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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plants are one of the most abundant sources of potentially useful chemical compounds and medicinal properties. As a result, over the last few decades, the market for plant research has been steadily increasing all over the world. The future pharmacological behaviours of *Cyperus rotundus Linn.* are assessed using a literature review as a basis. 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. 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. To determine which compounds are responsible for the current pharmacological activities, detailed pharmacological and phytochemical studies are needed. The phytochemical research revealed the presence of phytochemical constituents in various solvent fractions. The presence of these phytochemicals was found to be responsible for the plants' in-vitro thrombolytic action.

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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. Thrombolysis, as well known as thrombolytic healing, is a procedure that dissolves dangerous blood clots, improves blood stream, and prevents tissue and organ damage [1]. Thrombolysis, also known as cluster busting, is the pharmacological degradation (lysis) of blood clumps. *Cyprus rotandus* is also known as Koraikkizhangu in Siddha medicine and Naagarmothaa in Unani medicine. The word *Cyperus* derived from Greek “kuperos” and *rotundus* from Latin meaning “round.” The family *Cyperaceae* includes approximately 3000 species of which about 220 species are identified as weeds and of which 42% of these are in the genus *Cyperus* [2]. *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 [3]. The tubers have a distinctive fragrance and are almost blackish in shading resting on the external and ruddy white on the in the interior. The leaves are straight, dull green, and scored on the superior surface, and the stems cultivate to be about 25 cm long [4]. 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 [5].

The tuber, leaves, seeds, rhizomes, and oil of the *Cyperus* are used. The whole plant extract is utilised as an antinoceceptive, a liver and heart tonic, a digestion stimulant, and a hypertension treatment [6]. Antimalarial properties are found in the tuber part. As a food taste, the leaves were proven to enhance the sleeping time induced by common hypnotics, particularly in the Middle East and Southeast Asia [7].The rhizomes are used as a cooling, intellect promoting, nervine tonic, diuretic, antiperiodic, and analgesic, anti-inflammatory, antipyretic and to treat diarrhea, dysentery, leprosy, bronchitis, amenorrhea, and blood disorders. Fever, diarrhoea, dysentery, vomiting, and cholera can all be treated with the tuber portion, which has anti-obesity qualities, as an infusion or soup [8]. The use of drugs to break up or break up blood clots, which are the primary factor of heart attacks and strokes, is known as thrombolytic treatment. Thrombolytic drugs are approved for the treatment of strokes and heart attacks in the short term [9]. Tissue plasminogen activator (tPA) is one of the most often utilized thrombolytic medications, even though other medications can also be performed [10]. The aim of our present study was to investigate the thrombolytic activity of methanolic extracts of *Cyperus rotandus* by using an in vitro procedure.

2. MATERIALS AND METHODS

2.1 Plant Materials

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

2.2 Physicochemical Analysis

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

2.3 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 [11]. 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 [12]. The extractable matter content of air dried material (in percent w/w) was calculated as follows:

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

2.4 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 [13].

2.5 Phytochemical Screening

2.5.1 Test for tannins

One ml 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.

2.5.2 Test for saponin

Two 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.

2.5.3 Test for flavonoids

Five ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

2.5.4 Test for steroids

Two 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.

2.5.5 Test for terpenoids (Salkowski test)

Five 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.

2.5.6 Test for triterpenoids

One ml 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_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

2.5.7 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.

2.5.8 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.

2.5.9 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.

2.5.10 Test for cardiac glycosides

Five 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.

2.5.11 Test for coumarins

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

2.6 Quantitative Analysis of Phytochemicals

2.6.1 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 [14].

2.6.2 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 [15].

2.6.3 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 [16].

2.7 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 [17]. 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 [18].

2.8 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 [19].

2.9 Column Chromatography Analysis

2.9.1 Extraction of polyphenolic compounds

The following procedure was used to extract phenolic chemicals together with sugars. 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) [20]. 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 that remained on the filter paper was transferred back to dark glass bottles for extraction with 200 ml of the same extraction solution [21]. This method was repeated two more times, each time collecting the solution for analysis after 30 and 60 minutes of extraction. Supernatants were combined and evaporated using rotary vacuum evaporator to remove any remaining solvent; the water was then removed by lyophilization [22].

2.9.2 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 [23]. 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 $^{\circ}$C for 2 hours. 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 [24].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 ml 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 [25].

2.9.3 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 [26].

2.10 Thin Layer Chromatography

Thin layer Chromatography is based upon the principles of column and partition Chromatography. On a suitable flat surface, such as glass or plastic plate, a thin layer of the stationary phase is created. In this situation, a small coating of alumina or silica gel is used to separate the mixture, which is absorbed by different physical forces [27].

