



PHARMACOGNOSTIC, PHYTOCHEMICAL AND QUANTITATIVE EVALUATION OF HELICTERES ISORA LINN FRUIT EXTRACT

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Abstract: Medicinal plants are worth to nature. The bioactive compounds present in medicinal plants have various pharmaceutical importance. Helicteresisora commonly known as the Indian screw tree (Marorphali), has been reviewed for its phytochemical and pharmacological significance. Traditionally fruits, leaves, barks and roots of this plants has vital therapeutic potential. From traditional time fruits of Helicteresisora has been used for neonates for the dysentery. The plant contain number of active phyto constituents like glycosides, flavonoids, tannins, phenols, alkaloids, saponin etc. The plant has therapeutical importance and mainly shows antidiabetic, antioxidant, anticancer, antinociceptive, antimicrobial, antispasmodic, anti-inflammatory activities. The plant has been used in the traditional system of medicine to treat stomach-related disorders like stomach-ache, diarrhoea, wormicide, snakebite etc. The present study has done to investigate pharmacognostic characters of Helicteresisora macroscopic and microscopic characters, phytochemical screening for glycosides, tannins, saponin, flavonoids, alkaloids etc. Quantitative investigation has done for phenolic content determination by Folin –Ciocalteu Colorimetric method (50 µg/ml shows 51.06 ± 0.99), Flavonoid content determination by Aluminium Chloride Colorimetric method (1000 µg/ml shows 41.10 ± 0.20), and anti-oxidant potential has done by two methods that is DPPH radical scavenging potential (45.56 ± 0.56) and Nitric Oxide Reducing assay (16.49 ± 0.32). Thin layer chromatography of extract shows the presence of glycosides, flavonoids, tannins etc.

Keywords: Helicteresisora, Phenolic and Flavonoid content, TLC, Antioxidant, Pharmacognostic and phytochemical.

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INTRODUCTION

Several plant species have been considered as a source for developing therapeutic agents for nearly 1000 years and even today most of the drugs in practice are plant derived natural products. Both traditional Chinese medicine and Indian Ayurveda system was recorded over a millennium period of around 1st millennium. The vast range of medicinal plants distributed around the world is highly remarkable. According to reports, around 70,000 plant species from the lower level of lichens to higher level of trees are have been proven to have the potential for treating various illnesses. Based on WHO, 21,000 medicinal plants are in use for various medical applications.¹ Human knowledge of medicinal plants dates back 5000-3000 BC, written by Sumerians on clay tablets.² Presently, 88% of WHO member countries rely on herbal plants as traditional and complementary medicines³ The medicinal plants have been studied for their bioactive compounds for a long time.⁴⁻⁶

Helicteresisora Linn belonging to family Sterculiaceae/Malvaceae, commonly known as “East India screw tree” in English and “Avartani” in Sanskrit, is a sub-deciduous herb or small tree, which occur, often gregariously, throughout India and in dry forests. Almost all parts of H. isora are used in a traditional medicinal system for curing various ailments. This plant contains dietary ascorbic acid, which plays a central role in the prevention of cancer. Roots and bark of H. isora are expectorant, demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhea and dysentery. H. isora possess various

biological activities such as antidiabetic, hypolipidemic, antinociceptive, hepatoprotective, and cardiotoxic activities. Root juice of *H. isora* was claimed to be useful in fever, cough, asthma, stomach infections, and intestinal infections and was also useful for healing of cuts, wounds, and scabies. Betulinic acid, daucosterol, sitosterol, isorin, cucurbitacin B, and isocucurbitacin B were isolated from the roots of *H. isora*. Bark showed a significant hypoglycemic effect and lowering effect of hepatic enzymes. Fruits were used in alleviating griping and flatulence and also showed antispasmodic effects. The *Helicteres isora* L. is distributed in all district of Marathwada predominantly it is found in slopes of Mahur forest ranges⁷⁻⁸ Traditionally *Helicteres isora* Linn. as a medicinal plant mainly used in folk medicine in treating diarrhea, constipation of newborn babies, and snakebite. The plant possesses hypolipidaemic, antioxidant, antibacterial, cardiac antioxidant, brainantioxidation potency, antiperoxidative potency, hepatoprotective, antinociceptive, anticancer, anti-diarrheal, wormicidal, and antiplasmid activities.⁹⁻¹⁰.

