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# Study of Antioxidant, Cytotoxic and Analgesic Properties of the Stem of *Helicteres isora* Linn.

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**ABSTRACT**

*Helicteres isora* Linn., often referred to as the Indian screw tree, goes by the names Atmora or Rajot in Bangladesh. A genus of flowering plants in the Malvaceae family is called Helicteres (shrub). According to reports, extracts from fruits, roots, and bark have anti-cancer, hepatoprotective, anti-dysenteric, anti-diabetic, and antioxidant properties. To the best of our knowledge no attempts have been made for finding out therapeutic potentials of the stem part of this plant. From preliminary phytochemical screening we noticed the presence of different phytochemical constituents including the Alkaloids, glycoside, flavonoids, carbohydrates, tannins, fats & fixed oils. Where Alkaloids and fats & oils were moderately present. But steroids, saponins and Triterpenoids were absent in methanolic extract of *Helicteres isora* stem. The results of the present study provide scientific basis for the use of *Helicteres isora* in traditional medicine in the treatment of aforementioned diseases. Furthermore, some new pharmacological effects of the plant have been revealed. It is thus important that extensive phytochemical and pharmacological studies should be carried out on the basis of the results of the current study to eventually find bioactive compounds as new lead compounds.

**Keywords:** *Helicteres isora*; Cytotoxic; Analgesic; Antioxidant; Phytochemical; Calibration; DPPH; Glycosides; Pharmacological actions.

## 1. Introduction

In both modern and traditional medicine, a medicinal plant is one that is intended to treat a particular ailment, preserve health, or for both purposes. In 2002, the Food and Agriculture Organization made an estimate of the global usage of medicinal plants, which came to over 50,000. Out of about 30,000 plants for which a usage of any type is recorded, the Royal Botanic Gardens, Kew more cautiously assessed in 2016 that 17,810 plant species have a therapeutic function [1]. Since ancient times, medicinal plants—also known as medicinal herbs—have been identified and employed in conventional medical procedures. Hundreds of chemical compounds are synthesized by plants for a variety of purposes, such as defense against herbivorous mammals, fungus, insects, and illnesses. There are many phytochemicals that have been found to have biological activity, either demonstrated or potential. The consequences of taking a whole plant as medication are unknown, though, because a single plant contains a vast variety of phytochemicals. Furthermore, many plants with medicinal promise have not had their phyto-chemical content or any potential pharmacological activities evaluated by rigorous scientific research to determine safety and efficacy [2]. Traditional medicines represented the basis of healthcare throughout the world since the earliest days of mankind. Medicinal plants have been known for millennia as a rich source of therapeutic agents for the treatment and prevention of various diseases occupying an important place in the socio-cultural, spiritual and medicinal field. Throughout the past century, significant changes in human diet and lifestyle have given rise to a number of chronic illnesses. There has been a noticeable "renaissance" of herbs lately everywhere in the world, and it has been proposed that two thirds of plant species worldwide may have therapeutic benefit [3]. According to estimates from the World Health Organization, traditional medicine is the primary source of care for 80% of the populations in Africa and Asia. Furthermore, over 40% of Americans have reported utilizing complementary and

alternative therapies, such as dietary supplements containing botanicals. Utilizing plant medicine is a cheaper and more natural alternative to pharmaceuticals [4,5].

