QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL ANALYSIS OF TOTAL PHENOLIC CONTENT, FLAVONOID CONTENT OF VARIOUS SOLVENT EXTRACTS HELICTERES ISORA (STEM)

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ABSTRACT
This study validates the presence of pharmacologically relevant Flavonoids in Helicteres isora 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. Helicteres isora is a well-known medicinal plant widely used in traditional systems of medicine due to its diverse pharmacological properties. The present study was aimed at performing qualitative and quantitative phytochemical analysis of various solvent extracts of Helicteres isora, with special emphasis on the estimation of total phenolic content (TPC) and total flavonoid content (TFC). Different solvents such as methanol, ethanol, chloroform, and aqueous extracts were used for the extraction of phytoconstituents. Qualitative phytochemical screening revealed the presence of important secondary metabolites including phenols, flavonoids, alkaloids, tannins, saponins, and glycosides. Quantitative estimation of total phenolic content was carried out using the Folin–Ciocalteu method, while total flavonoid content was determined by the aluminium chloride colorimetric method. The results indicated that the methanolic extract exhibited the highest phenolic and flavonoid content compared to other solvent extracts, whereas aqueous and chloroform extracts showed comparatively lower concentrations. The higher levels of phenolic and flavonoid compounds suggest strong antioxidant potential of the plant extracts. The resently study revealed that the total weight of *Helicteresisora* (Stem,) Plants used were 300 After performing extraction of *Helicteres isora* (Stem) the percentage yield of leave extracts in petroleum ether solvent were found to be 0.29 % (0.897 gm). The percentage yield of leave extracts of *Helicteres isora* (Stem) in methanolic extracts was found to be 6.06 % (18.146gm).

KEYWORDS- *Helicteres isora*, Stem, Phytochemical Analysis, Quantitative and Qualitative Analysis, TPC, TFC, Solvent extraction, Folin- Ciocalteu method.

1. INTRODUCTION
Helicteres isora is a widely recognized medicinal plant belonging to the family *Sterculiaceae*. It is commonly distributed in tropical and subtropical regions and has been extensively used in traditional systems of medicine such as Ayurveda for the treatment of various ailments including gastrointestinal disorders, diabetes, and microbial infections. The therapeutic potential of this plant is mainly attributed to the presence of diverse bioactive phytoconstituents. Phytochemicals are naturally occurring secondary metabolites in plants that play a significant role in disease prevention and health promotion. Among these, phenolic compounds and flavonoids are of particular interest due to their strong antioxidant properties. These compounds help in neutralizing free radicals, thereby reducing oxidative stress and preventing various chronic diseases such as cancer, cardiovascular disorders, and inflammation. The extraction of phytochemicals from plant materials depends largely on the type of solvent used, as different solvents have varying polarities and extraction efficiencies. Commonly used solvents such as methanol, ethanol, chloroform, and water can extract different classes of phytochemicals in varying quantities. Therefore, comparative analysis of different solvent extracts is essential to determine the most effective extraction method for maximum yield of bioactive compounds.

Qualitative phytochemical analysis provides preliminary information about the presence or absence of various secondary metabolites such as alkaloids, tannins, saponins, glycosides, phenols, and flavonoids. On the other hand, quantitative analysis focuses on the measurement of specific compounds, particularly total phenolic content (TPC) and total flavonoid content (TFC), which are important indicators of antioxidant potential. Standard methods such as the Folin–Ciocalteu assay for phenolics and the aluminium chloride colorimetric method for flavonoids are widely employed for this purpose. The chemical investigations done by N.Saraswati Bhai,[4] led to the identification of pigments, phytosterols, hydroxyl carboxylic acid, orange-yellow colouring matter, saponins phlobotannis, sugar and lignins. Medicinal Plants have multiple biological effects on human system including antioxidant activity [6]. Due to presence of various compounds such as flavonoids, phenolic acid, tannins, coumarins, lignans and lignins [7-8]. The present study aims to perform both qualitative and quantitative phytochemical analysis of different solvent extracts of *Helicteres isora*, with special emphasis on the estimation of total phenolic and flavonoid contents. This investigation will contribute to understanding the phytochemical profile of the plant and its potential applications in pharmaceutical and nutraceutical fields.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The *Helicteres isora* was collected from Jhansi, Palera, Jatara, Prithvipur, Kakawani, Niwari, Bhopal, Chhindwara, Balaghat, in Madhya Pradesh and Uttar Pradesh.

