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Extraction, Phytochemical Screening And Quantitative Studies Of Phytoconstituents In Roots Extract Of *Triplophyllum Protensum*

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Introduction

The plant *Triplophyllum protensum* is a member of the family Polypodiaceae, commonly known for its rich diversity and significant medicinal properties. This fern species, found predominantly in tropical regions, has been traditionally used in various cultures for treating a range of ailments. Recent scientific interest in *Triplophyllum protensum* is driven by its potential pharmacological properties, which may include antioxidant, anti-inflammatory, and antimicrobial activities.

The extraction and phytochemical screening of plant roots are critical steps in identifying and isolating bioactive compounds. Phytochemicals such as alkaloids, flavonoids, phenols, and saponins are known for their therapeutic benefits, contributing to the plant's medicinal value (Parekh & Chanda, 2007). These compounds are secondary metabolites produced by plants as a defense mechanism against pathogens and environmental stressors, and they have been harnessed in traditional medicine and modern pharmaceuticals alike (Cowan, 1999).

Studies have shown that the bioactive compounds in plant extracts can vary significantly based on the extraction solvent used. Solvent extraction is process that influences the yield and purity of the phytoconstituents obtained. Common solvents like methanol, ethanol, chloroform, and

ethyl acetate are used to extract different types of phytochemicals, each solvent having varying efficacy depending on the polarity of the target compounds (Tiwari et al., 2011).

Quantitative analysis of phytochemicals provides valuable insights into the concentration of active compounds within the plant extracts. Techniques such as UV-visible spectrophotometry, are widely employed for this purpose. Accurate quantification is essential for correlating the biological activity of plant extracts with their chemical composition (Harborne, 1998).

This study aims to investigate the phytochemical profile and quantify the major phytoconstituents present in the roots of *Triplophyllum protensum*. By employing extraction methods and phytochemical screening techniques, this research seeks to identify and measure the active compounds that contribute to the medicinal properties of this fern species. Understanding the phytochemical composition will not only validate traditional uses of *Triplophyllum protensum* but also pave the way for its potential application in pharmaceutical development.

Material and Methods

Collection of plant materials

Roots of *Triplophyllum protensum* were collected from Subham nursery, Bhopal (M.P) in month of December, 2023. The fresh roots parts of this species were washed under running tap water, shade dried at room temperature and powdered following which they were ground to a fine powder, sieved through a 500-µm sieve, and stored until the extraction.

Figure 1: Collection of roots of *Triplophyllum protensum*

Extraction by maceration process

Defatting of plant material

50 grams of shade dried powdered of roots of *Triplophyllum protensum* subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction with successive solvent using maceration

Defatted powder was measured and mixed with successive solvents like chloroform, ethyl acetate, ethanol and water (Kokate, 1994). This was left for 2 days in sterile environment. The liquid extract was then filtered through Whatman filter paper no. 40. The filtrate was kept in water bath at 80-90ºC till the extract was dried out.

Phytochemical Screening

Preliminary screening of biochemical tests of all three extracts were done for testing various phytochemicals found in plants (Mukherjee, 2007). The crude extracts were tested for the presence or absence of secondary metabolites such as alkaloids, phenolic compound, flavonoids, saponins, tannins and glycosides. The following biochemical tests have been performed to confirm the presence or absence of the secondary metabolites in the plant extract.

Quantitative studies of phytoconstituents in roots extract of *Triplophyllum protensum*

Estimation of total phenol content

The total phenol content of the extracts was determined by the modified folin-ciocalteu method (Parkhe and Bharti, 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extracts was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of each extract and standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$

solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (John *et al.*, 2014).

Results and Discussion

The study on the roots extract of *Triplophyllum protensum* has provided significant insights into the plant's chemical composition and potential medicinal properties. The extraction yields, as presented in Table 1, showed varying efficiency with different solvents. The aqueous extract had the highest yield at 7.88% w/w, followed by the ethanol extract at 6.15% w/w, ethyl acetate extract at 2.50% w/w, and chloroform extract at 1.12% w/w. The high yields of the aqueous and ethanol extracts suggest that these solvents are more effective in extracting the soluble phytoconstituents from the roots of *Triplophyllum protensum*, which is likely due to their polarity and ability to dissolve a wide range of polar compounds present in the plant material.