2.10.1 R_f value

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

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

2.11 *In Vitro* Thrombolytic Activity

Three ml of venous blood were divided into four pre-weighted eppendorf tubes and incubated for 45 minutes at 37 ^{0}C. Following clot formation, serum was withdrawn entirely without disturbing the clot, and each clot-containing tube was measured again to calculate 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. For positive control, 100µl of streptokinase (SK) was added. After that, all of the tubes were incubated at 370°C for 90 minutes and clot lysis was observed. After incubation, the fluid released was removed and the tubes were weighed again to see if there was a difference in weight after the clot was disrupted. The weight difference obtained before and after clot lysis was expressed as a percentage of clot lysis [28]. The formula for calculating clot weight 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

3. RESULTS

3.1 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. Among the two extract value, alcohol extract (17%) value has higher than water extract (4%) value.

3.2 Qualitative and Quantitative Analysis

Qualitative and quantitative analysis of *Cyperus rotandus* rhizome was 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.

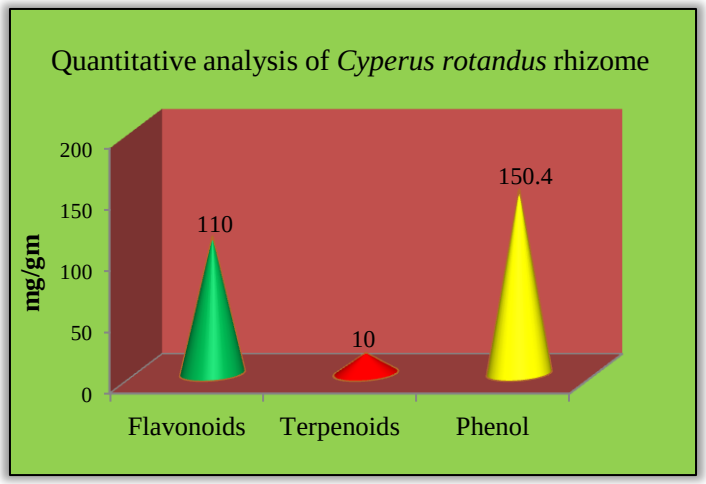


Fig. 1. Quantitative phytochemical analysis of *Cyperus rotundus* rhizome

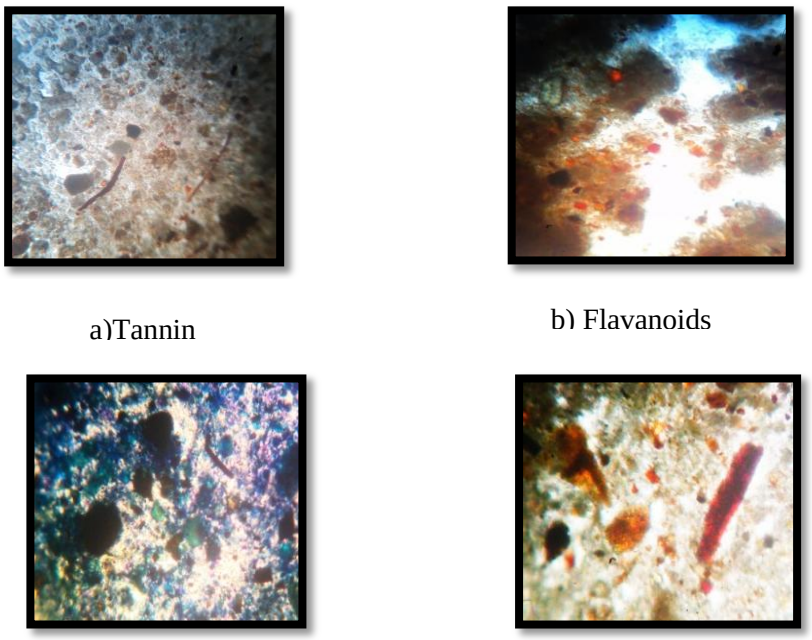
Table 1. Extractive value of *Cyperus rotundus* rhizome

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

Table 2. 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

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

3.3 Histochemical Analysis

Histochemical analysis of *Cyperus rotundus* rhizome powder was investigated. *Cyperus rotandus* rhizome powder treatment with different reagents and observation under microscope [29]. The results revealed the presence of Tannin, Flavonoids, Polyphenol and Terpenoids. In this results further confirmed the presence of phytochemicals (Table 2 and Fig. 2).

3.4 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 [30].

3.5 FTIR Analysis

FTIR also referred as FTIR spectroscopy is an FTIR evaluation is an analytical move toward used to perceive organic, polymeric and in some cases, inorganic substances. The FTIR analysis technique makes use of infrared mild to experiment take a look at samples and examine chemical residences [31].

