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Phytochemical Analysis And total Phenolic Content, Flavonoid Content of Various Solvent Extracts Uraria Picta

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ABSTRACT

This study validates the presence of pharmacologically relevant Flavonoids in Uraria Picta and supports its traditional medicinal applications. The identified compound can serve as a potential lead for future drug development due to its reported antioxidant and antimicrobial properties. The present study focuses on the advanced spectroscopic profiling and isolation of bioactive phytoconstituents from Uraria picta, a medicinal plant widely recognized for its therapeutic potential. The plant material was subjected to extraction using solvents of varying polarity to obtain a broad spectrum of phytochemicals. Preliminary phytochemical screening revealed the presence of important secondary metabolites such as flavonoids, phenolics, alkaloids, tannins, and saponins.. These techniques enabled the identification and structural elucidation of key phytoconstituents responsible for the plant's biological activity. Furthermore, the study highlights the correlation between the presence of phenolic and flavonoid compounds and the antioxidant potential of the extracts. The findings suggest that Uraria picta is a rich source of bioactive molecules with significant pharmacological importance.

The recently study revealed that the total weight of Uraria picta (UP) leaves used was 300 g. After performing the extraction of *Uraria picta*, the percentage yields of the extracts in petroleum ether (PE), ethyl acetate (EA), and methanol (MeOH) were found to be 2.44% (7.337 g, PE), 2.15% (6.320 g, EA), and 5.63% (16.144 g, MeOH). The ethyl acetate and methanol extracts of *Uraria picta* (UP) leaves were found to contain phytochemical constituents such as carbohydrates, alkaloids, flavonoids, terpenoids, steroids, glycosides, proteins and amino acids, tannins, phenols, and saponins, as revealed by phytochemical investigation using standard chemical tests.

KEYWORDS - Uraria Picta, Phytochemicals, Flavonoids, Leaves, TPC, TFC, Methonolic petroleum ether, extract, antioxidant Properties.

1. INTRODUCTION

Prisniperni (Uraria picta desv.) is one of the most important Ayurvedic plant herb. It is one among dashmoola. The Aim of this systematic review is to provide an in depth study of extraction, characterization, phytochemistry , pharmacological activities.

Traditionaly various species of Uraria Picta are used by the in habitat of Indian country for the treatment of variety of including asthma, cough, fever, cancer, renal diseases. Several pharmacological attributes of Uraria Picta species such as anti oxidant , anti viral, anti bacterial , anti tumor have already been provided.In the recent years , plenty of research has been conducted to explore the phytochemical composition and pharmacological activities crude extract and isolated compounds obtained from different parts of Uraria Picta. Its leaves and oil preparations have been attributed with a broad spectrum of biological activities such as antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, anticancer, neuroprotective, cardioprotective, antidiabetic, gastroprotective, nephroprotective, and hepatoprotective effects **(Yimer *et al.*, 2019).** Despite its rich history and wide usage, phytochemical investigations of Uraria Pictaremain limited. The current study focuses on the isolation and structural elucidation of novel flavonoids compounds from Uraria Picta, with the aim of uncovering potential

therapeutic agents and contributing to the scientific understanding of its medicinal value.
Phytochemicals are the naturally occurring compounds present in all plants parts which together with nutrients and flavonoids, phenolic compounds to plants and humans against diseases. Development of rapid and accurate methods of phytochemical analysis. This study adds to the fundamental scientific knowledge through qualitative analysis of phytochemicals constituents and quantification in plants parts of Uraria Picta. *Uraria picta Desv*., commonly known as Prishnaparni or Pithvan, is a perennial herb from the Leguminosae family, growing 90-180 cm tall. It features rough-haired stems and imparipinnate leaves with 5-9 leaflets that are lanceolate, variegated, and shiny above **(McNeill et al., 2007).** The inflorescence is a long, many-flowered raceme, with pink to reddish flowers and distinctive fruit. This plant is widely found in India, Bangladesh, Sri Lanka, and many parts of Asia and Africa **(Yusuf et al., 1994).** It is a key component of "Dashmula," an Ayurvedic formulation used for various ailments, including fatigue and gynecological disorders. Additionally, the leaves exhibit antianxiety effects **(Yadav et al., 2009).**

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The Uraria Picta was collected from Mauranipur Jhansi, Bhopal, Chhindwara, Balaghat in Madhya Pradesh and Uttar Pradesh.

