EVALUATION OF ANTIGOUT AND FREE RADICAL SCAVENGING ACTIVITY OF *THESPESIA POPULNEA*'S LEAF ETHANOLIC EXTRACT – AN INVITRO RESEARCH

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ABSTRACT

Gout is a widespread metabolic disorder caused by the dysregulation in the metabolism of the purines, which in turn causes the deposition of monosodium urate crystals in the joints and causes acute inflammatory arthritis. Plant-based medicines that are natural have been of interest in treating gout based on their ability to treat gout using a variety of mechanisms such as enzyme inhibition and antioxidant. *Thespesia populnea*, a medicinal plant with traditional medicinal use, is reputed to have various pharmacological activities including anti-inflammatory and antioxidant effects. The current research was designed to determine the in-vitro anti-gout effects and free radical scavenging capability of the ethanolic extract of *Thespesia populnea*, and the phytochemical constituents. The maceration method was used to prepare the extract, which was preliminarily screened in terms of phytochemicals. The xanthine oxidase inhibitory activity with hypoxanthine and xanthine as substrates was measured to determine the anti-gout activity. The standard reference drug was allopurinol. The ferric reducing antioxidant power (FRAP) assay was used to determine the antioxidant activity of the extract and ascorbic acid served as the positive control. The findings revealed that the ethanolic extract had a high xanthine oxidase inhibitory property, which suggested that it could help to lower uric acid synthesis. Also, the extract demonstrated a significant free radical scavenging property implying that it can alleviate the oxidative stress. Finally, *Thespesia populnea* has a good potential of anti-gout and antioxidant activity, which justifies its traditional application. The results indicate that it can be used as a natural remedial to gout and should be investigated further in-vivo and clinically.

Keywords: anti- gout, anti- oxidative, in vitro study, *thespesia populunea*, xanthine oxidase, xanthine.

INTRODUCTION

Gout is a chronic metabolic disease that is marked by chronic hyperuricemia that leads to deposition of monosodium urate (MSU) crystals in joints and in the surrounding tissues [1]. It is a condition which results in repeated attacks of acute inflammatory arthritis, which causes severe pains, deformation of joints and poor quality of life. During the last 10 years, the incidence of gout worldwide has grown tremendously, mainly attributed to aging, changes in diet and lifestyle [2]. The disease does not only impact physical health but also affects day to day functioning and social functioning.

The main treatment approach to gout is to reduce the level of serum uric acid. It is usually done by inhibiting xanthine oxidase (XO), one of the enzymes involved in the metabolism of hypoxanthine to xanthine and, subsequently, to uric acid [3]. The most common XO inhibitors prescribed include conventional medications like allopurinol and febuxostat, but their use is commonly linked to other adverse effects like hypersensitivity reactions, skin rashes, vasculitis, and renal complications. Such restrictions have led to seeking alternatives that are safer and more effective [4].

Medicinal plants have been of significant interest in recent years as possible sources of anti-gout agents because they have multi-target effects, are less toxic, and more cost-effective [5]. According to the literature, flavonoids and phenolic compounds present in plants have been shown to have pronounced XO-inhibitory and antioxidant properties [6], which subsequently lower the levels of uric acid and oxidative stresses caused by gout. A number of studies put forward the therapeutic prospect of plant-based compounds in the management of hyperuricemia and gout [7].

Thespesia populnea Soland. ex Correa or the Portia tree is a plant in the Malvaceae family. Thespesia is a Greek word: thespesios, which translates as divine or sacred. This perennial seashore shrub is very common in the tropical areas of India, Southeast Asia, Africa and the Pacific Islands. Traditionally, different parts of the plant such as bark, leaves, flowers, and fruits

have been used for treating various ailments including inflammation, skin diseases, liver disorders, diabetes, and infections [8,9]. The plant is also antioxidant, anti-inflammatory, hepatoprotective and antimicrobial. [10] Although *Thespesia populnea* has a broad spectrum of potential traditional applications and pharmacological actions, limited scientific data exists on the anti-gout effects of *Thespesia populnea*, specifically, its xanthine oxidase inhibitory effect. As the plant contains bioactive phytoconstituents like flavonoids and phenolics, the plant can potentially be of potential therapeutic use in gout management. Hence, the current research was intended to assess the phytochemical contents, in-vitro anti-gout effects in terms of xanthine oxidase inhibition and the free radical scavenging potential of the ethanol extract of the *Thespesia populnea* leaves with the aim of scientifically validating its possible application in gout treatment.