1.2 Preparation of extracts

300 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 Stem extract of Helicteres isora were carried out according to the methods described to qualitative analysis for various phytochemical constituents.

2.1 Soxhlet extraction:

Dried powered of *Helicteresisora* (Stem) were successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using methanolsolvent system at 40-60^{o}C temperature of the heating mantle for 8-12 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Obtained extracts were evaporated using rotary vacuum evaporator at 40°C. Extracts were collected in air tight container **(Evans. 2009 and Alara*et al.*, 2019).** Extraction yield of all extracts were calculated using the following equation below:

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

2.2 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of *Helicteresisora* (Stem) by using standard procedures **(Kokate*et al.*, 2006).** 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.

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.

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 of plant extract was determined using the Folin-Ciocalteu Assay. Methanol extracts of Stem, of *Helicteres isora* (0.2 mL from stock solution) was mixed separate in test tubes with 2.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 2 ml of a 7.5% Na_2CO_3 solution was added to the mixture and volume make up to 7 ml with deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 25°C, after which the absorbance was read at 760 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (20 to 100µg/ml). The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample. **(Saeed *et al.*, 2012).**

3.2 Spectrophotometric Quantification of Total Flavonoid Content: -

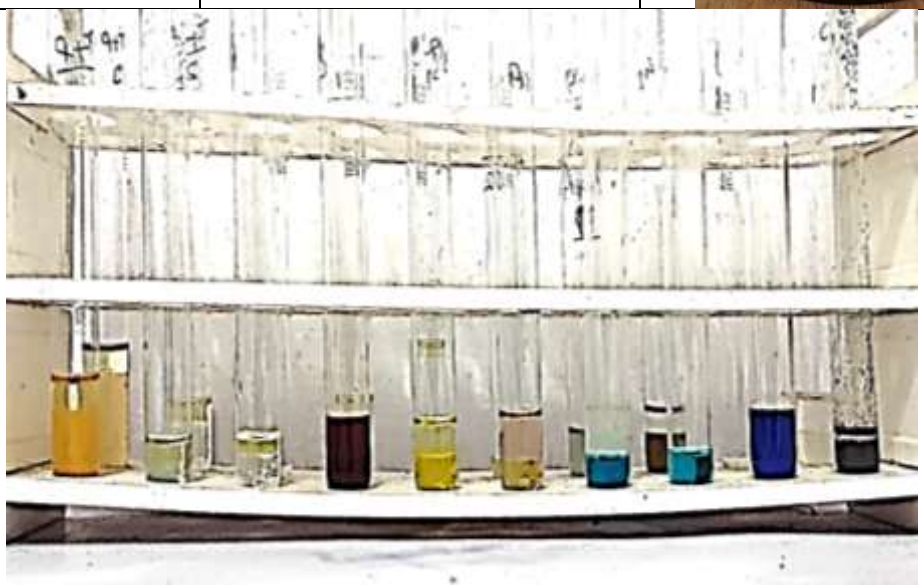
The flavonoid content was determined using Aluminium chloride method **(Chang *et al.*, 2002)**. In a 10 ml test tubes, 0.5 ml of Methanol extracts of Stem of *Helicteresisora*, 0.15 ml of $NaNO_2$ (5%) and 0.15 ml of $AlCl_3.6H_2O$ (10%) was mixed separate in test tubes. After 5 min, 2 ml of NaOH (4%) was added and volume up to 5ml with deionized distilled water. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoid was made using rutin standard solution (20 to 100µg/ml) under the same procedure as earlier described. The total flavonoid was expressed as milligrams of rutin equivalents per g of dried sample.**(Senguttuvan *et al.*, 2014).**

Soxhletation of *Helicteres isora* (Stem) with Pet. Ether and methanolic solvents

 ISSN: 2278-4632

Rotary vacuum evaporator

Sr. No.	Solvent	Extract
1	*Helicteresisora* (Stem) (Pet. Ether)	
2	*Helicteresisora* (Stem) (Methanol)	