*Helicteres isora* Linn. is a significant medicinal plant with notable nutritional and therapeutic properties among the many native medicinal herbs. This tropical shrub from Southeast Asia is grown all throughout India. The Indian System of Medicine (ISM) has traditionally used different components of the plant to treat different types of illnesses. Moreover, findings from current studies indicate that H. isora was a rich source of bioactive substances with medicinal properties, including tannins, polyphenols, and alkaloids. In addition, H. isora is said to be a good source of iron, calcium, phosphorus, proteins, fiber, and carbohydrates. According to a different study, certain antioxidant components such ascorbic acid, flavonoids, and phenolics (cucurbitacin B and iso-cucurbitacin B) are present. These findings are based on extraction and characterisation investigations [6]. *Helicteres isora* Linn., often known as the Indian screw tree, is a species of small tree or big shrub widespread in Asia, including the Indian Subcontinent, South China, the Malay Peninsula, Java, and Saudi Arabia. In Bangladesh, it is known by the common name Atmora or Rajot. Located in Australia as well. Sunbirds, also are the primary pollinators of the red flowers. It has a remarkable array of therapeutic and nutritional benefits. Rope is made from the bark's fibers. Hymenoptera and a large number of butterflies also visit them [7]. It is a good source of iron, calcium, phosphorus, fiber, proteins, and carbs. The indigenous medical system traditionally uses different sections of the plant to treat different kinds of illnesses. The bark and roots have expectorant, demulcent, constipating, and lactifuge properties; they are also helpful in the treatment of colic, scabies, gastropathy, diabetes, diarrhea, and dysentery. The fruits have antispasmodic, stomachachic, vermifuge, refrigerant, and astringent properties [8]. Leaf paste is said to be beneficial for treating a number of skin conditions, including scabies and eczema. Fruit pod extracts have been shown to be vermifuge (colic) and anti-dysentetic. It is also used as an astringent and for gout, stomach aches, and flatulence. In order to reduce pain, fruits are fried in mustard oil and applied to the bodies of newborns.

To help with postpartum health problems, new moms are given fruit powder blended with other herbs and spices as a delightful delicacy called laddoo [9]. It is also claimed to be used as expectorant, astringent, anti-galactagogue, to reduce gripping and a cure for snakebite [10]. The root and bark extract exhibits hypolipidemic action, insulin uptake sensitivity, and prospective application in the management of type 2 diabetes [11]. It has been observed that alcoholic and aqueous extracts from the fruits and bark of H.isora exhibit antioxidant activity, including the ability to scavenge free radicals, toxicity towards tumor cells, and protection against normal cells. However, in cell-free systems, the majority of them are restricted to initial evaluation [12,13]. Some researchers have demonstrated antimicrobial activity from aqueous and alcoholic of fruits of H. isora against a number of bacterial strains [14]. The traditional usage of this plant in the treatment of liver problems has a scientific basis thanks to research that suggests the ethanolic extract from the root and bark has hepatoprotective properties [15].

The present study's findings offer a scientific foundation for the traditional medical application of *Helicteres isora* in the management of the aforementioned conditions. Additionally, the plant's novel pharmacological activities have come to light. Based on the current study's findings, it is crucial to conduct comprehensive phytochemical and pharmacological investigations in order to identify bioactive molecules that could serve as novel lead compounds.

## 2. Methods

The present study was designed to identify the groups of chemical constituents that are present in the crude extract of *Helicteres isora* Linn. as well as to observe the pharmacological activities of the stem extracts of the plant.

The study protocol consists of the following steps:

| **Extraction** | **Part used** | **Solvent** |
|---|---|---|
| | Stem | Methanol |
| **Phytochemical Screening** | | Different qualitative tests to find out the presence of chemical constituents |
| **In-vitro Experiments** | | |
| Antioxidant Activity | | 1. Total Phenol Content Determination |
| | | 2. Total Flavonoid Content Determination |
| | | 3. Determination of Antioxidant Capacity |
| | | 4. DPPH Scavenging Assay |

### 2.1. Experimental animals

For the experiment Swiss albino mice of either sex, 6-7 weeks of age, weighing between 25-30gm, were collected from the animal research lab in the department of pharmacy, Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: 27 ± 1º C, relative humidity: 55-65% and 12 hours light/12 hour dark cycle) and had free access to feed and water ad libitum. All protocols for animal experiments were approved by the institutional animal ethical committee.