1.2 Preparation of extracts

250 mg dried and powdered plant materials was soaked over night in 20ml of different solvents namely waters, methanol, ethanol, petroleum, ether, diethyl ether, ethyl acetate. The different extract were filtered were used for qualitative , quantitative phytochemical analysis.

1.3 Preliminary phytochemical analysis

The Preliminary phytochemical analysis of leaves extract of Uraria Picta were carried out according to the methods described to qualitative analysis for various phytochemical constituents.

2.4 Soxhlet extraction:

Dried powdered leaves of *Uraria picta* were successively defatted with petroleum ether and then placed in a thimble of a Soxhlet apparatus. Extraction was carried out using ethyl acetate and methanol as solvent systems at a temperature range of 40–60°C for 8–10 hours using a heating mantle. After the extraction process, the extracts were filtered and concentrated to dryness. The obtained extracts were then evaporated using a rotary vacuum evaporator at 80°C and collected in air-tight containers**(Evans. 2009 and Alara *et al.*, 2019).** Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.5 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify the presence or absence of various phytoconstituents in the petroleum ether, ethyl acetate, and methanol extracts of *Uraria picta* using standard procedures**(Kokate*et al.*, 2006, Okuna *et al.*, 2024, Basumatary 2016, Shaikh and Patil 2020 and Prabhavathi *et al.*, 2016).**The extracts were subjected to following tests:

Tests for carbohydrates:

- **Molisch test:** To 1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Conc. sulphuric acid was added along the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which confirms the presence of carbohydrates in the test samples.

- **Fehling's test:** To 1 ml of extract, similar quantity of Fehling's solution A and B was added and heated on a water bath for few minutes. The development of brick red precipitate was observed.
- **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.
- **Barfoed's test:** 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.
- **Tollen's test:** 1 mL of Tollens' reagent was mixed with 1 mL of the plant extract and heated in a water bath. The formation of a silver mirror or a black precipitate on the test tube walls indicated the presence of reducing sugars, particularly aldoses
- **Iodine test:** 3mL extract solution and few drops of iodine solution. The formation of a blue colour, which disappears on boiling and reappears on cooling
- **Trommer test:** 3 mL of the aqueous extract add one mL of 2.5 % copper sulphate and 2 mL of 5 % sodium hydroxide and boil for 3 min. First, there is the blue precipitate that becomes red when heated; hence, it would indicate the presence of reducing sugars.

Test for alkaloids:

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filtrate of all the test extracts was exposed to following tests:

- **Mayer's test:** To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.
- **Hager's test:** To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.
- **Wagner's test:** To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.
- **Dragendorffs test:** Add 1 mL of reagent to 2 mL of plant extract. A positive result is the formation of a brownish or yellowish precipitate, which is a complex compound of potassium alkaloid.
- **Tannic acid test:** The appearance of brownish black or blue color shows the presence of tannins, by the addition of few drops of Tannic acid solution and 10% ferric chloride to 2 mL of extract sample**.**

Test for flavonoids:

- **Lead acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.
- **Alkaline reagent test:** The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

Test for glycosides:

- **Borntrager's test:** To 3 ml of extract, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.
- **Legal's test:** 1 ml of extract was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.
- **Keller-Killiani test:** To 2 ml of extract, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

Test for protein and amino acids:

- **Biuret's test:**The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.
- **Ninhydrin test:** 3 ml of the extractwas heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Test for saponins:

- **Froth test:** 1 ml of extract was dissolved in 20 ml of distilled water and shacked for 15min in a graduated cylinder. Formation of persistent foam around 1cm layer was observed.

