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IN VITRO EVALUATION OF METAL CHELATING PROPERTIES OF *T. CATAPPA* AND *T. KIRILOWII* LEAF EXTRACTS

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ABSTRACT

Thalassemia, a genetic blood disorder, often requires regular blood transfusions, leading to iron overload and severe organ damage if not managed properly. This study investigates the *in vitro* metal chelating properties of *Terminalia catappa* and *Trichosanthes kirilowii* leaf extracts, focusing on their potential to manage iron overload in thalassemia patients. The leaves were collected from Adilabad district, Telangana, and processed using a sequential maceration method with solvents like chloroform, ethyl acetate, and acetone. The extracts were then subjected to *in vitro* assays to assess their metal chelating activities. The findings could provide valuable insights into the potential of *T. catappa* and *T. kirilowii* as natural chelators, offering a safer and more cost-effective alternative for managing iron overload in thalassemia patients. At higher concentrations, *T. catappa* and *T. kirilowii* displayed chelating activities of 76.8% and 71.2% respectively at 1.6 mg/ml, compared to EDTA's 68.1%. At the highest concentration of 3.2 mg/ml, *T. catappa* and *T. kirilowii* exhibited superior activities of 88.3% and 84.5%, respectively, while EDTA showed 71.5%. Regression curve analysis revealed IC50 values of 0.28 mg/ml for *T. catappa* and 0.35 mg/ml for *T. kirilowii,* both lower than the 0.76 mg/ml for EDTA. The findings could provide valuable insights into the potential of *T. catappa* and *T. kirilowii* as natural chelators, offering a safer and more cost-effective alternative for managing iron overload in thalassemia patients. This research not only contributes to the growing body of knowledge on plant-based metal chelators but also underscores the therapeutic potential of these plants in treating iron overload and improving the quality of life for thalassemia patients This research highlights the potential of these plant extracts as natural chelators, offering a promising alternative for managing iron overload in thalassemia patients.

Keywords: *T. catappa, T. kirilowii,* Adilabad, Metal chelating, EDTA, Thalassemia.

INTRODUCTION

Thalassemia, a genetic blood disorder characterized by ineffective erythropoiesis and chronic anemia, often necessitates regular blood transfusions (Karimi et al., 2021). These transfusions, while life-saving, lead to iron overload in the body, which can cause severe organ damage if not managed properly (Chew et al., 2009). Metal chelating agents are essential in controlling this iron overload by binding excess iron and facilitating its excretion. Current chelators, such as deferoxamine, deferiprone, and deferasirox, have significantly improved the management of

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thalassemia by reducing iron levels and preventing iron-induced complications (Wong et al., 2012). However, these agents are not without limitations, including high costs, side effects, and the need for long-term adherence, which can be challenging for patients.

Terminalia catappa and *Trichosanthes kirilowii* are renowned for their extensive use in traditional medicine, particularly in India, where they are employed to treat a variety of ailments (Wong et al., 2014). These plants are rich in diverse phytochemicals, including flavonoids, tannins, and phenolic acids, which contribute to their therapeutic properties (Porika et al., 2014). Traditional healers have long utilized these plants to address conditions such as diarrhea, fever, skin diseases, and inflammation, leveraging their potent bioactive compounds (Mohan et al., 2012). Despite their widespread use and the known benefits of their phytochemicals, the specific metal chelating activity of these plants has not been thoroughly investigated. Metal chelation is a critical process for mitigating metal toxicity, which can lead to severe health issues such as thalassemia, neurodegenerative diseases, and organ damage (Patel et al., 2013). Recognizing the gap in research, this study aims to evaluate the in vitro metal chelating activity of crude leaf extracts from *T. catappa* and *T. kirilowii*.