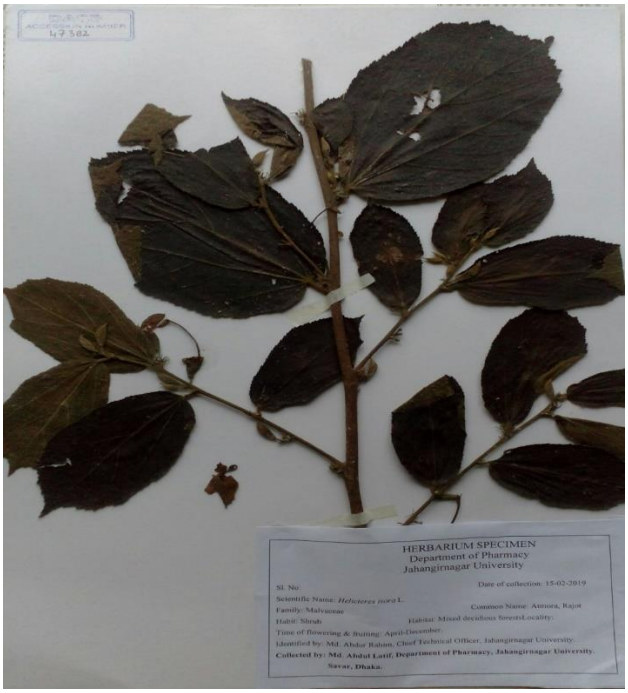


**Figure 1.** Identification of the stem of *Helicteres isora* Linn.

### 2.2. Preparation of plant extract

The stem of *Helicteres isora* Linn. was collected from Savar, Dhaka, Bangladesh and authenticated by Md. Abdur Rahim, technical officer, Department of Botany, Jahangirnagar University. A voucher specimen (DACB No. 47382) was deposited in the herbarium for future reference. The collected plant part (stem) was cleaned and washed well with water. The cleansed stem was then partially dried by fan aeration and then fully dried in the oven at below 40ºC for 4 days. The fully dried part was then grinded to a powdered form and stored in suitable condition for few days.

### 2.3. Extraction procedure

The powdered plant material of stem (800 gm) were used for extraction by Soxhlet apparatus at elevated temperature (65°C) using methanol consecutively (500 ml solvent). After each extraction the powder was dried and used again for the next extraction. Extraction was considered to be complete when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. All extract of stem were filtered individually through fresh cotton bed. The filtrates obtained were dried at temperature of 40±2ºC to have gummy concentrate of the crude extracts. The extract was kept in suitable container with proper labeling and stored in cold and dry place. The yield value for methanol extract of the stem of *Helicteres isora* Linn. was 4.9%.

### 2.4. Phytochemical screening test

The plant extracts were subjected to different qualitative tests to find out the presence of chemical constituents. These were identified by characteristic color changes using standard procedure. Phytochemical screening tests include Molisch's test (General test for Carbohydrates), Barfoed's test (General test for Monosaccharides), Fehling's test, Benedict's test, Saponification test, Tests for alkaloids, Ferric chloride test, Alkaline reagent test, were done.

**Table 1.** Phytochemical test

| **Constituents** | **Test** | **Procedure** | **Observation** |
|---|---|---|---|
| **Carbohydrates** | Molisch's test (General test for Carbohydrates) | Two drops of molisch's reagent (10% alcoholic solution of α-naphthol) were added to 2ml of aqueous extract. Allow 2ml of conc. sulfuric acid to flow down the side of the inclined test tube so that the acid forms a layer beneath the aqueous solution. | A red or reddish violet ring is formed at the junction of the two layers if a carbohydrate is present. On standing or shaking a dark purple solution is formed. |