PHYTOCHEMICAL COMPOSITION

The phytochemical studies of the plant are an important step to identify the bioactive compounds present in the medicinal plants. These compounds have various use in medicinal industries to prepare novel drugs. Phytochemical screening of crude extract and chloroform extract of the plant shows the presence of carbohydrates, saponin tannin, proteins, steroids, anthraquinon glycosides, cardiac glycosides, phenolic compounds, terpenoides, alkaloid salts, and free alkaloid¹¹. The phytochemicals analysis of fruit shows the presence of alkaloids, tannins, saponins, flavonoids, glycosides, cardiac glycosides, and anthraquinones. The fruit contains high amounts of polyphenols, ascorbic acid, carotenoids, and a high amount of phosphorus in form of nutrients¹²⁻¹³.

The spectroscopic analysis of the fruit revealed the presence of four compounds rosmarinic acid and three new compounds 4, 4'-O-di-β-D-glucopyranosyl rosmarinic acid, 4'-O-β-D-glucopyranosyl rosmarinic acid, and 4'-Oβ-D-glucopyranosyl isorinic acid. Besides, the fruit also contains 2-ethoxyphenethylamine, 2-hydroxy-5-methylbenzaldehyde, and 4-dihydroxy methyl ester Benenepropanoic acid. The spectroscopic and hydrolysis analysis yielded five flavonoid glucuronides compounds from the fruits of the plant mainly isoscutellarein 4'-methyl ether 8-O-beta-D-glucuronide 2", 4"-disulfate, isoscutellarein 4'-methyl ether 8-O-beta-D-glucuronide 6"- n-butyl ester, and isoscutellarein 8-O-beta-D-glucuronide 2",4"-disulfate.¹⁴

PLANT MATERIAL

Collections and Drying

Fruit of *Helicteres isora* Linn purchase from mogadpalli medical shop at Shri Nagar, Nanded Maharashtra, India, in the month of December - January.

Authentication

The plants, *Helicteres isora* Linn was authenticated by Botanist of Botanical Survey of India, Pune by comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune; with the Voucher specimen number 01-02 (Ref. No. BSI/WRC/Iden. /Cer. /2022/0502220000411. Dated 07/02/2022).

Preparation of Plant Material

Fruits of *Helicteres isora* Linn dried under shade and powdered. Powdered part was passed through 40# and stored in closed air tight container individually.

Pharmacognostic study

Fruits of *Helicteres isora* Linn pharmacognostic study were carried as per below procedure and methods.¹⁵.

Macroscopy

Organoleptic characters, extra feature and macroscopical details for all parts of plants were carried out.

Microscopy

Microscopical study was done according to method described by Khandelwal, (2008). Transverse section of stem and leaf was taken, stained with phloroglucinol: Hydrochloric acid (1:1) and observed under microscope at 10X, 45X.

Evaluation of Physical Constants

physical parameters has been checked for determination of foreign organic matter, determination of moisture content, ash value, Determination of Acid-insoluble ash, Determination of Water-soluble ash, Extractive values, Determination of water-soluble extractive value, Determination of Alcohol-soluble extractive value.¹⁶⁻¹⁷

Extraction Of Helicteres Is ora Linn Fruit Extract

Maceration:

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

Preparation Of Ethanolic Extract From Helicteres Isora Linn Fruit

The *Helicteres isora* linn fruit, washed well using clean water to remove adhering matter, dried under shade and powdered separately. 1kg of powder was transferred into conical flasks containing 100ml of methanol. The flasks were left for two days at room temperature with occasional stirrings. Later, the contents of the flasks were filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness and stored in refrigerator until use. The weight and colour of extracts was noted.¹⁸

Phytochemical Screening

The phytochemical studies of the plant are an important step to identify the bioactive compounds present in the medicinal plants. These compounds have various use in medicinal industries to prepare novel drugs. Phytochemical screening of ethanolic fruit extracts has done by the procedure as given in the book of Dr.