Phytochemical screening revealed a diverse range of bioactive compounds in the different extracts, with the ethanol and aqueous extracts showing a richer composition compared to the chloroform and ethyl acetate extracts. For alkaloids, only the ethanol extract tested positive using Wagner's Test, indicating the presence of nitrogenous compounds known for their therapeutic properties, including analgesic, antimalarial, and antibacterial activities. Glycosides were not detected in any of the extracts, suggesting that these compounds may not be prevalent or extractable using the solvents employed in this study.

The presence of flavonoids was confirmed in both the ethanol and aqueous extracts through the Alkaline Reagent and Lead acetate tests. Flavonoids are renowned for their antioxidant properties, which contribute to the plant's potential in combating oxidative stress-related

disorders. Diterpenes were not detected in any of the extracts, which could mean that they require different extraction techniques or solvents for proper isolation. The detection of phenols in the ethanol and aqueous extracts, as well as in the ethyl acetate extract via the Folin-ciocalteu Test, highlights the presence of these compounds known for their antioxidant and anti-inflammatory properties.

Proteins were absent in all extracts, while carbohydrates were present only in the aqueous extract, suggesting the presence of water-soluble polysaccharides or simple sugars. Saponins, which possess various pharmacological properties including expectorant, anti-inflammatory, and immunomodulatory effects, were found in both the ethanol and aqueous extracts. Tannins were not detected in any of the extracts, indicating that they might either be absent or not extractable by the solvents used. Sterols were detected only in the aqueous extract, indicating the presence of steroidal compounds with potential cholesterol-lowering and anti-inflammatory effects.

Quantitative analysis revealed significant concentrations of total phenols, flavonoids, and alkaloids in the extracts. The ethanol extract had the highest total phenol content at 1.883 mg/100 mg, followed by the aqueous extract at 0.994 mg/100 mg, and the chloroform extract at 0.388 mg/100 mg. This high phenolic content suggests strong antioxidant potential in the ethanol extract. The highest flavonoid content was found in the aqueous extract at 2.506 mg/100 mg, followed by the ethanol extract at 1.462 mg/100 mg, further indicating the antioxidant capabilities of these extracts. The total alkaloid content was significant in the ethanol extract at 5.833 mg/100 mg, while the chloroform and aqueous extracts did not show detectable alkaloid content in the quantitative analysis.

Table 1: % Yield of roots extract of *Triplophyllum protensum*

S. No.	Extracts	% Yield (W/W)
1.	Chloroform	1.12
2.	Ethyl acetate	2.50
3.	Ethanol	6.15
4.	Aqueous	7.88

Table 2: Result of phytochemical screening of extract of *Triplophyllum protensum*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract

1.	**Alkaloids**				
	Wagner's Test:	-ve	-ve	+ve	-ve
	Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides**				
	Cons. H_2SO_4	-ve	-ve	-ve	-ve
3.	**Flavonoids**				
	Alkaline Reagent	-ve	-ve	+ve	+ve
	Test:	-ve	-ve	+ve	+ve
	Lead acetate Test:				
4.	**Diterpenes**				
	Copper acetate Test:	-ve	-ve	-ve	-ve
5.	**Phenol**				
	Ferric Chloride Test:	-ve	-ve	+ve	+ve
	Folin-ciocalteu Test:	+ve	-ve	+ve	+ve
6.	**Proteins**				
	Xanthoproteic Test:	-ve	-ve	-ve	-ve
7.	**Carbohydrate**				
	Fehling's Test:	-ve	-ve	-ve	-ve
	Benedict Test:	-ve	-ve	+ve	+ve
8.	**Saponins**				
	Froth Test:	-ve	-ve	+ve	+ve
9.	**Tannins**				
	Gelatin test:	-ve	-ve	-ve	-ve
10.	**Sterols**				
	Salkowski's Test:	-ve	-ve	-ve	+ve