| | | | |
|---|---|---|---|
| | Barfoed's test (General test for Monosaccharides) | 1 ml of an aqueous extract of the plant material was added to 1 ml of Barfoed's reagent in a test tube and heat in a beaker for boiling water. | Red precipitate of cuprous oxide is formed within 2 minutes if a monosaccharide is present. |
| | Fehling's test | 2 ml of an aqueous extract of the plant material was added to 1ml of a mixture of equal volumes of Fehling's solutions A and B then boiled for a few minutes. | A red or brick-red precipitate is formed if a reducing sugar is present. |
| | Benedict's test | Test solution is treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath | Redish brown precipitate forms if reducing sugars are present. |
| **Glycosides** | General test | A small amount of an alcoholic extract of the fresh or dried plant material was dissolved in 1 ml of water and add few drops of aqueous sodium hydroxide solution. | A yellow color develops in the presence of glycoside. |
| | Test for glucosides (glycosides with glucose as the glycone) | A small amount of an alcoholic extract of the plant material was dissolved in water and alcohol, solution was divided into two portions and were treated in the following ways:<br>One of them was boiled with a mixture of equal volume of Fehling's solution A and B was boiled. Note any brick-red precipitate. Other portion was boiled with a few drops of dilute sulphuric acid for about 5 minutes, | Production of a brick-red precipitate in the second experiment (carried out with the hydrolyzed extract) and no production of such a precipitate in the first experiment show the presence of glucosides in the extract. |

| | | | |
|---|---|---|---|
| | | neutralized the mixture with sodium hydroxide solution, add an equal volume of mixture of Fehling's solution A and B was added and then boiled. | |
| **Fats & Fixed Oils** | Stain test | The small quantity of extract is pressed between two filter papers. | The stain on 1 filter paper indicates the presence offixed oils. |
| | Saponification test | A few drops of 0.5N of alcoholic potassium hydroxide was added to small quantities of various extracts along with a dropof Phenolphthalein separately and heated on a water bath for 1-2 hrs. | The formation of soap or partial neutralization of alkali indicates the presence of Fixed oils and Fats. |
| **Proteins & Amino Acids** | Ninhydrin test | A small amount of extract was boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate). | Violet colorappears indicates the presence of proteins and amino acid. |
| **Alkaloids** | Tests for alkaloids | A small volume of each extract is neutralized by adding 1 or 2 drops of dilute $H_2SO4$. This neutralized solution is treated with a very small amount of the following reagents and the respective color and precipitate formation is observed: | |
| | | a) Mayer's reagent (Potassium-mercuric iodide solution). | For Mayer's reagent white or creamy white precipitate. |
| | | b) Hager's reagent (1% solution of picric acid). | For Hager's reagent yellow crystalline precipitate. |

| | | | |
|---|---|---|---|
| | | c) Dragendorff's reagent (Bismuth potassium iodide solution). | For Dragendorff's reagent orange or orange-red precipitate is observed. |
| | | d) Wagner's reagent | For Wagner's reagent formation of brownish-black precipitate indicates the presence of alkaloids. |
| | | e) Tannic acid reagent (10% tannic acid solution). | For tannic acid reagent formation of buff color precipitate indicates presence of alkaloid. |
| **Triterpenoids** | Salkowski test | The extract is treated in Chloroform with few drops of cone. Sulfuric acid, was shaken well and allowed to stand for some time. | Red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of triterpenoid. |
| **Steroids** | Liebermann-Burchard's Test | A small amount of a petroleum ether extract of the plant material was dissolved in 1 ml of chloroform then 2 ml of acetic anhydride was added with 1 ml of conc. sulphuric acid. | A greenish color is produced which turns blue on standing if a steroid is present. |
| **Saponins** | Frothing test | About 0.5 ml of extract is shaken vigorously with water in a test tube. | Production of a persistent frothing (which remains stable in heating) indicates the presence of saponins. |
| **Tannins** | Lead acetate test | 5 ml of aqueous extract of the plant material was taken in a test tube and a few drops of a 1% solution of lead acetate were added. | A yellow or red precipitate is formed. |

| | | | |
|---|---|---|---|
| | Ferric chloride test | Test solution is treated with ferric chloride solution. | Test solution gives blue green color with ferric chloride. |
| | Alkaline reagent test | Test solution with sodium hydroxide solution gives yellow to red precipitate within short time. | Test solution with sodium hydroxide solution gives yellow to red precipitate within short time. |
| **Flavonoids** | Shinoda test (Magnesium Hydrochloride reduction test) | To the test Solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise. | Pink scarlet, crimson red or occasionally green to blue color appears after few minutes. |
| | Zinc Hydrochloride reduction test | To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. | Formation of red color after few minutes. |
| | Alkaline reagent test | To the test solution add few drops of sodium hydroxide solution; and then add dil. Acid. | Formation of an intense yellow color, which turns to colorless indicates presence flavonoids. |

