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Research Paper

Pharmacognostic Potential of *Thelypteris dentata* (Forssk.)

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ABSTRACT

Thelypteris dentata (Forssk.), a fern species rich in phytochemical compounds, has garnered attention for its potential health benefits. The study employed various assays to evaluate primary and secondary metabolites with reference to their pharmacognostic potential, such as alpha amylase inhibitory activity (AAI), antioxidant and anti-inflammatory activity. Fronds of Thelypteris dentata (Forssk.) possessed various phytochemicals, where important primary metabolites like total proteins, reducing sugars, soluble carbohydrates and Vit. C content as well as secondary metabolites such as total phenolics, flavonoids, tannins and coumarins were estimated with standard protocols. Results revealed a substantial amount of both primary and secondary metabolites. An in-vitro alpha amylase inhibitory, antioxidant and anti-inflammatory assay showed significant antioxidant activity in T. dentata extracts, with notable scavenging effects on DPPH radicals. Additionally, the extracts demonstrated potent anti-inflammatory properties. These findings underscore the potential of T. dentata as a natural source of antioxidants and anti-inflammatory agents, warranting further exploration for pharmaceutical and nutraceutical applications.

INTRODUCTION

Medicinal plants have long served as major components of traditional and modern healthcare systems, owing to their rich and diversified bioactive compounds with therapeutic potential. In recent years, there has been a growing interest in exploring neglected plant groups such as Pteridophytes (ferns and fern allies) for their pharmacological properties. Although angiosperms dominate ethnopharmacological research, ferns represent an underutilized group despite their widespread distribution and documented use in traditional medicinal systems.[1] Pteridophytes are known to synthesize a varied group of primary and secondary metabolites, including proteins, carbohydrates, phenolics, flavonoids, tannins, and coumarins, many of

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which exhibit significant biological activities such as antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects.[2] These phytochemicals play a vital role in plant defence mechanisms and also contribute to their therapeutic efficacy in humans.

Among all ferns, *Thelypteris dentata* (Forssk.), belonging to the family Thelypteridaceae, is abundantly available in tropical and subtropical regions. It thrives in moist habitats and has been traditionally used in folk medicine for treating various ailments, although its pharmacognostic profile remains unexplored. Preliminary phytochemical investigations of related species in the genus *Thelypteris* suggest the presence of phenolic compounds and flavonoids, which are known for their potent antioxidant properties.[3] Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defences, is implicated in the pathogenesis of numerous chronic diseases, including diabetes, inflammation, cardiovascular disorders, and cancer.[4] Natural antioxidants derived from plant sources are therefore gaining attention as safer alternatives to synthetic compounds. In addition, the inhibition of enzymes such as α-amylase is a promising strategy in the management of postprandial hyperglycemia in diabetic patients.[5] Similarly, plant derived anti-inflammatory agents are increasingly preferred due to fewer side effects compared to synthetic drugs.

Though *T. dentata* is abundantly available, there is limited scientific data available regarding its phytochemical composition and pharmacological activities. Therefore, systematic pharmacognostic evaluation, including the estimation of primary and secondary metabolites along with assessment of antioxidant, anti-inflammatory, and α-amylase inhibitory activities, is essential to validate its therapeutic potential.

Thus, the present study aims to investigate the pharmacognostic properties of *Thelypteris dentata* fronds by analyzing their primary and secondary biochemicals and evaluating their biological activities. This research not only contributes to the scientific validation of this fern but also highlights its potential as a natural source of bioactive compounds for pharmaceutical and nutraceutical applications.

MATERIALS AND METHODS

Fresh fronds of *Thelypteris dentata* (Forssk.) were collected from Pune and nearby areas. It was authenticated by the Botanical Survey of India (BSI), WRC, Pune and a voucher specimen was deposited in the herbarium for future reference. The collected fronds were washed thoroughly with distilled water to remove dust and debris. Fresh fronds were extracted with various solvents such as buffer, distilled water, ethanol and methanol. Clear supernatants after centrifugation were considered as crude extract and stored at 4 $^{\circ}$C for further estimations of primary and secondary metabolites as well as biological analysis. [6]

Total Protein Content

Total soluble protein content was estimated using the Lowry method. Briefly, plant extract was mixed with alkaline copper reagent, followed by Folin Ciocalteu reagent, and absorbance was recorded at 660 nm using a spectrophotometer. Bovine serum albumin (BSA) at 1mg /ml was used as a control.[7]

Reducing Sugars

Total reducing sugars were estimated using the DNS (3,5-dinitrosalicylic acid) method. The reaction mixture was heated in a boiling water bath and absorbance was measured at 540 nm. Maltose was used as a control. [8]