Test for triterpenoids and steroids:

- **Salkowski's test:** The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turn red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.
- **Libermann-Burchard's test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Test for tannin and phenolic compounds:

- **Ferric chloride test:** Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.
- **Lead acetate test:** Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

- **Gelatin Test:** Into the distilled water some quantity of extract was dissolved. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate depicts the presence of phenolic compounds.

3. QUANTITATIVE PHYTOCHEMICAL ESTIMATION

3.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content (TPC) of the plant extract was determined using the Folin–Ciocalteu assay. Ethyl acetate and methanolic extracts of *Uraria picta* (0.2 mL from the stock solution) were separately mixed in test tubes with 2.5 mL of Folin–Ciocalteu's phenol reagent. After 5 minutes, 2 mL of a 7.5% Na_2CO_3 solution was added to each mixture, and the volume was made up to 7 mL with deionized distilled water, then mixed thoroughly. The mixtures were kept in the dark for 90 minutes at 25°C, after which the absorbance was measured at 760 nm. The TPC was determined from the calibration curve prepared using gallic acid standard solutions (20–100 µg/mL). The estimation of phenolic compounds was carried out in triplicate, and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dried sample **(Kupina *et al.*, 2018).**

3.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The total flavonoid content was determined using the aluminum chloride method **(Chang *et al.*, 2002).** In 10 mL test tubes, 0.5 mL each of the ethyl acetate and methanolic extracts of *Uraria picta* were mixed separately with 0.15 mL of 5% $NaNO_2$ and 0.15 mL of 10% $AlCl_3 \cdot 6H_2O$. After 5 minutes, 2 mL of 4% NaOH was added, and the volume was made up to 5 mL with deionized distilled water. The solutions were mixed thoroughly, and the absorbance was measured at 510 nm against a reagent blank. A standard calibration curve was prepared using rutin standard solutions (20–100 µg/mL) under the same procedure as described above. The total flavonoid content (TFC) was expressed as milligrams of rutin equivalents (mg RE) per gram of dried sample **(Shraim *et al.*, 2021).**

Soxhletation of *Uraria picta* with Petroleum Ether

Soxhletation of *Uraria picta* with Ethyl acetate

Soxhletation of *Uraria picta* with Methanol

Rotary vacuum evaporator of Ethyl acetate

Sr. No.	Solvent	Extract
1	***Uraria picta*** **(Petroleum Ether)**	
2	***Uraria picta*** **(Ethyl acetate)**	
3.	***Uraria picta*** ***(Methanol)***	

4. RESULTS

4.1 Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Uraria picta*	Leaf	300.00gm

4.2 Percentage yield

Table 2 Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Uraria picta*	Petroleum ether	Dark greenish	300.00gm	7.337gm	2.44 %
2.	*Uraria picta*	Ethyl acetate	Dark green to brownish	292.60 gm	6.320gm	2.15 %
3.	*Uraria picta*	Methanol	Dark brownish	286.30 gm	16.144gm	5.63 %

4.3 Qualitative Phytochemical Analysis of different extracts

Table 3 Phytochemical analysis of *Uraria picta*Extracts

S. No.	Experiment	Result		
		Petroleum Ether	Ethyl acetate	Methanol
Test for Carbohydrates				
1.	Molisch's Test	Positive	Positive	Positive
2.	Fehling's Test	Positive	Positive	Positive
3.	Benedict's Test	Positive	Positive	Positive
4.	Barfoed's test	Positive	Positive	Positive
5.	Tollen's test	Positive	Positive	Positive
6.	Iodine test	Positive	Positive	Positive
7.	Tommer's test	Positive	Positive	Positive
Test for Alkaloids				
1.	Mayer's Test	Positive	Positive	Positive
2.	Hager's Test	Positive	Positive	Positive
3.	Wagner's Test	Positive	Positive	Positive
4.	Dragendorffs's test	Positive	Positive	Positive
5.	Tannic test	Positive	Positive	Positive
Test for Terpenoids				
1.	Salkowski Test	Positive	Positive	Positive
2.	Libermann-Burchard's Test	Positive	Positive	Positive
Test for Flavonoids				
1.	Lead Acetate Test	Positive	Positive	Positive
2.	Alkaline Reagent Test	Positive	Positive	Positive