(+ve= Positive, -ve= Negative)

Table 3: Estimation of total phenol, flavonoids and alkaloid content of *Triplophyllum protensum*

S. No.	Extracts	Total phenol content	Total flavonoids content	Total alkaloid content
		(mg/ 100 mg of dried extract)		
1	Chloroform	0.388	-	-
2	Ethanol	1.883	1.462	5.833

3	Aqueous	0.994	2.506	-

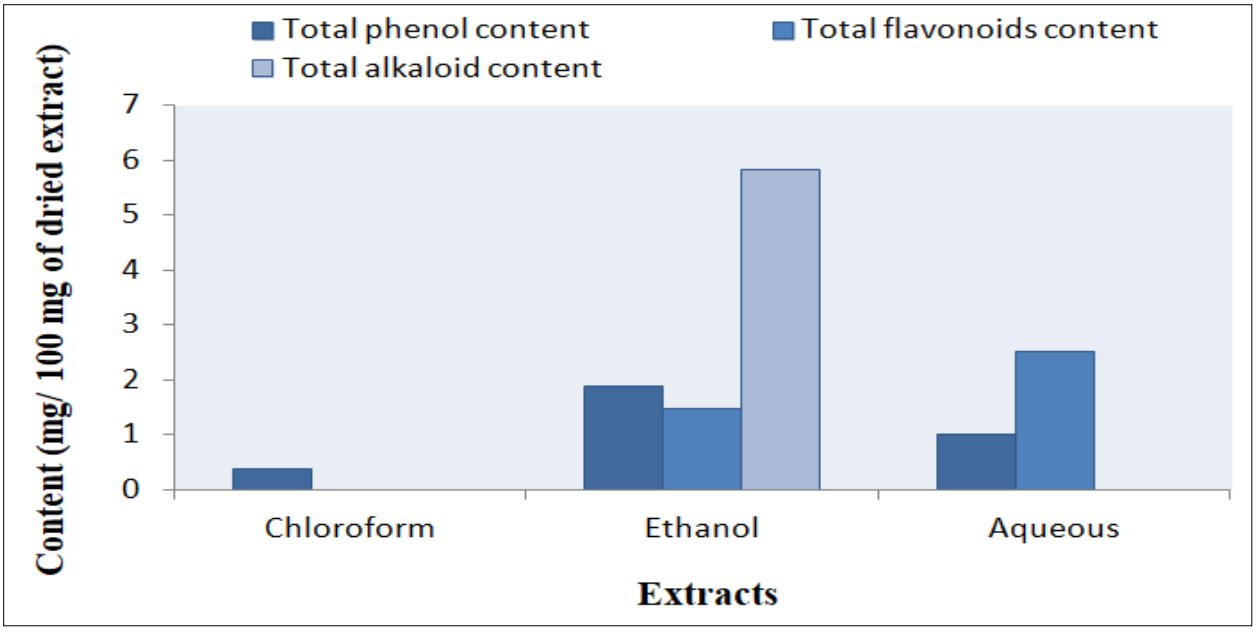

Figure 2: Graph of Estimation of total phenol, flavonoids and alkaloid content of *Triplophyllum protensum*

Conclusion

The findings indicate that the ethanol extract of *Triplophyllum protensum* roots are rich in bioactive compounds such as phenols, flavonoids, and alkaloids, which are known for their diverse pharmacological properties. The presence of these compounds supports the traditional use of *Triplophyllum protensum* in medicinal applications. The high yield and significant phytochemical content of the ethanol and aqueous extracts suggest that these solvents are effective for extracting valuable phytoconstituents from the roots. These results provide a scientific basis for the medicinal use of *Triplophyllum protensum*, and further research could focus on isolating specific compounds and evaluating their biological activities in detail. The comprehensive phytochemical profile and quantification also pave the way for potential pharmaceutical applications of this plant species in developing natural therapeutic agents.

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