### 2.5. Determination of Total Phenolics content

The content of total phenolic compounds in plant methanolic extracts was determined as described previously using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent.

### 2.6. Determination of Total Flavonoids Content

The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. The wavelength 415 nm is chosen for absorbance measurement.

### 2.7. Determination of Total Antioxidant Capacity

The phosphor-molybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The phosphor-molybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acid pH.

In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI) and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm.

### 2.8. Pharmacological Investigation & study design

High dosages of plant extract are administered to different groups of mice to see the effects of plant extract on their physiology. Acute toxicity study is a well-known method to determine LC50 value of the plant extract. Animals were divided into 4 groups of 5 animals each. All doses are administered per orally and here HIM means *Helicteres isora* extract in methanol.

| **Group** | **Administered substance with their dose** |
|---|---|
| Group I | Control (normal water) |
| Group II | Standard (Diclofenac Sodium) |
| Group III | HIM (250 mg/kg) |
| Group IV | HIM (500 mg/kg) |

### 2.9. Ethical consideration

All experimental animals were used according to predefined ethical consideration and following the guideline of research ethics committee of Jahangirnagar University.

### 2.10. Data analysis

P< 0.05, P< 0.01 and P< 0.001 were considered statistically significant, highly significant and very highly significant respectively. Independent samples T test was performed to analyze this data set. SPSS version 20.0 and Microsoft excel software is used to analyze data.

## 3. Result and Discussion

### 3.1. Phytochemical screening

Preliminary phytochemical screening of the extract of the stem of *Helicteres isora* Linn. revealed the presence of different kind of phytochemical constituents that are summarized in table 2.

**Table 2.** Result of phytochemical screening tests of the extract of the stem part of *Helicteres isora* Linn.

| **Phytochemicals** | **Name of the test** | **Observed changes** | **Result** |
|---|---|---|---|
| **Alkaloids** | Mayer's test | Creamy white precipitate | - |
| | Hager's test | Yellow crystalline precipitate | - |
| | Wagner's test | Brown or deep brown precipitate | - |

| | | | |
|---|---|---|---|
| | Dragendorff's test | Orange or orange-red precipitate | + |
| | Tannic acid reagent (10% tannic acid solution). | buff color precipitate | + |
| **Carbohydrates** | Molisch's test (General test for Carbohydrates). | A red or reddish violet ring is formed at the junction of two layer and on shaking a dark purple solution is formed. | - |
| | Barfoed's test (General test for Monosaccharids. | The red precipitate of cuprous oxide is formed within 2 minutes if a monosaccharide is present. | + |
| | Fehling's test | A red or brick-red precipitate is formed if a reducing sugar is present. | + |
| **Glycosides** | General test | Yellow color | + |
| | Test for Glucoside | Production of brick-red precipitation. | + |
| **Flavonoids** | Zinc Hydrochloride reduction test. | Red color after few minutes. | + |
| | Alkaline reagent test. | intense yellow color, which turns to colorless. | + |
| **Saponins** | Frothing test | Formation of stable foam | - |
| **Steroids** | Libermann-Burchard 's test. | A greenish color is produced which turns blue. | - |
| **Tannins** | Lead acetate test | A yellow or red precipitate | - |
| | Ferric chloride test | Blue green color | + |
| | Alkaline reagent test | Yellow to red precipitate | + |
| **Triterpenoids** | Salkowski test | Yellow color appears at the lower layer. | - |
| **Fats & Fixed Oils** | Stain test | The stain on 1 filter paper indicates the presence of fixed oils. | + |
| | Saponification test | The formation of soap or partial neutralization of alkali indicates the presence of Fixed oils and Fats. | - |

