

Phytochemical Screening of Methanolic, Pet Ether and Ethyl Acetate extracts of *Helicteres isora* Linn

Sunny Sharma¹, Seema Bhargava²

^{1,2}Department of Biotechnology, Mewar University Gangrar, Chittorgarh, Rajasthan, India

Abstract: *Helicteres isora* is well known for its use in herbal medicine and it is said to possess many medicinal values. The present study was designed for screening phytochemicals in three different extracts (Pet Ether, Ethyl Acetate, and Methanol). Phytochemical screening was performed to detect the presence of alkaloids, carbohydrates, reducing sugar, flavonoids, glycoside, tannin, phenolic compound, saponin, proteins, amino acid, triterpenoids and steroids. Ethyl acetate extract was found to be phytochemically richer as compared to Pet Ether and Methanolic extract. The reducing sugar and saponin were not detected in these extracts. A good quantity of the phytochemicals may contribute to the medicinal and nutritive values of *Helicteres isora*.

Keywords: *Helicteres isora*, Methanol, Pet ether, Ethyl acetate, Soxhlet

1. Introduction

Therapeutic use of herbs and their phytochemicals have always fascinated researchers all over the world. Herbs and their agents have been a source of medicine since, the Vedic period. Modern medicines are mostly isolated from plant based agents, depending on the traditional medical knowledge¹. Thus, plants and herbs contribute a major share in traditional and modern medicinal system for health care. The bioactive compounds present in plants like alkaloids, flavonoids, tannins and phenolic compounds are the source of its medicinal value that produces a definite physiological and biochemical action on the body². *Helicteres isora* linn belongs to the family Sterculiaceae. It has been frequently used as an astringent and blood-purifier³. It is commonly found in India from Jammu eastwards to Nepal, Bihar, Central, Western and Southern India, West Bengal, and the Andaman islands. Due to screw like appearance of its fruit it is commonly known as Enthani, Marodphali, Marorphali, etc. It is a medium size tree attaining a maximum high of 5m. It is shown in Literature that *Helicteres isora* plant shows antioxidant, anti-inflammatory, antipyretic, and antispasmodic⁴⁻⁵ activities. presence of flavones⁶, triterpenoids⁷, cucurbitacin⁸, phytosterols, saponins, sugars and phlobatannins. The root and stem barks are considered to be expectorant, demulcent, astringent and anti-galactagogue and are useful in colic, scabies, empyema, gastropathy, diabetes, diarrhea and dysentery⁹. The fruits are astringent, acrid, refrigerant, demulcent, constipating, stomachic, vermifuge, vulnerary, haemostatic and urinary astringent. They are useful in vitiated conditions of pitta ophthalmitis, colic, flatulence, diarrhea, dysentery, verminosis, wounds, ulcers, hemorrhages, epistaxis and diabetes¹⁰.

2. Material and Methods

Helicteres isora plant material was collected from central India. Herbarium of plant was prepared and submitted for authentication. The plant material was shade dried and was further processed from extraction processes.

2.1 Extraction

Dried and powdered raw material was defatted with petroleum ether than soxhleted with ethyl acetate and methanol. The extraction was carried out by soxhletion to complete extraction.

2.2 Phytochemical screening

Detailed phytochemical testing was performed to identify presence or absence of different phyto-constituents. We used more than one test for confirmation. Tests are carried out as per the procedure mention in the lab protocol¹¹ which are internationally referenced procedures.

2.2.1 Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shake it well and filtered. With the filtrate, the following tests were performed.

2.2.2 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.

2.2.3 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.

2.2.4 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.

2.3 Tests for Carbohydrates

2.3.1 Molish Test: 2 ml of aqueous extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicate the presence of carbohydrates.

2.3.2 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.

2.4 Tests for Reducing Sugar

2.4.1 Fehling's Test

To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

2.4.2 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.

2.5 Tests for Flavonoids

2.5.1 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 colorless on addition of few drops of dilute acid, indicate presence of flavonoids.