K.R. Khandelwal. Screening of extract has done for glycosides, flavonoids, tannins, saponins, proteins, sugars etc.¹⁹.

Thin Layer Chromatography

Principle

Thin layer chromatography, like other chromatography techniques, is based on the idea of separation (TLC). The difference is based on the relative affinity of each phases' chemicals. During the mobile phase, the compounds travel over the surface of the stationary phase. The movement occurs in such a manner that compounds with a higher affinity travel slowly while others rush to the stationary process. Following that, the combination will be degraded. The various components of the combination show on the plates as spots at their respective phases once the separation procedure is completed. Appropriate identification techniques categories their personality and characteristics.²⁰⁻²².

Steps involved in performing TLC of extracts

- **Preparation of TLC plate:** Prepared the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass plates to obtain a thin layer.
- **Activation of TLC plate:** TLC plate was activated by heating in oven for 30min at 105°C.
- **Sample application:** Dipping the capillary into the solution to be examined and applied the sample by capillary touched to the thin layer plate at a point about 2cm from the bottom. Air-dried the spot.
- **Chamber saturation:** The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid. Allowed saturating about 30 min
- **Chromatogram development:** After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15cm on the silica plate

Visualization: Plates were removed and were examined visually, under UV and suitable visualizing agent (ethanol, sulphuric acid, glacial acetic acid and anisaldehyde reagent (135:5:1:3.7)) after that R_f was calculated by formula

Total Phenolic Content Determination

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging.

Helicteresisoralinn fruit extract

1. Total phenolic content was analysed by Folin –Ciocalteu Colorimetric method.
2. The gallic acid was used as standard, solution (5,10,15,20 &20 µg/ml) was prepared in methanol and 100 µg/ml stock solution of test sample were also prepared.
3. Test sample was prepared by adding 0.3ml of distilled water, 0.4ml of Folin – Ciocalteu reagent and sample were allowed to stand for 6min before adding 4ml of 7 % sodium carbonate (Na_2CO_3).
4. The water was used to adjust the volume up to 10ml. After incubation at 90min, the absorbance was recorded 765nm. Reference curve were prepared using (5-25 µg/ml) of gallic acid and results were prepared are presented at amount of phenolic content (Gallic acid Equivalent) per dry weight.
5. The blank solution was prepared using 0.4ml of Folin- Ciocalteu reagent, 4ml of 7 % of sodium carbonate and water was used to adjust the volume up to 10ml.

Total Flavonoid Content Determination

1. Total Flavonoid Content was determined by Aluminum Chloride Colorimetric Method
2. The solution standard sample of quercetin was prepared using 50% methanol to produce concentration of 1000 µg/ml.
3. The total flavonoid contents were determined in the test samples a with minor modification.
4. Briefly, 40 of µl test samples and quercetin at various concentrations were separately mixed with 200 µl of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (2%, w/v) in 96 well plate.
5. The resultants mixtures were incubated for 10 minutes at normal temperature.
6. The optical density was measured at 440 nm using microplate reader (EPOCH, BioTek-Agilent, USA).
7. The standard calibration curve for quercetin standard was prepared from stock solution and used for calculation of total flavonoid contents for test samples. The results are expressed as quercetin content in 1 g of test sample.²²⁻²³

In Vitro Antioxidant Activity By Dpph Method

Principle: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical and is commonly used to evaluate the radical scavenging activity of antioxidant agents. The principle of this method is based on the

fact that, decrement of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H.