METHODOLOGY

Collection of Plant Material

Fresh leaves of Thespesia populnea were collected in November 2025 from Nadupatty, Tiruchirappalli District, Tamil Nadu, India.

Authentication of Plant Material

The plant specimen was identified using *Flora of the Presidency of Madras* (Gamble, Vol. I, p.101) and authenticated by Dr. S.P. Anand, Associate Professor, Department of Botany, National College, Tiruchirappalli (Authentication No: CT/BOT/SPA/PI/25/00005).

Preparation of Plant Extract

The collected leaves were shade-dried and coarsely powdered. The powdered material was extracted with ethanol by cold maceration at room temperature for seven days. The extract was filtered and used for further analysis.

Preliminary Phytochemical Screening

The ethanolic extract was subjected to phytochemical screening to identify the presence of alkaloids, flavonoids, glycosides, carbohydrates, proteins, phytosterols and phenolic compounds through qualitative phytochemical analysis. The tests included Wagner, Dragendorff, Mayer (alkaloids), Keller-Killiani (glycosides), Molisch and Benedict (carbohydrates), Shinoda and alkaline reagent (flavonoids), Biuret and Ninhydrin (proteins) as specified tests conducted in accordance with standard procedures [11,12]

In-vitro Xanthine Oxidase Inhibitory Activity

The anti-gout effect was assessed by determining the xanthine oxidase (XO) inhibitory activity through a spectrophotometric technique [13]. This reaction mixture was kept at 25ºC with phosphate buffer (50 mM, pH 7.8), enzyme solution (XO), and various concentrations of plant extract (10-500 ug/mL) and incubated at this temperature over 10 minutes. Then the substrate (hypoxanthine/xanthine) was put in and left to incubate at 37ºC. The reaction was stopped with 1N HCl and the absorbance was recorded at 290 nm in ELISA microplate reader. Allopurinol was used as the standard drug. Percentage inhibition and IC_{50} values were calculated.

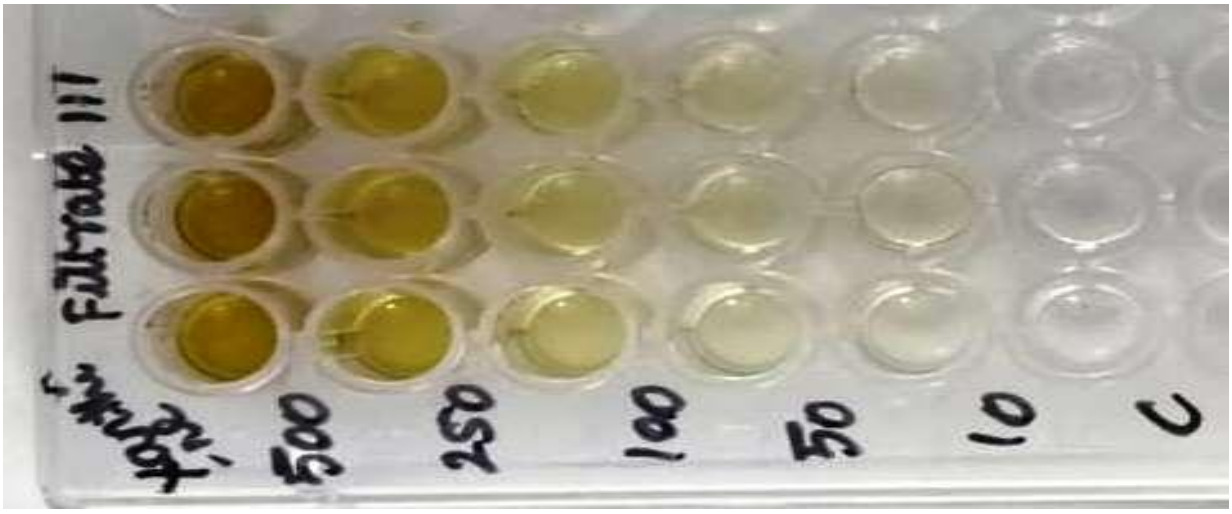

Figure: 1 ELISA (enzyme-linked immunosorbent assay) microplate reader; Fitrate-III-*Thespesia populunea* plant's ethanolic extract at various concentration; Xanthine as control.