Total Soluble Carbohydrates

Total soluble carbohydrates were estimated by the Anthrone method. The reaction mixture was heated, and absorbance was recorded at 620 nm. Glucose served as the control. [9]

Ascorbic Acid (Vitamin C)

Ascorbic acid content was determined using the 2,6-dichlorophenol indophenol dye by the titrimetric method. [10]

Estimation of Secondary Metabolites

Total Phenolic Content

Total phenolics were determined using the Folin–Ciocalteu reagent method. Absorbance was measured at 760 nm, and results were expressed as mg tannic acid equivalents. [11]

Total Flavonoid Content

Total flavonoid content was estimated using the aluminium chloride colorimetric method, and absorbance was recorded at 415 nm. Results were expressed as mg quercetin equivalents (QE)/g extract.[12]

Total Tannin Content

Tannins were estimated using Folin–Denis reagent, and absorbance was measured at 700 nm.[13]

Coumarin Content

Total coumarin content was determined spectrophotometrically following standard protocols described by Harborne (1998). [6]

Alpha-Amylase Inhibitory Activity

The salivary alpha amylase inhibitory activity was determined using the dinitrosalicylic acid (DNSA) method. Plant extract was incubated with α-amylase enzyme, followed by the addition of starch solution. The reaction was stopped using the DNS reagent, and absorbance was recorded at 540 nm. Acarbose (Glucobay tablet) was used as the standard inhibitor. [14]

Radical Scavenging Activity (DPPH Assay)

The free radical scavenging activity of the extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Different concentrations of methanolic plant extract were mixed with DPPH solution and incubated in the dark. Absorbance was measured at 517 nm. Ascorbic acid was used as a standard antioxidant.[15]

Anti-inflammatory Activity

Anti-inflammatory activity was assessed using a protein denaturation assay. The reaction mixture containing plant extract and bovine serum albumin was incubated and heated. Absorbance was read at 660 nm. [16]

Statistical Analysis

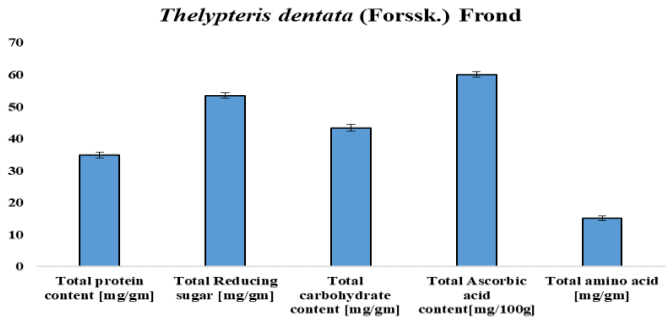
All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD).

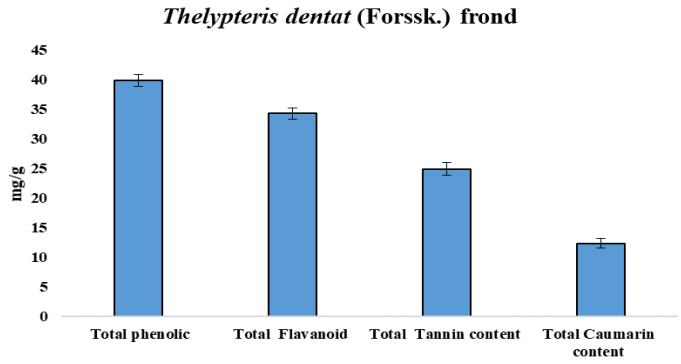
Sec. Metabolites	Reagent [chemical] test	Aqueous Ext.	Methanol Ext.	Ethanol Ext.	Colour Obs.
Alkaloid	Mayers	-	+	++	Greenish
	Hagers	++	+++	+	Yellow
	Dragendroff	+	++	+++	Reddish

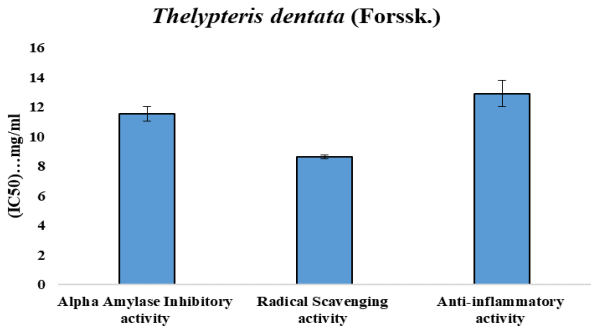
Flavanoid	Sodium hydroxide	+	++	-	Mild-yellow
	Ferric chloride	+	++	+++	Reddish yellow
Phenols	Ferric chloride	-	+	++	Greenish-black
Terpenoids	Chloroform	-	+	+++	Reddish-black
Tannins	Ferric chloride	-	-	+	Brownish-green
Caumarin	Sodium hydroxide	+	++	-	Yellow
Glycosides	Sulphuric acid	++	+	-	Reddish-yellow
Saponins	distilled water	+	++	-	Whitish foam