Given these challenges, there is an urgent need to explore new metal chelating agents from natural sources, such as medicinal plants, which may offer safer and more cost-effective alternatives. The leaves of *T. catappa* and *T. kirilowii*, with their rich phytochemical profiles, present a promising avenue for such exploration (Bouriche et al., 2011. The evaluation of their metal chelating activities through in vitro assays can provide valuable insights into their potential as natural chelators. Developing effective plant-based chelating agents could revolutionize the treatment of thalassemia, offering patients a more sustainable and accessible option for managing iron overload and improving their quality of life (Porika et al., 2023). This research not only contributes to the growing body of knowledge on plant-based metal chelators but also underscores the therapeutic potential of *T. catappa* and *T. kirilowii.*

MATERIALS AND METHODS

Plant Collection

The leaves of *Terminalia catappa* and *Trichosanthes kirilowii* were collected from the forest area of Adilabad district, Telangana state. The plant voucher specimens were identified with the help of taxonomist Dr. Sreenivas, Department of Botany, Government Degree & PG College, Adilabad.

Preparation of Plant Extracts

The selected plant parts were collected, washed, and shade-dried at room temperature until they were free from moisture. The dried leaves of these plants were subjected to size reduction to get a coarse powder (200 g), which was then stored in a clean, dry, airtight container and extracted

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by sequential maceration method using non-polar to polar solvents (chloroform, ethyl acetate, and acetone).

The powder (200 g) was treated with 400 ml of chloroform and kept for 24 hours. After 24 hours, the extract was filtered, and the residue was added with 400 ml of ethyl acetate solvent and kept for another 24 hours. The ethyl acetate extract was filtered, and the residue was then added with 400 ml of ethanol and kept for 24 hours. All the extracts were filtered using Whatman filter paper #41, and the filtrates were collected in a beaker. The filtrates were then kept for solvent evaporation. The weight of the residual extract was measured, and the percent yield was calculated using the following formula:

Percent yield = (Weight of the residual extract / Weight of the plant powder) × 100
Extract yield % = W1/W2 X 100; Where

W1= Net wt of powder in grams after extraction
W2= total wt of powder in grams taken for extraction.

In vitro Metal chelating activity of Crude Extracts:

The crude extracts which obtained high yield were selected to assess *in vitro* Metal chelating activity. The chelation of ferrous ions by extracts was estimated by method of Sinha et al. (1972). 50 µl of 2 mM FeCl2 was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm.

The percentage inhibition of ferrozine–Fe2+ complex formation was calculated a [(A0- As)/ As]x100, where;
A0 was the absorbance of the control, and
As was the absorbance of the extract/standard. Na_2EDTA was used as positive control

Statistical Analysis

Results are presented as mean ± SD. Statistical analyses were performed by Student's t-test with the use of MS excel software. The values of $p < 0.05$ were considered significant**.**

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RESULTS AND DISCUSSION

Percentage of Extract Yield

The yield of sequential extracts of *T. catappa* is shown in (Table-1). The chloroform extract resulted in a yield of 1.850 grams, which corresponds to a percentage yield of 0.925% (w/w) based on the initial weight of 200 grams. The ethyl acetate extract had the highest yield among the three solvents, with 2.570 grams, translating to a percentage yield of 1.285% (w/w). The acetone extract had a yield of 2.220 grams, which equates to a percentage yield of 1.110% (w/w).

The sequential extracts of the leaves of the *T. kirilowii* plant show some interesting characteristics based on the provided data in Table-2. The chloroform extract yielded 1.970 grams, which corresponds to a percentage yield of 0.985% (w/w) from the initial weight of 200 grams. The ethyl acetate extract had a yield of 2.130 grams, resulting in a percentage yield of 1.065% (w/w). The acetone extract had the highest yield among the three solvents, with 2.780 grams, translating to a percentage yield of 1.390% (w/w).

The high yields obtained from these specific solvent extracts imply that they likely contain a greater concentration and diversity of the target phytochemicals or bioactive compounds of interest. Therefore, selecting these two extracts, the *T. catappa* ethyl acetate and the *T. kirilowii* acetone, for further in-depth studies and analyses would be a logical and well-informed decision, as they have demonstrated the optimal extraction efficiency among the solvents tested. Based on the high yields obtained from the different solvent extracts, the ethyl acetate crude extract of *T. catappa* and the acetone crude extract of *T. kirilowii* were selected for further studies.