Free Radical Scavenging Activity (FRAP Assay)

The Ferric Reducing Antioxidant Power (FRAP) assay was used to determine the antioxidant activity. Different concentrations of the extract were combined with phosphate buffer and potassium ferricyanide and then incubated at 50ºC, 20 minutes. Trichloroacetic acid and centrifugation were added to the supernatant followed by the use of distilled water and ferric chloride. The colorimeter was used to measure the absorbance at 700 nm. As the positive control, ascorbic acid was used. [14]

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of the ethanolic leaf extract of *Thespesia populnea* revealed the presence of a wide spectrum of biologically active secondary metabolites including alkaloids, glycosides, steroids, carbohydrates, phenolic compounds, flavonoids, and proteins (Table 1). The extract was particularly rich in steroidal and flavonoid content, which is consistent with earlier phytochemical reports on the same species.

The presence of alkaloids was confirmed by positive reactions with Wagner's reagent (reddish-brown precipitate), Dragendorff's reagent (reddish-brown precipitate), and Mayer's reagent (cream-coloured precipitate). Alkaloids are nitrogen-containing compounds known to exhibit significant XO inhibitory and anti-inflammatory activities. Glycosides were detected by the Keller–Killiani test, Molisch test, and concentrated sulphuric acid test. Steroidal content was confirmed by the Salkowski test, indicating the presence of triterpenoids and phytosterols that contribute to anti-inflammatory mechanisms.

Phenolic compounds were identified by positive ferric chloride, lead acetate, and dilute iodine tests. Phenolics are well-established inhibitors of xanthine oxidase and potent antioxidants that reduce uric acid biosynthesis by chelating the molybdenum cofactor of the XO active site. Flavonoids were confirmed by the Shinoda test (magenta colour), Zinc-HCl test (magenta colour), alkaline reagent test (intense yellow colouration), and lead acetate test (orange to

crimson colour). Flavonoids are among the most studied natural XO inhibitors, with quercetin, kaempferol, and luteolin reported to suppress urate production more potently than allopurinol in some studies. The co-occurrence of flavonoids and phenolic compounds in the extract further supports the synergistic anti-gout potential of *T. populnea* leaf extract.

Table 1: Phytochemical Screening of Ethanolic Leaf Extract of *Thespesia populnea*

S. No.	Phytochemical Class	Test	Observation	Result
1	Alkaloids	Wagner's reagent	Reddish-brown precipitate	Positive
		Dragendorff's reagent	Reddish-brown precipitate	Positive
		Mayer's reagent	Cream-colour precipitate	Positive
2	Glycosides	Keller–Killiani test	Reddish-brown ring at junction of two layers	Positive
		Conc. H_2SO_4 test	Reddish-colour precipitate	Positive
		Molisch test	Reddish-purple ring at junction	Positive
3	Steroids	Salkowski test	Brown/red ring on H_2SO_4 layer	Positive
4	Carbohydrates	Benedict's test	Green/yellow/orange/red colouration	Positive
5	Phenolic Compounds	Ferric chloride test	Blue, green or violet colour	Positive
		Lead acetate test	White precipitate	Positive
		Dilute iodine solution	Red colour	Positive
6	Flavonoids	Ammonia test	Yellow colouration (fades with time)	Positive
		Shinoda's test	Magenta colour	Positive
		Zinc-HCl test	Magenta colour	Positive
		Lead acetate test	Orange to crimson colour	Positive
		Alkaline reagent test	Intense yellow (colourless on dil. HCl)	Positive

7	Proteins	Ninhydrin test	Purple/blue colour	Positive
		Biuret test	Blue colour	Positive

Conc. = Concentrated; H_2SO_4 = Sulphuric acid; HCl = Hydrochloric acid

In Vitro Anti-Gout Activity: Xanthine Oxidase Inhibitory Assay

The *in vitro* anti-gout activity of the ethanolic leaf extract of *Thespesia populnea* was evaluated by measuring its ability to inhibit xanthine oxidase (XO) enzyme activity at five concentrations (10, 50, 100, 250, and 500 µg/mL). The absorbance values at 540 nm for the control and test concentrations are presented in Table 2, and the corresponding percentage inhibition values are shown in Table 3.