The Phytochemical screening and qualitative estimation of the crude extract of *Helicteres isora* Linn. stem shown to possess phytoconstituents including the Alkaloids, glycoside, flavonoids, carbohydrates, tannins, fats & fixed oils. Where Alkaloids and fats & oils are moderately present. Steroids, saponins and Triterpenoids are absent in methanol extract of *Helicteres isora* stem. According to literature review *Helicteres isora* fruit contain alkaloids, glycosides, flavonoids, carbohydrates, tannins, saponins, fats & fixed oils.

Apart from their anti-oxidant qualities, flavonoids have biological roles in preventing allergies, inflammation, free radicals, platelet aggregation, bacteria, ulcers, hepatotoxins, viruses, and cancers [16, 17]. This may plausibly corroborate and explain the data about the application of phytochemicals that were extracted from the solvent extract in the current investigation. Numerous phytochemicals found in *Helicteres isora* attest to this species' ability to serve as a powerful source of contemporary medications.

### 3.2. Phenol content determination

The Total phenolic content of the crude extract of the stem of *Helicteres isora* was determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid (y = 0.0066x + 0.0336; R2 = 0.9978). The methanol extract of the stem of *Helicteres isora* was found to contain the remarkable amount of phenols (Figure 2).

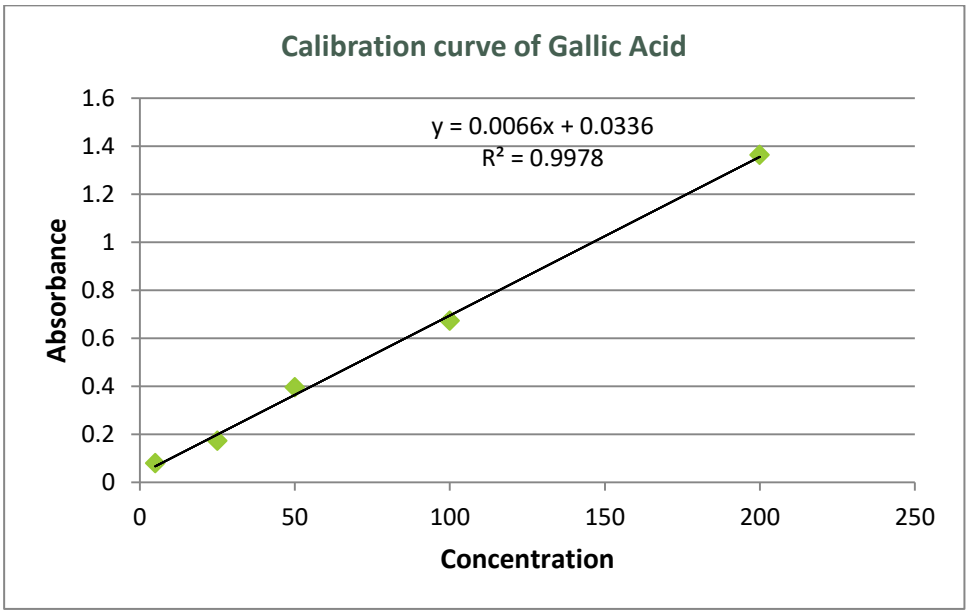


**Figure 2.** Calibration Curve of Gallic Acid

Phenolics are widely distributed in the kingdom of plants and are made up of anthocyanins, catechins, and flavonoids (flavones, isoflavones, and flavanones). Their structural chemistry is perfect for scavenging free radicals. The ability of polyphenols to chelate metal ions (ending the Fenton reaction) and their strong reactivity as hydrogen or electron donors, which can stabilize and delocalize the unpaired electron (chain-breaking function), are the sources of their anti-oxidative qualities. Polyphenols have been shown to block LDL oxidation, decrease the formation of atherosclerotic plaques and reduce arterial stiffness leaving arteries more responsive to

endogenous stimuli of vasodilation. The results suggest that phenolics are important components of the tested plant extracts.

