammed Abu Sayeed and Sekendar Ali.Evaluation of Thrombolytic Activity of Different Fractions of *Viburnum foetidum* L.World Journal of Medical Sciences,2015; Vol 12(4).
3. Bajpay A, Nainwal R.C, Singh D and Tewari. Medicinal value of *Cyperus rotundus* Linn. Indian journal of medicinal plants, 2018; Vol 10(3).
4. Bhaskar Das, Dilipkumar Pal and Arindam Haldar.A review on *Cyperus rotundus* as a tremendous source of pharmacologically active Herbal medicine. International Journal of Green Pharmacy, 2015; Vol 9(4).
5. Binkowski TA, Naghibzadeg S and Liang. CASTp computed atlas of surface topography of proteins. International journal of green pharmacy, 2013; Vol 31.
6. Edeoga H.O, Okwu D. E and Mbaebie. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 2005; Vol 4 (7).
7. Elumalai A, Eswariah M china, Chowdary CH Screening of Thrombolytic Activity of *Bougainvillea glabra* Leaves Extract by *In-Vitro*.Asian Journal of Research in Pharmaceutical Science,2014; Vol 2(4).
8. Al Amin Sikder, Abu Bakar Siddique, Nawshad Hossian A.K.M, Kowser Miah, Mohammad A. Kaisar and Mohammad A. Rashid Evaluation of Thrombolytic Activity of Four Bangladeshi Medicinal Plants, as a Possible Renewable Source for Thrombolytic Compounds. Journal of Pharmacy and Nutrition Sciences, 2011; Vol 1.
9. Midori A. Yenari, James T.Palmer, Bracci P.M and Gary K.Steinberg .Thrombolysis with tissue plasminogen activator (TPA) is temperature dependent. Thrombosis research, 1995; Vol 77(5).
10. Collen D and Lijnen H.R.Tissue- type plasminogen activator: a historical perspective and personal account. Journal of thrombosis and haemostosis, 2004; Vol 2(4).
11. Swaroop S. Kumar and Abdulhameed Sabu. Fibrinolytic Enzymes for Thrombolytic Function and clinical Implications, 2015; Vol 3(2).
12. Prashanthi Gandhamalla, Shiva Goud Buddola, Pravalika Rachakonada, Ramya Manga and Narender Boggula. Thrombolytic screening of *luffa cylindrica* linn. Fruits in-vitro study. International journal of pharmaceutical Sciences and Research, 2018; Vol 6(1).
13. Barton G. M, Evans R. S. and Gardener. Paperchromatography of phenolic substances. Journal of Nature, 1952; Vol 8(3).
14. Sikandar Khan Sherwani, Muhammad Mumta Khan, Arif Zubair, Muhammad Ajmal Shah and Shahana Urooj Kazmi. Evaluation of In Vitro Thrombolytic Activity of *Bougainvillea Spectabilis* Leaf Extract. International Journal of Pharmaceutical Sciences Review and Research, 2013; Vol 21(1).
15. Dibyajyoti S and Swati P. Phytochemical screening and thrombolytic activity of chloroform extract of *Urena sinuata* L. Journal of fundamental and applied science, Vol 6(2).
16. Sai Sandeep.Y, Mamata panigrahi, Divya and Beena. Evaluation of in vitro thrombolytic activity of phytochemicals in *Bacopa monnieri* Linn. Journal of Pharmacy Research, 2012; Vol 5(1).

17. Azad A.K, Ohidul Islam,Emratunnesa Rima, Mohaiminul Islam, Chand Sultana, Jeb-Un Nesa, Firoj Ahmed .Phytochemical Screenining and in-vitro Thrombolytic Activity of Methanolic Leaf Extract of *Zanthoxylum rhetsa*. Journal of pharmaceutical science and research, 2015; Vol 7(6).
18. Kamrul Islam Siddique, Mir Muhammad Nasir Uddin, Md. Siddiqul Islam, Salma Parvin and Mohammad Shahriar. Phytochemical screenings, thrombolytic activity and antimicrobial properties of the bark extracts of *Averrhoa bilimbi*. Journal of Applied Pharmaceutical Science, 2013; Vol 3(3).
19. Saddam Hussain Md, Mohammad Salim Hossain, Mohammad Tohidul Amin and Shalahuddin Millat Md (2016), 'In vitro thrombolytic potentials of methanolic extract of *Vigna unguiculata* Linn (seed)', Journal of Pharmacognosy and Phytochemistry, Vol 5(3), pp. 129-133.
20. Rajeswari S and Vidhya R (2017), 'Evaluation of In *Vitro* Thrombolytic and Antiproteinase Activities of *Wedelia Trilobata* (Linn.)', Innovare Journal of Life Sciences, Vol 5(3), pp. 359-367.
21. Hossan sakib Md, Monalisha Sharma, Rana Dhar, Naymul karim, Asif Al Mahmood, Mohammad Shahin Alam, Sadequr Rahman (2015),'Study on in-vitro thrombolytic activity of chloroform extract of leaves Of *Dioscorea bulbifera*' International Journal of Medical and Health Research, Vol 1(2), pp. 60-62.