Procedure

1. The free radical scavenging activity of test sample was determined by DPPH scavenging method.
2. 0.1mM DPPH solution was prepared in methanol by adding 39.4 mg of DPPH in 1000 ml of methanol, and to 0.5 mL of this solution, 1.5 mL of test sample dissolved in DMSO was added at various concentrations (1000, 500, 250, 125, 62.5, 15.62, 1.95ug/ml).
3. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes.
4. Then the absorbance was measured at 517nm using non-coated 96 well plate on microplate reader (EPOCH, Agilent BioTek, US). Ascorbic acid was used as standard compound.
5. Reduction in absorbance by test compounds indicates radical scavenging activity.
6. The scavenging activity by the DPPH radical was determined by

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A_1)/A_0\} \times 100\}$$

Where, A₀ is the absorbance of the control reaction, and A₁ is the absorbance test compound or Ascorbic acid

In Vitro Antioxidant Activity By Nitric Oxide Method

Principle

Nitric oxide (NO) is a free radical that produced by interaction of NO with oxygen or reactive oxygen species. It is free radical due to its unpaired electron and exhibits similar properties like superoxide free radicals. This assay is based on the reduction of nitrate to nitrite by a reducing agent at 37 0C. Converted nitrite and endogenous nitrite are collectively converted by Griess reagent to a blue coloured azo compound. This compound can be measured spectroscopically between 580-630 nm and absorbance is directly proportional to the total nitric oxide concentration.

Procedure

1. Nitric oxide (NO) radical scavenging activity of test extracts *Helicteresisoraliin* were done, various concentrations of test compounds test extract *Helicteresisoraliin*, such as 1000, 500, 250, 125, 62.5, 15.62, 1.95 ug/ml were dissolved in dimethylsulphoxide (DMSO).
2. To which, 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was added, then, 1 ml of various concentrations 1000, 500, 250, 125, 62.5, 15.62, 1.95 of test compounds were mixed, and to this equal volume of freshly prepared Griess reagent was added, solution was then incubated at 25°C for 3 hours.
3. From above solution, 100 µl of the reaction mixture was transferred to a noncoated 96-well plate, and the absorbance was read at 546 nm using a microplate reader (EPOCH, Agilent BioTek, US). Ascorbic acid was used as standard control.
4. The percentage of nitrite radical scavenging activity of test compounds was calculated by

Pharmacognostic Study

Macroscopy

The fruits of *Helicteresisora* is greenish coloured when fresh turned to greenish brown when dried, cylindrical, pubescent with five follicles twisted spirally, 2-6 cm length and 2mm to 1 cm diameter. The seeds are tuberculated with characteristic odour and taste slightly bitter.

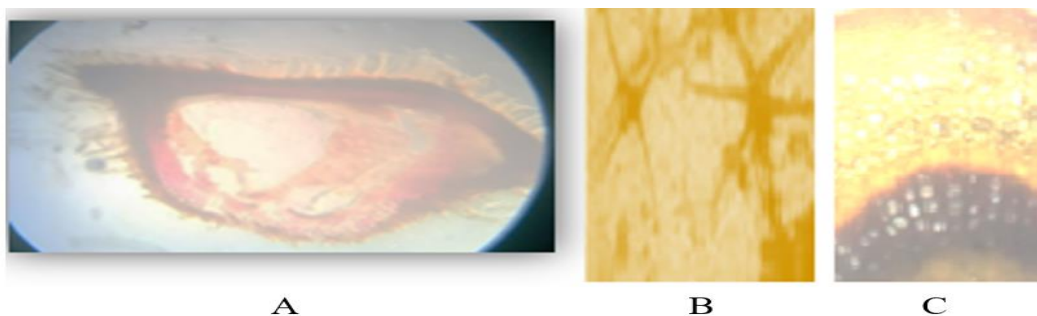
Microscopy

T. S. of *Helictres isora* fruit

The transverse section of fruit shows the presence of epicarp, endocarp, and mesocarp. Along with this it shows brown epicarp covered by stellate lignified trichomes, stone cells and vascular bundles and endocarp is composed of fibers.