Table 2: Optical Density (OD) Values at 540 nm for *Xanthine Oxidase* Inhibitory Assay

S. No.	**Sample / Concentration (µg/mL)**	**OD_1 (540 nm)**	**OD_2 (540 nm)**	**OD_3 (540 nm)**	**Mean OD**
1	Control	0.868	0.882	0.802	0.850
2	500	0.373	0.385	0.348	0.369
3	250	0.449	0.458	0.432	0.446
4	100	0.623	0.637	0.615	0.625
5	50	0.652	0.648	0.623	0.641
6	10	0.777	0.771	0.716	0.755

OD = Optical Density; Control mean OD = 0.850; Values represent triplicates

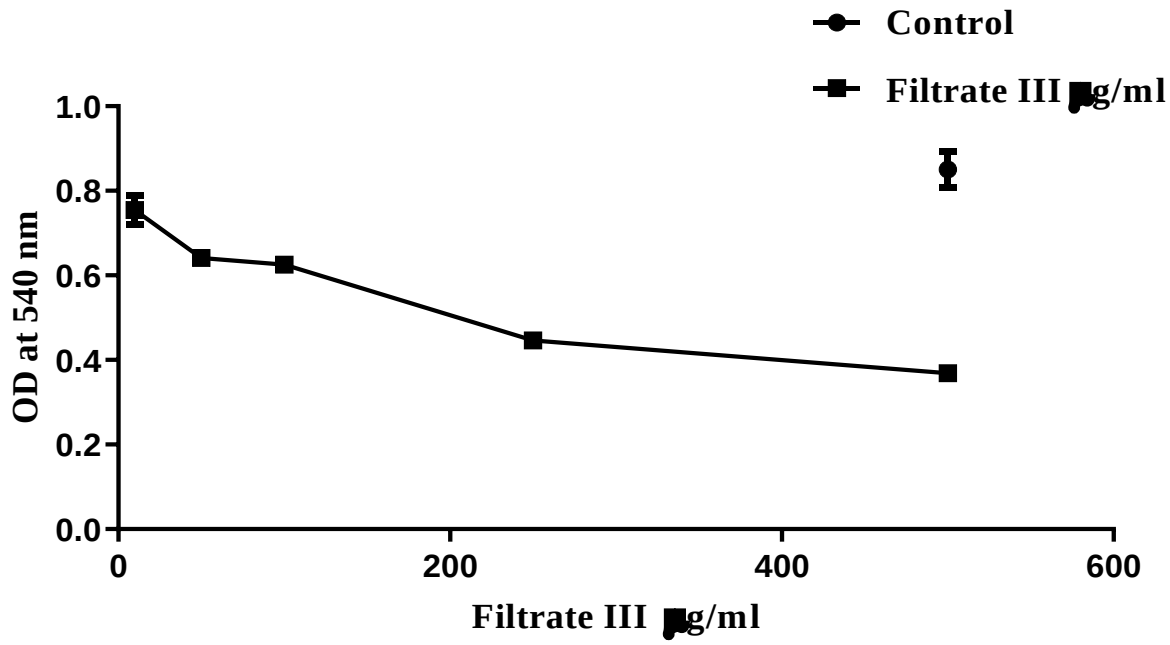

Figure:2 OD values of control and Filtrate

The extract demonstrated a clear concentration-dependent inhibition of XO activity across all tested concentrations. As the concentration of the extract increased from 10 to 500 µg/mL, a progressive reduction in OD values was observed, directly corresponding to increased enzyme inhibition. The mean OD decreased from 0.755 at 10 µg/mL to 0.369 at 500 µg/mL, compared to the control mean OD of 0.850, confirming dose-dependent suppression of XO activity.

Table 3: Percentage Inhibition of Xanthine Oxidase by Thespesia populnea Ethanolic Leaf Extract

S. No.	Concentration (µg/mL)	% Inhibition – Trial 1	% Inhibition – Trial 2	% Inhibition – Trial 3	Mean % Inhibition
1	500	56.12	54.71	59.06	56.63
2	250	47.18	46.12	49.18	47.49
3	100	26.71	25.06	27.65	26.47

4	50	23.29	23.76	26.71	24.59
5	10	8.59	9.29	15.76	11.22

% Inhibition = [(Control OD − Sample OD) / Control OD] × 100; Values are mean of triplicates

The percentage of XO inhibition ranged from 11.22% at the lowest concentration (10 µg/mL) to 56.63% at the highest concentration (500 µg/mL), with all three replicates showing consistent inhibitory responses, as evidenced by a high coefficient of determination ($R^2 = 0.9657$) from the dose–response curve analysis (Table 4). This strong linear correlation between concentration and percentage inhibition further validates the reliability of the assay and confirms the authentic XO inhibitory potential of the extract.