Table-1. Extractive values of different crude extracts of *T. catappa* *And T. kirilowii* leaves

S. No	Solvent Extract	*T. catappa*		*T. kirilowii*	
		Yield of the extract (in gm)	Percentage yield (%w/w)	Yield of the extract (in gm)	Percentage yield (%w/w)
1	Chloroform	1.850	0.925%	1.970	0.985%
2	Ethyl acetate	2.570	1.285%	2.130	1.065%
3	Acetone	2.220	1.110%	2.780	1.390%

In vitro Metal chelating activity of Crude Extracts:

Based on the high yields obtained from the different solvent extracts, the ethyl acetate crude extract of *T. catappa* and the acetone crude extract of *T. kirilowii* were selected for *in vitro* metal

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http://magellanes.com/

chelating activity. The chelating of Fe2+ by extracts was estimated by the method of Dinis et al. (1994). Ferrozine can quantitatively form complexes with Fe2+. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased.

In vitro Metal Chelating Activity of *T. catappa* crude extract:

The ethyl acetate crude extract of *T. catappa* demonstrated a substantial metal chelating activity across all tested concentrations. At the lowest concentration of 0.2 mg/ml, it exhibited a chelating activity of 48.5%. As the concentration increased to 0.4 mg/ml, the chelating activity also increased significantly to 61.2%. This upward trend continued at 0.8 mg/ml, where the activity reached 68.4%. At a concentration of 1.6 mg/ml, *T. catappa* exhibited a metal chelating activity of 76.8%, showcasing its potency. The highest tested concentration of 3.2 mg/ml showed the most pronounced activity, with an impressive chelating activity of 88.3%. These results highlight the strong and concentration-dependent metal chelating capability of *T. catappa* ethyl acetate crude extract.

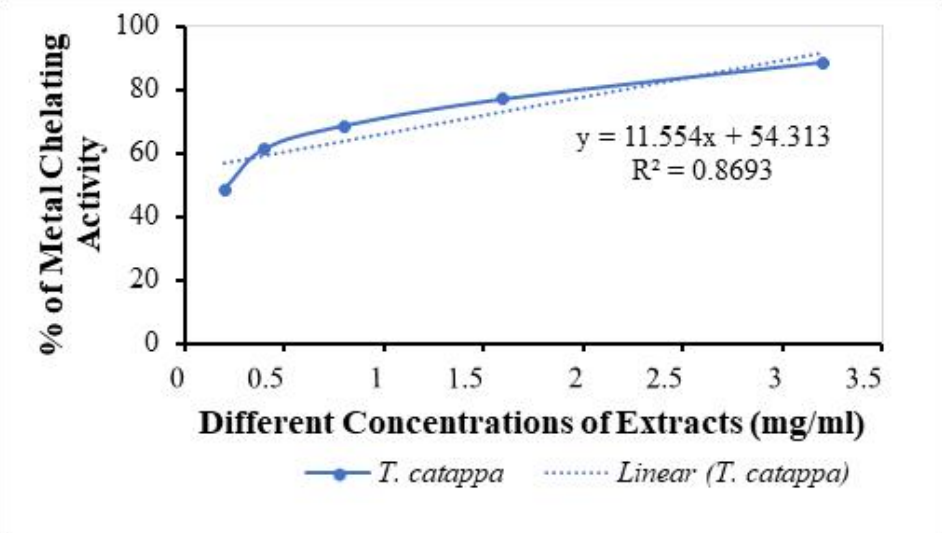

Figure-1. Metal Chelating Activity of *Terminalia catappa* Ethyl Acetate Crude Extract at Different Concentrations

The linear trendline showed in Figure-1 suggests a consistent enhancement in chelating ability as the extract concentration increases, making *T. catappa* a promising candidate for applications requiring effective metal chelation. Based on the regression curve analysis, the IC50 value for the ethyl acetate crude extract of *T. catappa* was determined to be 0.28 mg/ml. The IC50 (half-maximal inhibitory concentration) is a crucial pharmacological metric that indicates the concentration of a substance needed to inhibit a biological process by 50%.