Figure 1: Macroscopy



A
Figure 2:A: T. S. of Helicteresisorafruit
B:Stellate trichomes
C: Endo. Meso.Epid.andVasc. bundles

Powder Microscopy

The powder is brownish in colour with characteristic odour and slightly bitter taste. Powder shows the characteristic cells of epicarp, stellate trichomes, fragments of mesocarp and endocarp.

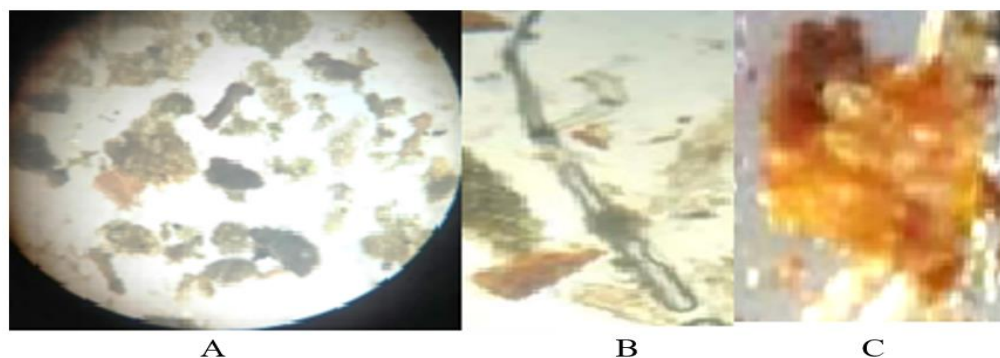


Figure 3:A: Microscopy of powder
B: Stellate trichomes
C:Endocarp with prismatic crystals

Evaluation of physical constants

The drug material has been evaluated for various physical constants like determined of foreign organic matter, moisture content, ash value, Acid-insoluble ash, Water-soluble ash, Extractive values, water-soluble extractive value and Alcohol-soluble extractive value as procedure described in IP1996, shows the significant result as shown in table no. 1.

Table 1: Evaluation of Physical Constants

| Parameters | Helicteresisoralinn fruit extract |
|--|-----------------------------------|
| Chloroform Soluble Extractives Values % (w/w) | 16.29 + 0.38 |
| Methanol Soluble Extractives Values % (w/w) | 54.21+0.27 |
| Ethanol Soluble Extractives Values % (w/w) | 79.42+0.51 |
| Ethyl Acetate Soluble Extractives Values % (w/w) | 10.5 +0.61 |
| Water Soluble Extractives Values % (w/w) | 7.35 +0.52 |
| Total Ash Values % (w/w) | 3.4 +0.59 |
| Acid Insoluble Ash Values % (w/w) | 3.8 +0.44 |
| Water Soluble Ash Values % (w/w) | 2.6±0.66 |
| Moisture Content % (w/w) | 1.14±0.66 |

Phytochemical Screening

Phytochemical study helps to know the composition vital phytochemical present in the drug which will help to design new drugs and development of formulation. Phytochemical screening of Helicteresisoralinn Ethanolic fruit extract shows the presence of glycosides, flavonoids, saponin glycosides, proteins and tannins and phenols as shown in table no. 2

| Sr.No. | Test For | Helicteresisoralinn fruit extract | Ethanolic |
|--------|---------------|--------------------------------------|-----------|
| 1. | Alkaloids | - | |
| 2. | Carbohydrate | - | |
| 3. | Glycosides | + | |
| 4. | Flavonoids | + | |
| 5. | Proteins | - | |
| 6 | Fats and Oils | + | |

| | | | | |
|--------------|----------|--------------------|---|-----------|
| Table | 7 | Tannins & Phenols | + | 2: |
| | 8 | Saponin Glycosides | + | |