22. Arifur Rahman Chowdhury Md, Rasadul Islam Md,Ataul Gani Muktadir Md (2017), 'Thrombolytic activity of *Lagerstroemia speciosa* Leaves', Journal of discovery phytomedicine, Vol 4(4),pp. 41-45.
23. Rashaduz Zaman, Mohammad Parvez, Jakaria Md, Mohammed Abu Sayeed and Minhajul Islam (2015), 'In vitro Clot Lysis Activity of Different Extracts of *Mangifera sylvatica Roxb*. Leaves', Research Journal of Medicinal Plant, Vol 9(3),pp. 135-140.
24. Anand Prakash, Divya Jain, Rashmi Tripathi and Pracheta (2019), 'Pharmacognostical analysis of different parts of *Cyperus rotundus* L.', Plant Science Today, Vol 6(1),pp. 607-612.
25. Pushpendra kumar jain and Debajyoti das(2016),'Ethno pharmacological study of Cyperus rotundus a herb used by tribal community as a traditional medicine for treating various diseases', Innovare journal of Ayurvedic sciences, Vol 4(2),pp. 5-8.
26. Vikas Kumar (2016), 'Medicinal Uses, Phytochemistry and Pharmacological Activities of Motha (*Cyperus rotundus* Linn.): A Potential Herb', International journal of pharmaceutical research, Vol 7(3), pp. 23-30.
27. Sri Ranjani Sivapalan (2013), 'Medicinal uses and Pharmacological activities of *Cyperus rotundus* Linn' International Journal of Scientific and Research Publications, Vol 5(3), pp. 25-29.
28. Ashokkumar R and Ramaswamy M (2014), 'Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants', International journal of current microbiology and Applied science, Vol 3(2), pp. 395-406.
29. Dibyajyoti S and Swati P (2014), 'Phytochemical screening and thrombolytic activity of chloroform extract of *Urena sinuata* L, Journal of fundamental and applied science, Vol 6(2), pp 208-219.
30. Sikandar Khan Sherwani, Muhammad Mumtaz Khan, Arif Zubair, Muhammad Ajmal Shah and Shahana Urooj Kazmi (2013),' Evaluation of In Vitro Thrombolytic Activity of *Bougainvillea Spectabilis* Leaf Extract, International Journal of Pharmaceutical Sciences Review and Research, Vol 21(1), pp 6-9.
31. Barton G. M, Evans R. S. and Gardener (1952), 'Paperchromatography of phenolic substances', Journal of Nature, Vol 8(3), pp 249-250.
32. Fatema Tabassum, Somaia Haque Chadni, Kamrun Nahar Mou,Imrul Hasif KM, Tareque Ahmed and Mahbuba Akter (2017), '*In-vitro* thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of *Asteraceae* family', Journal of Pharmacognosy and Phytochemistry, Vol 6(4),pp 1166-1169.
33. Ghose AK and Crippen GM (1987), 'Atomic physicochemical parameters for three dimensional-structure-directed quantitative structure activity relationships,Modelling dispersive and hydrophobic interactions', Journal of Chemistry Information Computational Science, Vol 27, pp 21–35.
34. Shruthisrivastava (2012),' Evaluation of anti- arthritic potential of the methanolic extract of the aerial parts of costus speciosus', Journal of ayurvedha &Integrative medicine, Vol 3(4), pp 345-350.
35. Trott.O and Olson A. J (2010), 'AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading', Journal of computational chemistry, Vol 31, pp 455–461.

36. Binkowski TA, Naghibzadeg S and Liang J (2003), 'CASTp computed atlas of surface topography of proteins', International journal of green pharmacy, Vol 31,pp 3352– 3355.
37. Boham B.A and Kocipai-Abyazan R (1974), 'Flavonoids and condensed tannins from leaves of Vaccinium *vaticulatum* and V. *calycinium*', Journal of Pacific Science, Vol 48, pp 458-463.
38. Beers R and Sizer I (1952), 'A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase', Journal of Biological Chemistry, Vol 3(1), pp 195- 133.
39. Beuge JA and Aust SD (1978), 'The thiobarbituric acid assay', Methods in Enzymology, Vol 52, pp 306-307.
40. Prashanthi Gandhamalla, Shiva Goud Buddola, Pravalika Rachakonada, Ramya Manga and Narender Boggula (2018), 'Thrombolytic screening of *luffa cylindrica* linn. Fruits and in-vitro study', International journal of pharmaceutical Sciences and Research, Vol 6(1), pp 61-74.
41. Sheikh Anwar Md , Irfan Newaz Khan, Mominul Islam Sarkar , Sabuj Barua , Mostafa Kamal A.T.M , Zahid Hosen S.M(2011), 'Thrombolytic & cytotoxic effect of different herbal extracts',International journal of Pharmaceutical Sciences And Research, Vol 11(6),pp. 3118-3121.