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http://magellanes.com/

***In vitro* Metal Chelating Activity of *T. kirilowii* crude extract:**

The acetone crude extract of *T. kirilowii* also showed notable metal chelating activity, though slightly lower than that of *T. catappa*. At a concentration of 0.2 mg/ml, *T. kirilowii* exhibited a chelating activity of 49.4%, which is marginally higher than that of *T. catappa*. (Figure-2). At 0.4 mg/ml, the chelating activity of *T. kirilowii* increased to 55%, demonstrating its effectiveness. This increase continued at 0.8 mg/ml, where the activity reached 64.6%. At a concentration of 1.6 mg/ml, *T. kirilowii* showed a metal chelating activity of 71.2%. The highest concentration of 3.2 mg/ml resulted in an activity of 84.5%, indicating a strong chelating capability, although slightly less than that of *T. catappa.* These values reflect the considerable metal chelating potential of *T. kirilowii* acetone crude extract. The IC50 value of the *T. kirilowii* Ethyl Acetate Crude Extract is 0.35, which indicates the concentration of the extract required to inhibit 50% of a specific activity.

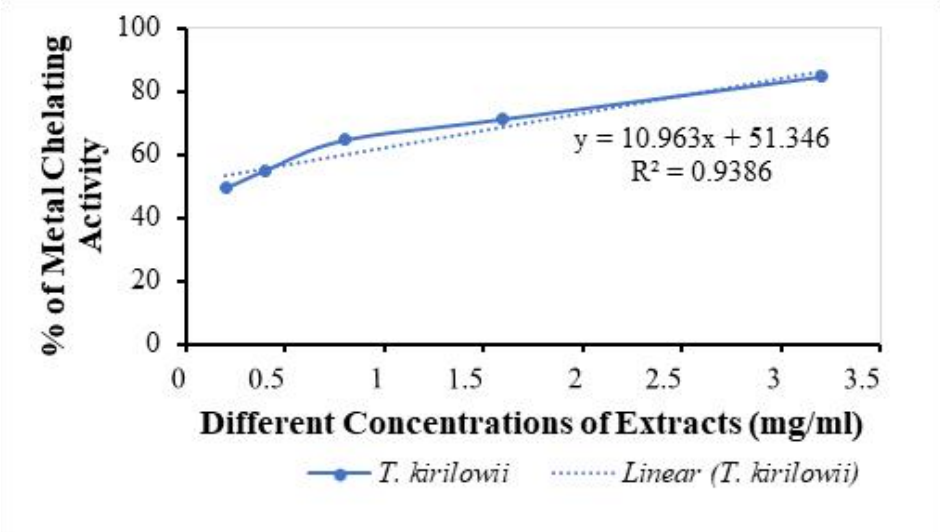

Figure-2. Metal Chelating Activity of *T. kirilowii* Ethyl Acetate Crude Extract at Different Concentrations

The standard EDTA exhibited metal chelating activity that was consistently lower than both plant extracts across all tested concentrations. The metal chelating activities of the three samples- *T. catappa* ethyl acetate crude extract, *T. kirilowii* acetone crude extract, and standard EDTA—demonstrated distinct patterns across various concentrations in Figure-1,2 and 3. *T. catappa* showed the highest chelating activity, starting at 48.5% at 0.2 mg/ml and reaching 88.3% at 3.2 mg/ml. *T. kirilowii* followed closely, with initial activity at 49.4% at 0.2 mg/ml and peaking at 84.5% at 3.2 mg/ml. In comparison, EDTA exhibited consistently lower activity, starting at 42.6% at 0.2 mg/ml and increasing to 71.5% at 3.2 mg/ml. Both plant extracts outperformed EDTA at all concentrations, particularly at higher concentrations where *T. catappa* and *T*

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http://magellanes.com/

kirilowii displayed chelating activities of 76.8% and 71.2% respectively at 1.6 mg/ml, compared to EDTA's 68.1%. At the highest concentration of 3.2 mg/ml, *T. catappa* and *T. kirilowii* exhibited superior activities of 88.3% and 84.5%, respectively, while EDTA showed 71.5%. These results indicate that both plant extracts have a stronger and more effective metal chelating ability compared to the standard EDTA, with *T. catappa* demonstrating the highest potency.