Phytochemical screening of extract

Thin Layer Chromatography

Ethanollic fruit extract, was performed chromatographed on pre-coated silica gel plates with solvent mixture Dichloromethane: Methanol (7:3), Toluene: Ethyl acetate: Formic acid: Water (3:3:1:0.2), and Chloroform: Ethyl acetate: Formic acid (5:4:1). The spots were visualized by spraying the plates with a mixture of ethanol, sulphuric acid, glacial acetic acid and anis-aldehyde reagent (135:5:1:3.7) and heating them in an oven for 3–5 min at 90 °C. The Rf value of extracted residue was in agreement corresponding to standard diosgenin, (Rf 0.56) which confirmed the presence of diosgenin. The Rf value of extracted residue was in agreement corresponding to standard diosgenin, (Rf 0.5) which confirmed the presence of rosmarinic acid.

Total Phenolic Content

Total phenolic content was analysed by Folin –Ciocalteu Colorimetric method. Gallic acid was used as standard by taking concentration (5, 10, 15, 20, & 25 ug/ml) and calibration curve has drawn concentration vs absorbance. HIET concentration (10, 20, 30, 40 & 50 ug/ml) shown significant concentration of phenols. Data expressed as (\pm) SD (n=3). ($\mu\text{g GAE}/ \mu\text{g DW}$ denote Gallic acid equivalent per dry weight of extract.



Table 3: Total Phenolic Content

| Concentration of Gallic acid (ug/ml) | Absorbance at 765nm | TPC of Gallic acid ($\mu\text{g Quercetin}/ \mu\text{g DW}$) | Concentration of (HIET) (ug/ml) | Absorbance 765nm (HIET) | TPC of (HIET) ($\mu\text{g GAE}/ \mu\text{g DW}$) |
|--------------------------------------|---------------------|--|---------------------------------|-------------------------|---|
| 5 | 0.25 | 45.09 \pm 0.12 | 10 | 0.392 | 39.08 \pm 0.00 |
| 10 | 0.399 | 51.03 \pm 0.19 | 20 | 0.599 | 41.03 \pm 0.3 |
| 15 | 0.591 | 53.05 \pm 0.00 | 30 | 0.735 | 45.12 \pm 0.10 |
| 20 | 0.752 | 56.08 \pm 0.22 | 40 | 0.898 | 47.07 \pm 0.05 |
| 25 | 0.916 | 65.09 \pm 0.60 | 50 | 0.102 | 51.06 \pm 0.99 |

Note: Data expressed as (±) SD (n=3). (µg GAE/ µg DW denote Gallic acid equivalent per dry weight of extract

