

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).

Cyperus 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 rotandus* 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

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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 and 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 H₂SO₄. 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 H₂SO₄. 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 H₂SO₄ (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 H₂SO₄. 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 H₂SO₄ 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 underlayered with 1 ml of concentrated H₂SO₄. 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.

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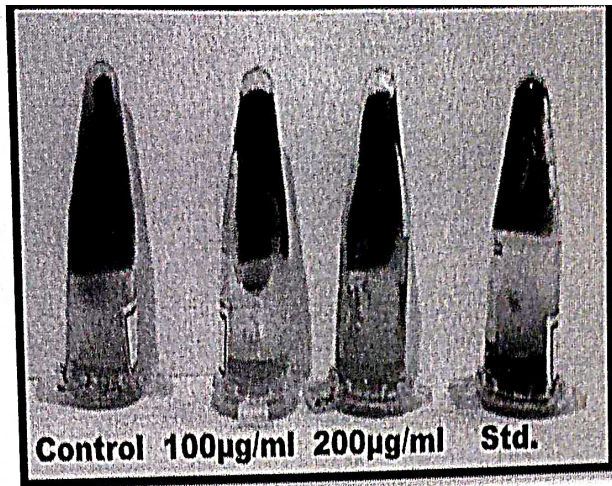


Figure 8: Experiment of thrombolytic activity of *Cyperus rotundus* rhizome extract

The present study a significant thrombolytic activity was observed after treating the clots with *Cyperus rotundus* rhizome ethanolic extract shows $60.00 \pm 5.18\%$ in $200\mu\text{g/ml}$ of clot lysis while Streptokinase shows the $70 \pm 4.46\%$ 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 rotundus* 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 rotundus* 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 rotundus* analysis such as UV-Visible spectroscopy, FTIR, Column chromatography and TLC were showed high activity compared to another plants (Trott.O *et al.*, 2010). The methanol extract of *Cyperus rotundus* 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 rotundus* was investigated in this research. Along with aim of determining the phytochemical screening and thrombolytic involvement of *Cyperus rotundus* in this research. Overall, it concluded that the above results suggest that the extract of *Cyperus rotundus* has rich source of phytochemicals confirmed by qualitative and quantitative. The present investigation provides. The experimental studies of *Cyperus rotundus* 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.

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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.

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