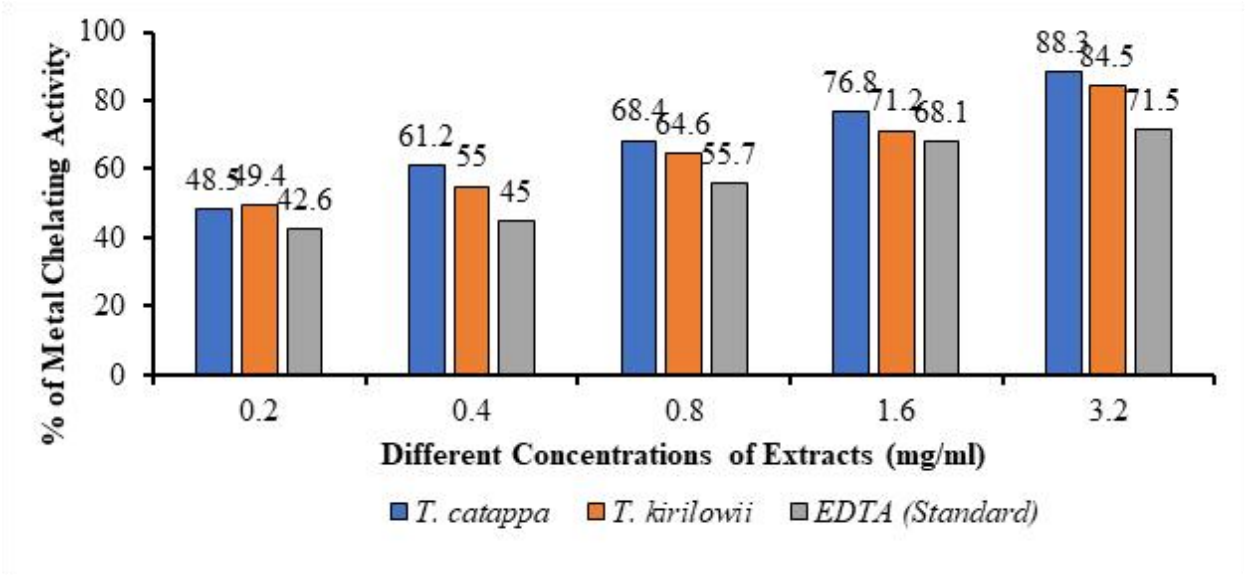

Figure-3. Metal chelating activity of selected plant extracts and EDTA standard

The regression curve analysis further revealed that the IC50 values, the concentration required to inhibit 50% of the metal chelation, were 0.28 mg/ml for *T. catappa* and 0.35 mg/ml for *T. kirilowii*, both lower than the 0.76 mg/ml for EDTA. These findings indicate that the bioactive compounds in the plant extracts have a stronger and more effective metal chelating ability compared to the standard EDTA, with *T. catappa* demonstrating the highest potency.

These findings align with previous research by Babri et al. (2015), who reported the medicinal plant extracts often show superior metal chelating activities compared to synthetic chelators like EDTA. Similarly, the work of Sarkar et al., (2012) supports these results, highlighting the potent chelating abilities of various plant-derived compounds. Furthermore, the study by Ebrahimzadeh et al,. (2008) demonstrated that natural extracts could outperform traditional chelators in various applications, reinforcing the potential of Terminalia species effective alternatives to EDTA. Additionally, the research by Vijayagiri et al. (2012) corroborates these findings, emphasizing the enhanced chelating properties of plant extracts due to their complex mixture of bioactive compounds.

The superior metal chelating activities of *T. catappa* and *T. kirilowii* extracts compared to EDTA suggest that these plant extracts could serve as more effective and natural alternatives for

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applications requiring metal chelation. This study, along with the corroborative findings from Gujjeti et al. (2013), Mamidala et al., (2013), Sarkar et al. (2012), and Ebrahimzadeh et al. (2008), underscores the potential of plant-based chelators in various industrial and environmental contexts.

CONCLUSION

The study demonstrates that *T. catappa* ethyl acetate crude extract and *T. kirilowii* acetone crude extract possess significantly higher metal chelating activities compared to the standard EDTA across all tested concentrations. *T. catappa* exhibited the highest chelating activity, followed closely by *T. kirilowii*, both outperforming EDTA, especially at higher concentrations. These findings suggest that plant extracts, particularly *T. catappa* and *T. kirilowii*, could serve as more effective and natural alternatives to synthetic chelators like EDTA. The results are consistent with previous research, highlighting the potential of plant-based chelators in various industrial and environmental applications, offering a promising avenue for future studies and practical implementations.

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