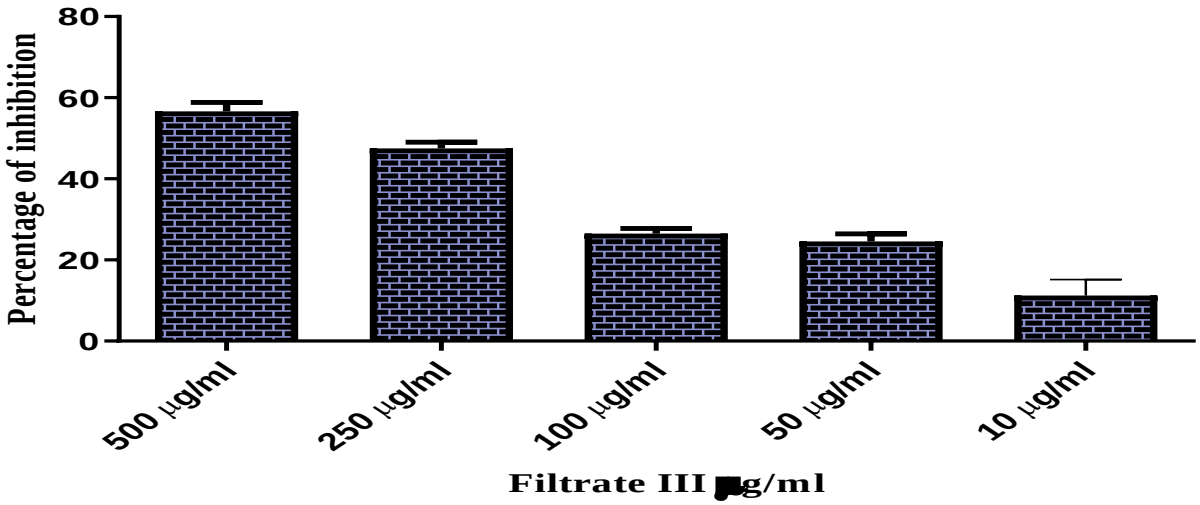

Figure: 3 % inhibition of XO in *Thespesia populnea* Ethanolic Leaf Extract

Table 4: IC_{50} Determination by Variable-Slope Dose–Response Analysis

Parameter	**Value**
IC_{50}	117.2 µg/mL
Log EC_{50}	2.069
Hill Slope	−1.715
95% CI for EC_{50}	96.59 to 142.2 µg/mL

R^2	0.9657
Degrees of Freedom	13
Number of Data Points Analyzed	15

IC_{50} = Half-maximal Inhibitory Concentration; EC_{50} = Effective Concentration 50%; CI = Confidence Interval.

The IC_{50} value of the ethanolic leaf extract of *Thespesia populnea* was determined to be 117.2 µg/mL using the variable-slope sigmoidal dose–response model (log[agonist] vs. normalized response). The normal reference IC_{50} range for allopurinol, the gold-standard XO inhibitor, is reported to be 0.2–50 µM. While the IC_{50} of the present extract is higher than that of the pure synthetic drug, it demonstrates moderate and scientifically relevant anti-gout activity for a crude plant extract. The observed XO inhibitory activity of *T. populnea* extract may be attributed to the rich content of flavonoids and phenolic compounds identified during phytochemical screening. These compounds are known to inhibit XO through non-covalent interaction with the molybdopterin cofactor at the enzyme's active site, thereby preventing the conversion of hypoxanthine to xanthine and xanthine to uric acid.

Free Radical Scavenging Activity: Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant potential of the ethanolic leaf extract of *Thespesia populnea* was assessed by the Ferric Reducing Antioxidant Power (FRAP) assay. The absorbance values at increasing concentrations of the extract are presented in Table 5. The positive control and negative control recorded absorbance values of 0.30 and 0.50, respectively.