3.	Shinoda test	Negative	Negative	Positive
Test for Tannins and Phenolic Compounds				
1.	Ferric chlorideTest	Positive	Positive	Positive
2.	Lead Acetate Test	Positive	Positive	Positive
3.	Gelatine Test	Positive	Positive	Positive
Test for Saponins				
1.	Froth Test	Positive	Positive	Positive
Test for Protein and Amino acids				
1.	Ninhydrin Test	Negative	Negative	Negative
2.	Biuret's Test	Negative	Positive	Positive
Test for Glycosides				
1.	Legal's Test	Negative	Negative	Negative
2.	Keller Killani Test	Positive	Positive	Positive
3.	Borntrager's Test	Negative	Negative	Positive

4.4 Quantitative Phytochemical estimation-

4.4.1 Total Phenolic Content (TPC) Estimation

Table 4 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.110
2.	40	0.237
3.	60	0.351
4.	80	0.459
5.	100	0.559

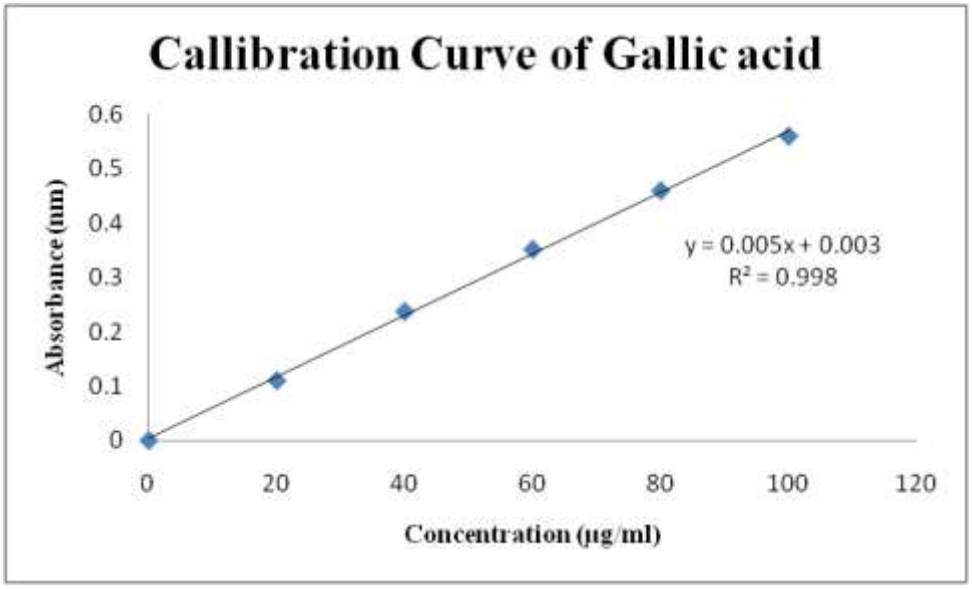

Figure 1 Graph represent standard curve of Gallic acid

Table 5 Total Phenolic Content in*Uraria picta*extracts -

Total Phenolic content (mg/gm equivalent to Gallic acid)		
Extracts	**Ethyl acetate**	**Methanol**
Absorbance Mean±SD	0.1670±0.002	0.4629±0.003
TPC	32.80	91.98

4.4.2. Total Flavonoid Content (TFC) Estimation:

Table 6 Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.086
2.	40	0.166
3.	60	0.234
4.	80	0.310
5.	100	0.393