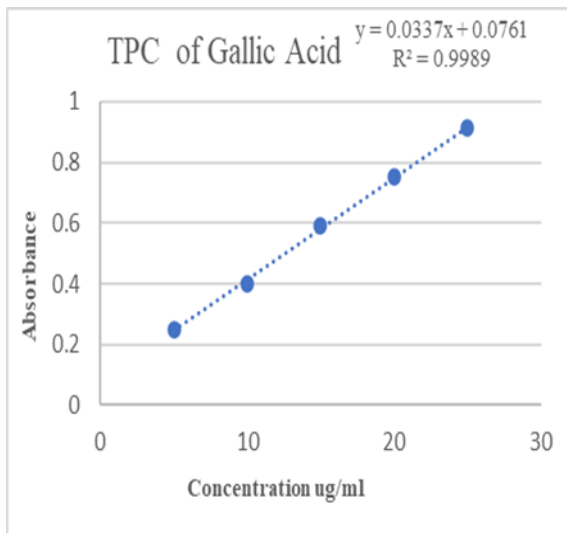


Figure 4: Calibration curve of Gallic Acid

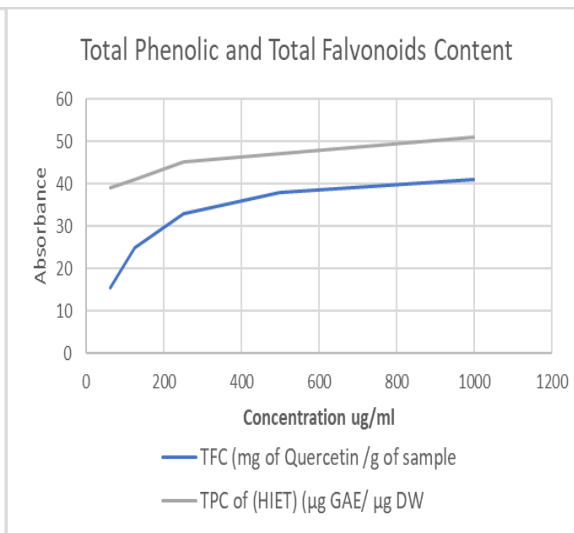


Figure 5: Chart for Total Phenolic and Total Flavonoids Content

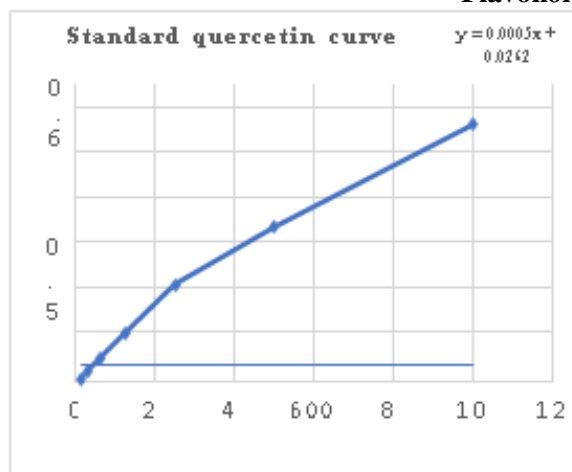


Figure 6 : Calibration curve for Quercetin

Total Flavonoid Content

Total Flavonoid Content was determined by Aluminium Chloride Colorimetric Method. Quercetin was taken as standard at the concentration (6.25, 15, 250, 500, & 1000 ug/ml). And the extract concentration was taken same as standard shows significant concentration of flavonoids present in the extract. Data expressed as (±) SD (n=3). (µg Quercetin/ µg) denote Quercetin equivalent per dry weight of quercetin.

Table 4: Total Flavonoids Content

| Conc. of Quercetin (µg/ml) | Abs. at 517nm | TFC (µg of Quercetin /µg DW) | Conc. of (HIET) (ug/ml) | Abs. at 517nm (HIET) | TFC (µg of Quercetin/ µg of sample) |
|----------------------------|---------------|------------------------------|-------------------------|----------------------|-------------------------------------|
| 62.5 | 0.049 | 25.03 ± 0.55 | 62.5 | 0.019 | 15.49±0.01 |

| | | | | | |
|------|-------|--------------|------|-------|-------------|
| 125 | 0.099 | 27.01 ± 1.00 | 125 | 0.031 | 25.00± 0.80 |
| 250 | 0.197 | 31.50 ±0.90 | 250 | 0.040 | 33.07± 0.33 |
| 500 | 0.312 | 49.03 ± 0.33 | 500 | 0.048 | 38.00± 0.50 |
| 1000 | 0.519 | 60.00 ± 0.65 | 1000 | 0.053 | 41.10± 0.20 |

Note: Data expressed as (±) SD (n=3). (µg Quercetin / µg DW denote quercetin equivalent per dry weight of extract

In Vitro Antioxidant Activity By Dpph Method

- The free radical scavenging activity of test sample was determined by DPPH scavenging method, Ascorbic acid was used as standard compound at concentration (1.92, 15.62, 62.5, 125, 250, 500, & 1000 µg/ml) and test concentrations has also been taken same as standard. Test sample shows significant radical scavenging potential. Data expressed as mean ± SD (n=3). µg Vit.C/ µg DW per dry weight.