Phytochemical study of Pet. Ether extract of *Helicteresisora (Stem)*

 ISSN: 2278-4632

Phytochemical study of methanolic extract of *Helicteresisora (Stem)*

4. RESULTS

4.1 Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	***Helicteres isora***	Stem	300.00gm

4.2 Percentage yield

Table 2 Percentage yield of extracts

S. No.	Plant name and Part	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Helicteres isora* (Stem)	Petroleum Ether	Dark yellow to brown	300.00 gm	0.897	0.29
2.	*Helicteres isora* (Stem)	Methanol	Brown	299.10 gm	18.146	6.06

4.3 Qualitative Phytochemical Analysis of different extracts

Table 3 Phytochemical analysis of *Helicteresisora (Stem)* Extracts

S. No.	Experiment	Result	
		Petroleum ether	Methanol
Test for Carbohydrates			
1.	Molisch's Test	+	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	+	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	+	+
3.	Wagner's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	-	-

Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	-	+
Test for Tannins and Phenolic Compounds			
1.	Ferric chlorideTest	-	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	-	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	+	+
2.	Biuret's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	-	-
3.	Borntrager's Test	+	+

4.4 Solubility determination of methanolic extracts of Methanol extracts of Stem of *Helicteres isora*-

Table 4 Solubility Determination of methanolic extract of Stem of *Helicteresisora*

S. No.	Solvent	Result
1.	Water	Sparingly Soluble
2.	Ethanol	Soluble
3.	Ethyl Acetate	Sparingly soluble
4.	DMSO	Soluble
5.	Petroleum Ether	Insoluble
6	Methanol	Soluble
7	Chloroform	Sparingly soluble
8	Acetone	Slightly soluble

4.5 Quantitative Phytochemical analysis of Methanol extracts of Stem of *Helicteresisora*-

4.5.1 Total Phenolic Content (TPC) Estimation

Table 5 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.111
2.	40	0.236
3.	60	0.353
4.	80	0.445
5.	100	0.577

 ISSN: 2278-4632

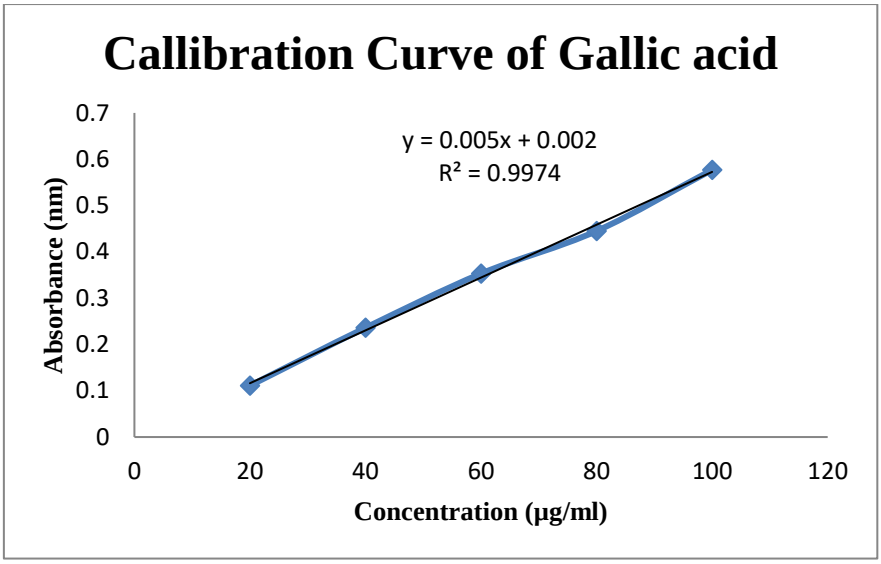


Figure 1 Graph represent standard curve of Gallic acid

Table 6 Total Phenolic Content in Methanolic *Helicteres isora* extracts-

Total Phenolic content (mg/gm equivalent to Gallic acid)	
Extracts	**Stem**
Absorbance Mean±SD	0.5301 ±0.003
TPC	**105.62**

4.5.2. Total Flavonoid Content (TFC) Estimation:

Table 7 Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.087
2.	40	0.158
3.	60	0.227
4.	80	0.307
5.	100	0.395