Table 5: Ferric Reducing Antioxidant Power (FRAP) Activity of Thespesia populnea Leaf Extract

S. No.	Concentration (µL)	Absorbance
1	10	0.53
2	20	0.87
3	40	1.87
4	60	2.00

5	80	0.65
6	Positive Control	0.30
7	Negative Control	0.50

Absorbance measured at 593 nm; Higher absorbance indicates greater reducing power.

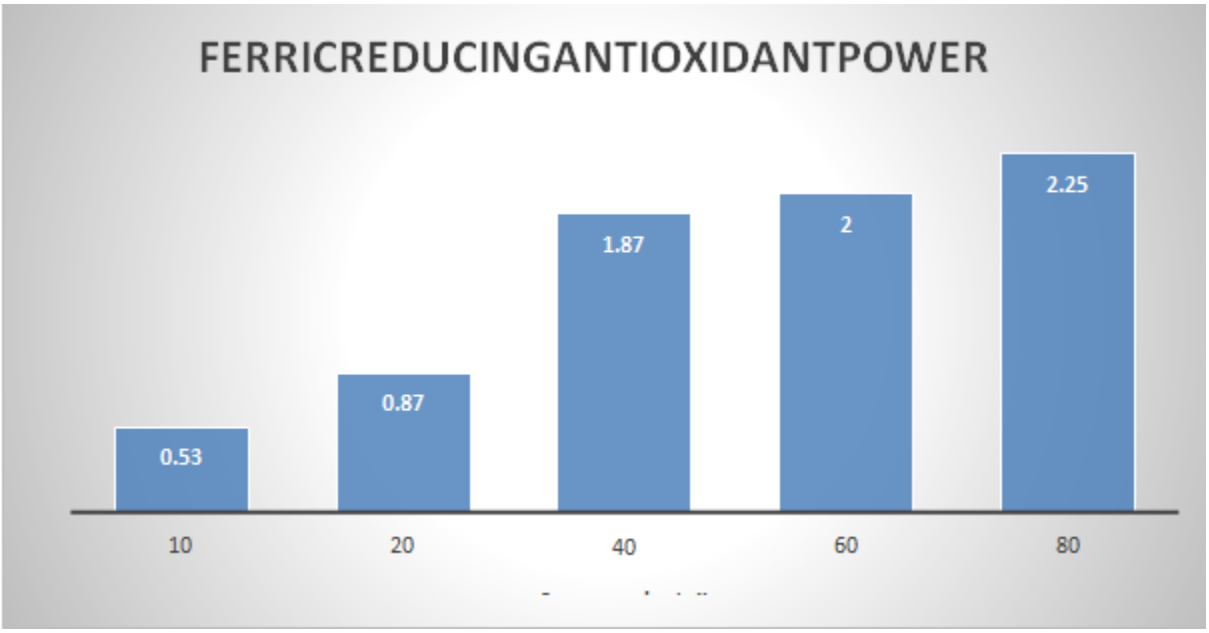

Figure: 4 FRAP assay of plant extract

The FRAP assay results demonstrate that the extract possesses significant ferric ion reducing capacity, indicating the presence of strong electron-donating constituents. A progressive increase in absorbance was observed from 10 µL (0.53) through 60 µL (2.00), confirming a concentration-dependent increase in antioxidant activity. The slight decline in absorbance at 80 µL may reflect a saturation phenomenon or a prooxidant effect at high concentrations, which has been reported for crude plant extracts.

The high antioxidant capacity observed is consistent with the rich phenolic and flavonoid content of the extract, as these compounds are the primary contributors to free radical scavenging and metal ion chelation activities in plant extracts. In the context of gout pathophysiology, this finding is particularly significant: xanthine oxidase catalysis generates superoxide anions and hydrogen peroxide as byproducts that exacerbate inflammatory tissue damage in gouty joints. The demonstrated free radical scavenging ability of *T. populnea* leaf extract therefore provides a complementary protective mechanism, reducing oxidative stress-mediated tissue injury alongside its primary XO inhibitory activity.