2.5.2 Lead Acetate Test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

2.5.3 Shinoda test

To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicate presence of flavonoids.

2.6 Tests for Glycosides

2.6.1 Borntrager's Test

To 3 ml of test solution, 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 well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammoniacal layer indicates presence of anthraquinone glycosides.

2.6.2 Legal's Test:

1 ml of test solution 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.

2.6.3 Keller-Killiani Test

To 2 ml of test solution, 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.

2.7 Tests for Tannin and Phenolic compounds

2.7.1 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.

2.7.2 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.

2.7.3 Dilute Iodine Solution test

To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicates presence of phenolic compounds.

2.8 Tests for Saponins

2.8.1 Froth Test

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

2.9 Tests for Proteins and Amino Acids

2.9.1 Ninhydrin Test

3 ml of the test solution was 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.

2.9.2 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.

2.9.3 Million's Test

3 ml of extract was mixed with 5 ml of Million's reagent. White precipitate formed which on heating turned to brick red, indicating the presence of proteins.

2.10 Tests for Triterpenoids and Steroids

2.10.1 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 layer turns red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

2.10.2 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.

3. Results

Table 1: Phytochemical evaluation

	PEE	EAE	ME
1. Alkaloids			
Mayer's reagent test	+	+	+
Wagner's reagent test	+	+	+
Hager's reagent test	+	+	+
2. Carbohydrates			
Molish's test	-	+	+
Barfoed's test	-	+	+
3. Reducing Sugar's			
Fehling's test	-	-	-
Benedict's test	-	-	-
4. Flavonoids			
Alkaline reagent test	-	+	+
Shinoda test	-	+	-
Lead acetate test	-	+	-
5. Glycoside			
Borntrager test	-	-	+
Legal's test	-	-	+
Killer- Killiani test	-	-	+
6. Tannin and Phenolic compound			
Ferric chloride test	-	+	-
Lead Acetate test	-	+	-
Dilute Iodine solution	-	+	-
7. Saponin			
Foam Test	-	-	-
8. Proteins and amino acid			
Ninhydrin test	-	-	-
Biuret's Test	-	+	+
Million's Test	+	+	+
9. Triterpenoids and Steroids			
Salwonski Test	-	+	+
Libberman and Burchard's test	-	+	+

PEE Pet ether extract, EAE- Ethyl acetate Extract, ME- Methanolic extract.

(+) indicates presence and (-) indicates absence

Above table shows the results of phytochemical analysis of *Helicteres isora*.

4. Discussion

Different medicinal plants and their products have been reported to be good sources of phytochemicals. The phytochemical analysis conducted on the Pet ether, Ethyl acetate and methanolic extract of *H. Isora* revealed the presence of alkaloids, carbohydrates, flavonoids, glycoside, tannin and phenolic compounds, triterpenoids, proteins and amino acids. Whereas, reducing sugar and saponin were not detected in all these extracts; flavonoids and phenolic compound were absent in Pet ether extract. These phytochemicals have been shown to play protective roles against various chronic diseases¹². The phytochemicals including polyphenols, flavonoids and triterpenoids were found to be more important for study as they have profound effects on human health¹³. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and acts as natural anticancer

agents¹⁴. Phenolic compounds show the ability as a potent chain breaking antioxidants¹⁵. It was shown that polyphenolic compounds are anti-mutagenic and inhibits carcinogenesis in humans, when ingested up to 1g daily from a diet rich in fruits and vegetables¹⁶. Flavonoids present in food of plant origin are also potential antioxidants¹⁷. The results show good quantities of phytochemicals which may help in formulating new drugs.

5. Conclusion

There is enormous amount of phytochemicals present in *Helicteres isora* and can be used as an effective drug against various chronic diseases. In addition to this, the methanolic and ethyl acetate extract of *Helicteres isora* contains significant amounts of phenols and flavonoids, which can play a major role in management of oxidation. The data found in this work might be useful for further study of the *Helicteres isora* plant products and plant as whole on various applications such as nutritive supplement and pharmaceutical benefits.

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