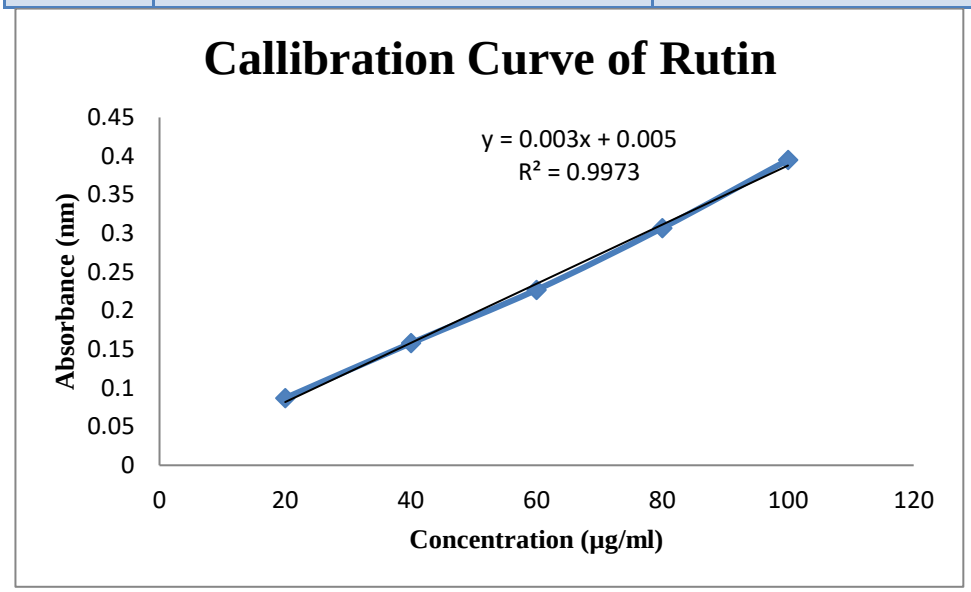


Figure 2 Graph represent standard curve of Rutin

Table 8 Total Flavonoid Content in Methanolic *Helicteresisora* extracts -

Total Flavonoid content (mg/gm equivalent to Rutin)	
Extracts	**Stem**
Absorbance Mean±SD	0.7491±0.004
TPC	248.03

5. CONCLUSION

The present study on Helicteres isora demonstrated that the plant is a rich source of bioactive phytochemicals. Qualitative phytochemical screening confirmed the presence of important secondary metabolites such as phenols, flavonoids, alkaloids, tannins, saponins, and glycosides in various solvent extracts. Quantitative analysis revealed significant variation in total phenolic content (TPC) and total flavonoid content (TFC) depending on the solvent used for extraction. Among the different extracts, the methanolic extract exhibited the highest concentration of phenolic and flavonoid compounds, indicating its superior extraction efficiency compared to ethanol, aqueous, and chloroform extracts. The high levels of phenolic and flavonoid content suggest strong antioxidant potential of Helicteres isora, supporting its traditional medicinal uses. These findings highlight the importance of selecting appropriate solvents for maximizing the yield of bioactive compounds. Helicteres isora can be considered a valuable natural source of antioxidants and holds promising potential for applications in pharmaceutical and nutraceutical industries. Further studies involving isolation, characterization, and biological evaluation of specific compounds are recommended to explore its full therapeutic potential. The methanolic extracts of *Helicteresisora (Leaves, Stem, Root and Fruit)* contains Phytochemical constituents like carbohydrates, alkaloids, flavonoids, terpenoids, steroid, glycosides, protein & amino acid, tannins, phenolic, saponin and compounds by phytochemical investigation with respect to chemical tests. The determination of the total phenolic content of methanolic extracts of *Helicteres isora* (Stem) were expressed as mg gallic acid equivalents and per mg/gram dry weight of sample. TPC of methanolic extracts of *Helicteres isora* (Stem) showed the content values of 105.62mg/gm . The total flavonoids content of the methanolic extracts of *Helicteres isora* (Stem) were expressed as percentage of Rutin equivalent per mg/gm dry weight of sample. The total flavonoids content of methanolic extracts of *Helicteres isora* (Stem) showed the content values of 248.03 mg/gm respectively.

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