+++: Represents higher concentrations; ++: Represents moderate concentrations; +: Represents less concentrations and -: Represents not detected

Estimation of important primary metabolites:







RESULTS AND DISCUSSION

The present study revealed that *Thelypteris dentata* is a rich source of various bioactive metabolites and exhibited significant α-amylase inhibitory, antioxidant and anti-inflammatory activities. These findings are consistent with recent advances highlighting ferns (pteridophytes) as underexplored reservoirs of pharmacologically active compounds. Recent reviews indicated that ferns contain varied phytochemicals such as polyphenols, flavonoids, and terpenoids that contribute to multiple therapeutic effects, including anti-hyperglycemic and anti-inflammatory activities. [17]The substantial amounts of phenolic (39.84 mg/g) and flavonoids (34.27 mg/g) contents observed in the present study strongly correlate with the effective DPPH radical scavenging activity (IC_{50} = 8.62 mg/ml). This relationship can be recognized as polyphenols act as primary antioxidants through hydrogen donation and electron transfer mechanisms. Recent studies emphasize that plant synthesized phenolics play a crucial role in overcoming oxidative stress and preventing chronic diseases by regulating redox homeostasis. Similarly, various fern derived extracts have been shown to restrain oxidative stress markers and inflammatory mediators such as IL-1β and iNOS pathways, supporting their biological relevance. The same was explained by Moussa *et. al.,* 2024. [17]The major anti-inflammatory activity (IC_{50} = 12.9 mg/ml) observed in *T. dentata* may be attributed to the synergistic action of flavonoids and tannins. Recent experimental evidence given by Lenka Langhansova *et. al.,*[18] that fern metabolites can inhibit key inflammatory enzymes such as COX and LOX, with some species demonstrating activity comparable to standard drugs. This strongly supports the current findings and the hypothesis that pteridophytes can serve as potential sources of safer anti-inflammatory agents with minimal side effects. The α-amylase inhibitory activity (IC_{50} = 11.54 mg/ml) further showed the potential antidiabetic potential, particularly in Type 2 diabetes of *T. dentata*. Thus, existing research highlighted that plant derived amylase enzyme inhibitors, particularly proteins as well as polyphenolic compounds, effectively regulate postprandial hyperglycemia by modulating carbohydrate metabolism.[19] Many scientists have recently reported that ferns possess antihyperglycemic properties and therefore emphasize their relevance in metabolic disorder management.[19]Apart from that, the considerable levels of primary metabolites such as proteins, carbohydrates, and ascorbic acid suggest that *T. dentata* is not only pharmacologically important but also nutritionally valuable. Presently, many researchers have recorded the number of phytochemicals from fern species and emphasized their dual role as functional foods and therapeutic agents due to their rich biochemical composition and health promoting properties.[18]Hence, the results confirmed the effective use of ferns as promising agents for drug discovery and nutraceutical development. However, despite encouraging *in-vitro* findings, further studies involving compound isolation, mechanistic pathways, and *in-vivo* validation are necessary to fully establish the therapeutic efficacy of *T. dentata*.

CONCLUSION

Thus, the present study confirms that *Thelypteris dentata* is a potent source of bioactive phytochemicals with substantial pharmacognostic value. The fronds exhibited substantial levels of primary metabolites such as proteins, carbohydrates, and ascorbic acid, along with significant concentrations of secondary metabolites including phenolics, flavonoids,

tannins, and coumarins. These biochemical constituents are directly associated with the various biological activities. The crude extract demonstrated notable effective α-amylase inhibition, antioxidant activity and significant anti-inflammatory potential which indicated its possible role in managing oxidative stress, inflammation, and metabolic disorders such as type II diabetes. These findings clearly revealed the therapeutic relevance of *T. dentata* and support its traditional use as a medicinal plant.Overall, this study offers scientific validation for the pharmacological potential of *T. dentata* and suggests its promising application in the development of natural antioxidants, antidiabetic agents, and anti-inflammatory formulations. However, further studies focusing on isolation of active compounds, elucidation of molecular mechanisms, toxicity and in-vivo validation are crucial to fully explore its clinical potential.

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