In Vitro Antioxidant Activity By Nitric Oxide Method

- Nitric oxide (NO) radical scavenging activity of test extracts Helicteresisoraliin were done, various concentrations of test compounds test extract Helicteresisoraliin , such as 1000, 500, 250, 125, 62.5, 15.62, 1.95 µg/ml were dissolved in dimethylsulphoxide (DMSO). The standard was used same Vitamin C.

$$\text{Nitric oxide scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Table 5: Anti-oxidant activity by DPPH method & Nitric oxide

| Conc. of Vit. C (µg/ml) | Abs. 517 nm | (%) Scavenging activity DPPH | Conc. of HIET (µg/ml) | Abs. of HIET at 517 nm | (%) Scavenging activity of (HIET)by DPPH | Conc. (µg/ml) | Abs. at 596 nm | (%) Scavenging activity,of (HIET) by NO |
|-------------------------|-------------|------------------------------|-----------------------|------------------------|--|---------------|----------------|---|
| 1.95 | 0.055 | 40.22± 0.03 | 1.95 | 0.078 | 1.09±0.01 | 1.95 | 0.112 | 1.03± 0.03 |
| 15.62 | 0.041 | 55.43± 0.05 | 15.62 | 0.071 | 3.088±0.22 | 15.62 | 0.102 | 2.150 ± 0.50 |
| 62.5 | 0.021 | 77.17± 0.30 | 62.5 | 0.061 | 8.055± 0.33 | 62.5 | 0.097 | 2.99± 0.65 |
| 125 | 0.017 | 81.52± 0.50 | 125 | 0.06 | 10.05±0.00 | 125 | 0.094 | 3.09± 0.54 |
| 250 | 0.011 | 88.04± 0.23 | 250 | 0.058 | 13.05± 0.36 | 250 | 0.09 | 7.22± 0.56 |
| 500 | 0.009 | 90.22 | 500 | 0.044 | 25.26± 0.25 | 500 | 0.082 | 15.46±0.20 |
| 1000 | 0.008 | 91.3 | 1000 | 0.032 | 45.56± 0.56 | 1000 | 0.081 | 16.49± 0.32 |

Note: Data expressed as mean ± SD (n=3). µg Vit.c µg DWper dry weigh

CONCLUSION

The ethanolic extract of Helicteresisoralinn has evaluated for microscopic. Macroscopic, physical evaluation, phytochemical screening, quantitative evaluation for total phenolic content, total flavonoids content, thin layer chromatography for phytochemical identification and antioxidant activity by two method that is DPPH radical scavenging activity and Nitric oxide activity. The result indicated that the plant is rich in biochemical constituents like calcium, magnesium and tannin. Based on the ethnobotanical

survey, phytochemical screening shows the presence of vital phytoconstituents like glycosides, alkaloids, tannins, phenols, proteins etc. Quantitative investigation, for phenolic content (50 ug shows 51.06 ± 0.99 GAE/ $\mu\text{g DW}$), Total flavonoids content (1000 ug shows 41.10 ± 0.00 $\mu\text{g Quercetin/ } \mu\text{gDW}$), antioxidant activity by DPPH radical scavenging activity (1000 ug shows 45.56 ± 0.56 $\mu\text{g Vit.C/ } \mu\text{g DW}$) and radical scavenging activity by Nitric oxide method (1000 ug shows 16.49 ± 0.32 $\mu\text{g Vit.C/ } \mu\text{g DW}$) results of the present investigation, it could be said that the fruit extracts possess potent medicinal values and contain chemical constituents of pharmacological significance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened can yield compounds of pharmaceutical importance. The biochemical analysis revealed that fruit has diosgenin and rosmarinic acid in good amount. Further research can be carried out to isolate, purify and characterize the detail chemical constituents in the fruit with a view to utilise these bioactive compounds in drug development. This study is an attempt to validate the tribal claims of medicinal value of *H. isora* through experimental observation.

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