DISCUSSION

The current research shows that ethanolic extract of Thespesia populnea is rich in phytochemicals, xanthine oxidase inhibitory and antioxidant properties. The phytochemical screening established the presence of flavonoids and phenolic compounds which have been highly reported to help in xanthine oxidase inhibition and antioxidant properties [15]. It has been demonstrated that these compounds have the potential to bind to the active site of XO and inhibit the production of uric acid. The results of the anti-gout activity indicated the presence of a dose-dependent effect on xanthine oxidase inhibition (maximum inhibition of 56.62) with the highest dose of 500 ug/mL. The IC50 (117.2 µg/mL) is moderate against allopurinol but validates the all-plant-based options. The same was found by a study who emphasized that plant extracts tend to present moderate XO inhibition as a result of synergistic phytoconstituents [16]. The results of the FRAP assay proved the presence of strong reducing power and antioxidant activity whose intensity rose with the concentration. Oxidative stress plays a key role in gout pathogenesis, and antioxidants help in reducing inflammation and tissue damage [17]. The antioxidant activity observed is in line with previous report of *Thespesia populnea* [18]. In general, the results of the study confirm that *Thespesia populnea* has dual therapeutic activity, lowering the synthesis of uric acid and oxidative stress. Though it is weaker than the standard drugs, as a promising natural and safer alternative, it should be subjected to additional in-vivo and clinical research.

CONCLUSION

The current research shows that the ethanolic extract of the leaves of *Thespesia populnea* have significant anti-gout and antioxidant properties. The extract exhibited high dose-dependent xanthine oxidase inhibitory activity and a good level of free radical scavenging potential, potentially useful in preventing uric acid production and oxidative stress related to gout. These effects are probably due to the presence of bioactive phytoconstituents like the flavonoids and phenolic compounds. The findings indicate the promise of the use of *Thespesia populnea* as a natural, safer alternative that can be used to manage gout although the activity was moderate as compared to standard drugs. More research such as isolation of the acting compounds and in-vivo testing should be encouraged to confirm its therapeutic effectiveness.

REFERENCES

1. Richette P, Bardin T. Gout. *Lancet.* 2010;375(9711):318–328.
2. Perez-Ruiz F, Dalbeth N, Bardin T. A review of uric acid and gout. *Adv Ther.* 2014;32(1):31–41.
3. Kannan CS, Muthupandian S. *Thespesia populnea*: A multipurpose tree species. *Int J Curr Res.* 2023;15(8):25561–25565.
4. Panchal H, Shah M. *Thespesia populnea* Linn: A review. *Int J Pharmacogn.* 2017;4(1):1–5.
5. Muthukumar S, Veerappa NS. Phytochemical analysis of *Thespesia populnea*. *J Pharmacogn Phytochem.* 2018;7(1):414–417.
6. Dipti P, Suthar S, Naskar A, Jain SK. *Thespesia populnea* for management of diseases: An overview. *Int J Pharm Sci Res.* 2024;15(4):990–1005.
7. Sarvaiya VN, et al. Evaluation of anti-gout activity of plant extracts. *Vet World.* 2015;8(10):1230–1236.
8. Zeng JX, Huang WH, Wan JY, Yao HQ, Yuan CS. Antigout effects via XO inhibition. *Evid Based Complement Alternat Med.* 2018; 1364617.
9. Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. *Biosci Biotechnol Biochem.* 1999;63(10):1787–1790.
10. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. *J Nutr Sci.* 2016;5:e47.
11. Harborne JB. *Phytochemical Methods.* 3rd ed. London: Springer; 1998.
12. Trease GE, Evans WC. *Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
13. Nile SH, Park SW. HPTLC analysis, antioxidant and antigout activity of Indian plants. *Iran J Pharm Res.* 2014;13:531–539.
14. Anandjiwala S, Bansal M, Parabia M, Rajani M. Evaluation of free radical scavenging activity. *Indian J Pharm Sci.* 2008;70:31–35.
15. Roohbakhsh A, Shamsara J, Khayyat MH, Karimi G. XO inhibition by plant remedies. *Pharmacologyonline.* 2009;3:1031–1036.
16. Mohamed DA, Al-Okbi SY. Evaluation of anti-gout activity of plant extracts. *Pol J Food Nutr Sci.* 2008;58(3):389–395.
17. Jayavarsha V, Gayathri R, Vishnu Priya V, Selvaraj J, Kavitha S. Antioxidant and anti-gout potential of plant extract. *J Pharm Res Int.* 2021;33(55B):21–28.

18. Shah SP, Bhagwat A. Evaluation of phytochemicals and antioxidant activity of *Thespesia populnea*. *Int J Pharm Pharm Sci.* 2015;7(Suppl 1):117–121.