3.6 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 (Fig. 5).The collected fractions further tested in phenol (Table 6) by qualitatively [32]. 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 (Fig. 5). The results of the study concluded that *Cyperus rotundus* rhizome extract contain Gallic acid and quercetin.

Table 3. 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

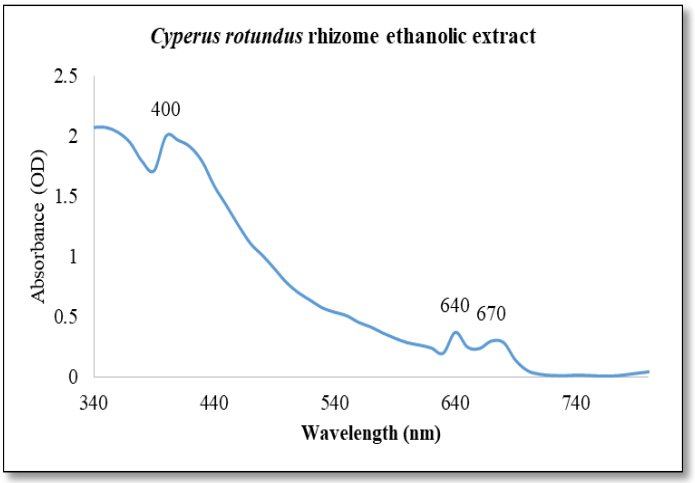


Fig. 3. UV-Visible analysis of *Cyperus rotundus* rhizome ethanolic extract

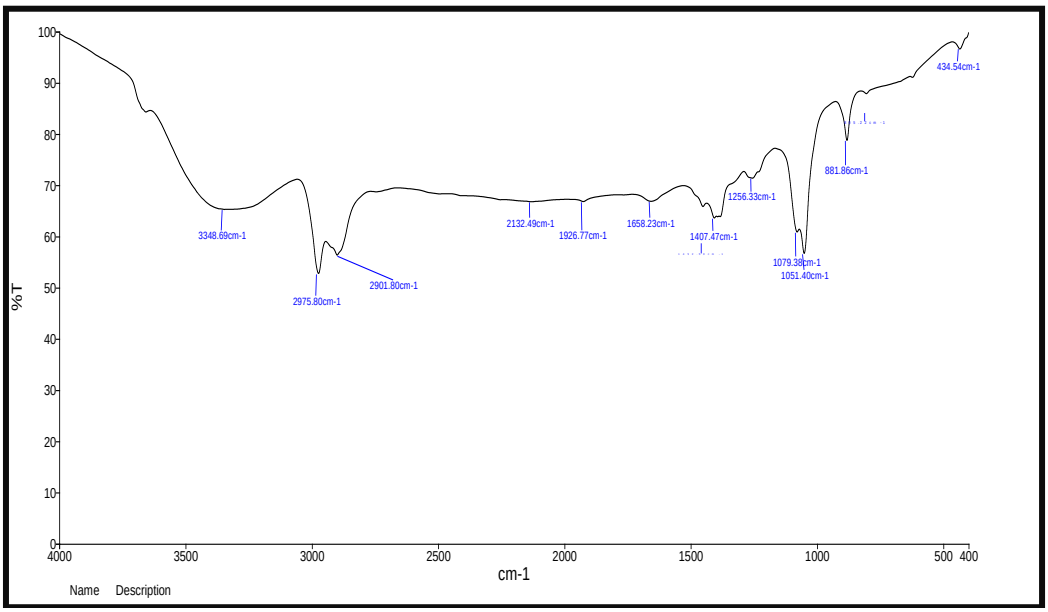


Fig. 4. FTIR peak of *Cyperus rotandus*

Table 4. 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

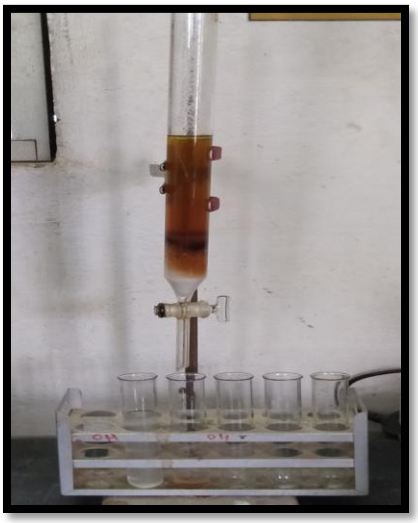


Fig. 5. Column chromatography

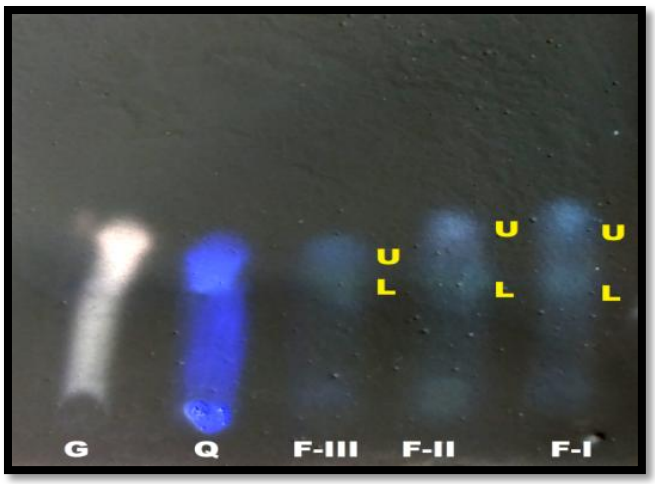


Fig. 6. Identification of the phenolic compound using Column Chromatography eluting and TLC

Table 5. 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

3.7 Thin Layer Chromotography

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

3.8 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 6). So it can be concluded as significant anti-coagulant agent.

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 100 µg/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 100 µg/ml, 200 µg/ml and Standard was statistically significance deference from α 0.05 significant level compared with Control.

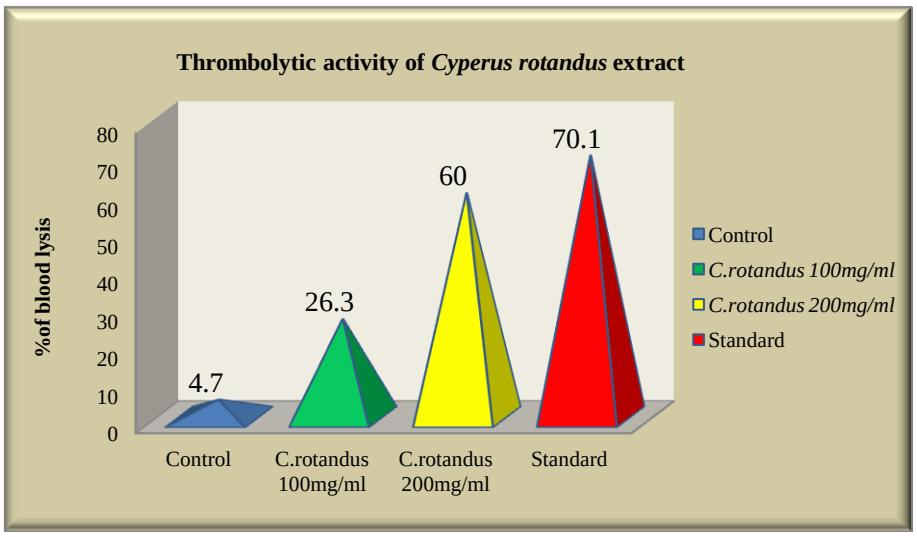


Fig. 7. Thrombolytic activity of *Cyperus rotundus* rhizome extract

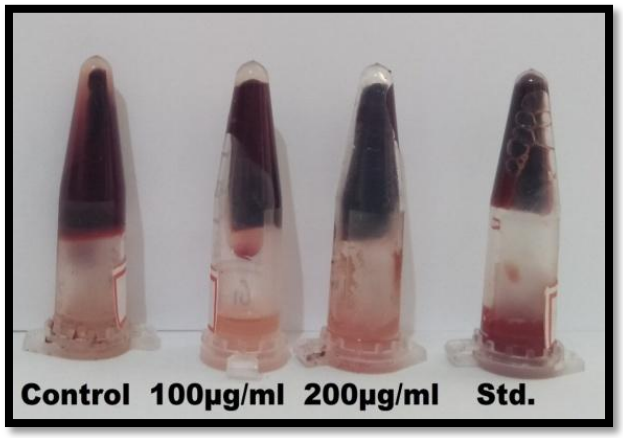


Fig. 8. Experiment of thrombolytic activity of *Cyperus rotundus* rhizome extract

Table 6. 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*

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 200 µg/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.

4. 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 [33]. 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 [34]. The present study indicates high level phytochemical constituents of Tannin, Flavonoids, Polyphenol and Terpenoids from *Cyperus rotandus* like other plants. The study evaluated the polyphenol is a high level Phytochemical in *Cyperus rotandus.* The results showed that *Cyperus rotundus*, when extracted with alcohol, obtained higher polyphenol content, while the aqueous extract. 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 [35]. 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 [36]. 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. 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 [37,38].

5. 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. 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. 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. Along with aim of determining the phytochemical screening and thrombolytic involvement of *Cyperus rotandus* in this research. The phytochemical screening of *Cyperus rotandus* extract was choose based on high concentration of phytochemicals. The presence of tannin, flavonoids, terpenoids, and polyphenol was also verified by histochemical analyses. The extract of *Cyperus rotandus* had excellent thrombolytic activity. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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