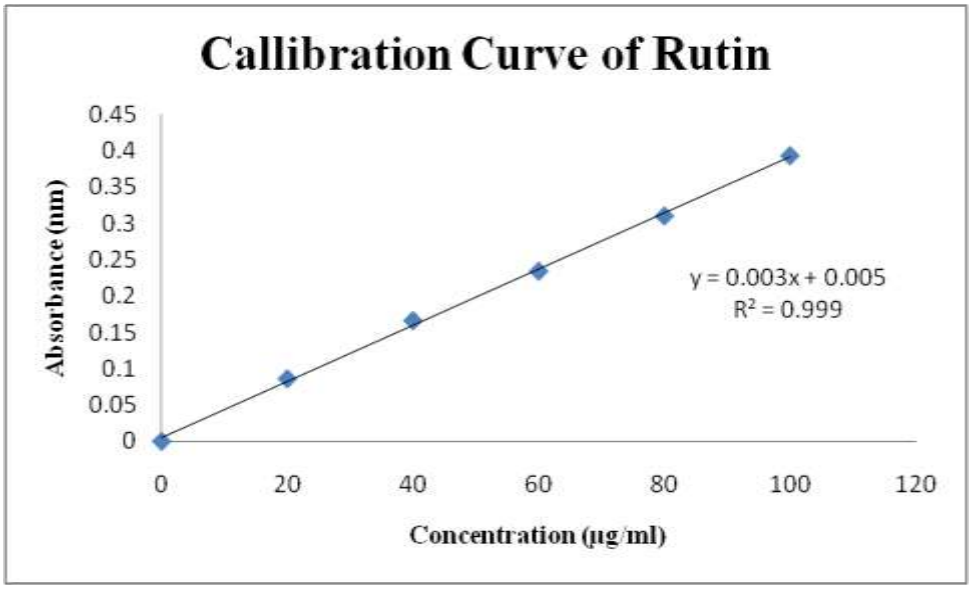

Figure 2 Graph represent standard curve of Rutin

Table 7 Total Flavonoid Content in*Uraria picta*extracts -

Total Flavonoid content (mg/gm equivalent to Rutin)		
Extracts	**Ethyl acetate**	**Methanol**
Absorbance Mean±SD	0.6403±0.003	1.0275±0.002
TFC	211.76	340.83

5. CONCLUSION

Phytochemical analysis of various solvent extracts of Uraria picta revealed the presence of several important bioactive compounds such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides. The estimation of total phenolic content (TPC) and total flavonoid content (TFC) demonstrated that polar solvents, particularly methanol and ethanol extracts, contained significantly higher amounts of phenolic and flavonoid compounds compared to non-polar solvents like chloroform and petroleum ether. The higher concentration of these compounds suggests strong antioxidant potential of the plant extracts, as phenolics and flavonoids are known to play a crucial role in scavenging free radicals. Overall, the study concludes that Uraria picta is a promising medicinal plant with significant phytochemical constituents and antioxidant properties. Its polar solvent extracts, especially methanolic and ethanolic extracts, may be further explored for therapeutic applications.

The total phenolic content of the ethyl acetate (EA) and methanol (MeOH) extracts of *Uraria picta* (UP) leaves was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight of the sample. The total phenolic content of the EA and MeOH extracts of *Uraria picta* leaves was found to be 32.80 mg GAE/g and 91.98 mg GAE/g.The total flavonoid content of the ethyl acetate (EA) and methanol (MeOH) extracts of *Uraria picta* (UP) leaves was expressed as milligrams of rutin equivalents (mg RE) per gram of dry weight of the sample. The total flavonoid content of the EA and MeOH extracts of *Uraria picta* leaves was found to be 211.76 mg RE/g and 340.83 mg RE/g. These finding substantiate the traditional medicinal value of Uraria Picta and highlight its potential as a source of pharmacologically active flavonoids compound. The identified compound can serve as a lead molecule for further pharmacological studies and potential drug development.

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