### 3.3. Flavonoid content determination

Aluminum chloride colorimetric method was used to determine the total flavonoid contents of methanolic extract of the stem of *Helicteres isora*. The total flavonoid content was calculated using the standard curve of quercetin (y = 0.0104x-0.0828; R2 = 0.9904) and was expressed as quercetin equivalents (QE) per gram of the plant extract. Methanolic extract of the stem of *Helicteres isora* was found to contain a significant amount of flavonoid (Figure 3).

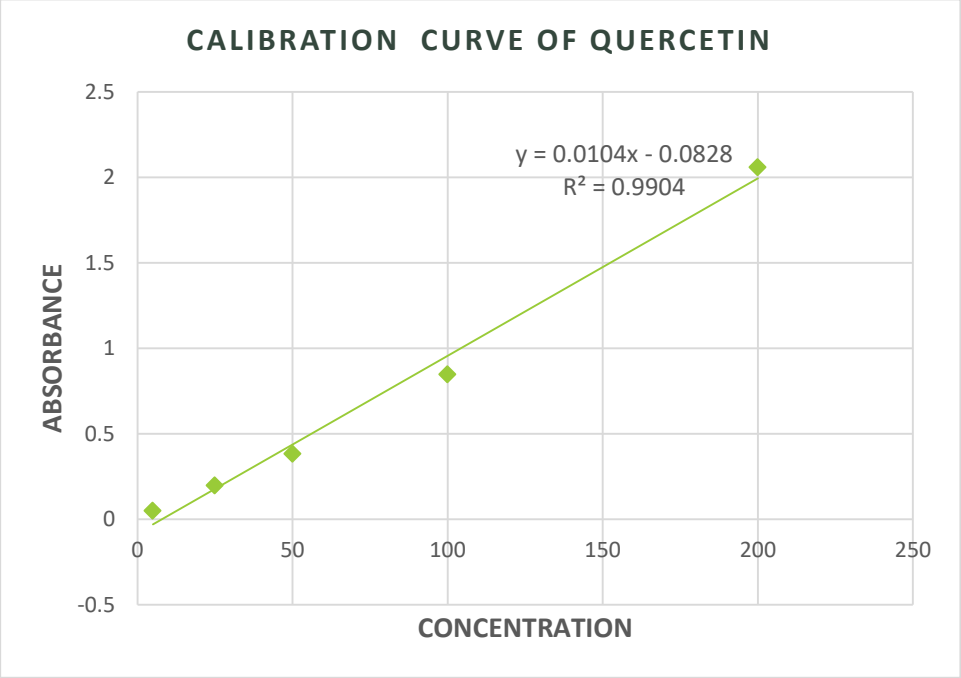


**Figure 3.** Calibration Curve of Quercetin

Plants' immune system is significantly influenced by flavonoids. Flavonoids exhibit anti-oxidative characteristics through multiple mechanisms, including the scavenging of free radicals, chelation of metal ions like copper and iron, and inhibition of enzymes that generate free radicals [18, 19]. Depending on their molecular makeup, flavonoids can scavenge almost every known ROS. Given that *Helicteres isora* stem extract has been demonstrated to contain a considerable quantity of flavonoids, but less than fruit, it may be presumed that this extract can scavenge almost all known ROS.

### 3.4. Total antioxidant capacity

Total antioxidant capacity of the methanolic extract of the stem of *Helicteres isora* evaluated by the phospho-molybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid (y = 0.0017x+ 0.0947; R2= 0.9792). The methanolic extract of the stem of *Helicteres isora* was found to possess the significant amount of total antioxidant capacity (Figure 4).

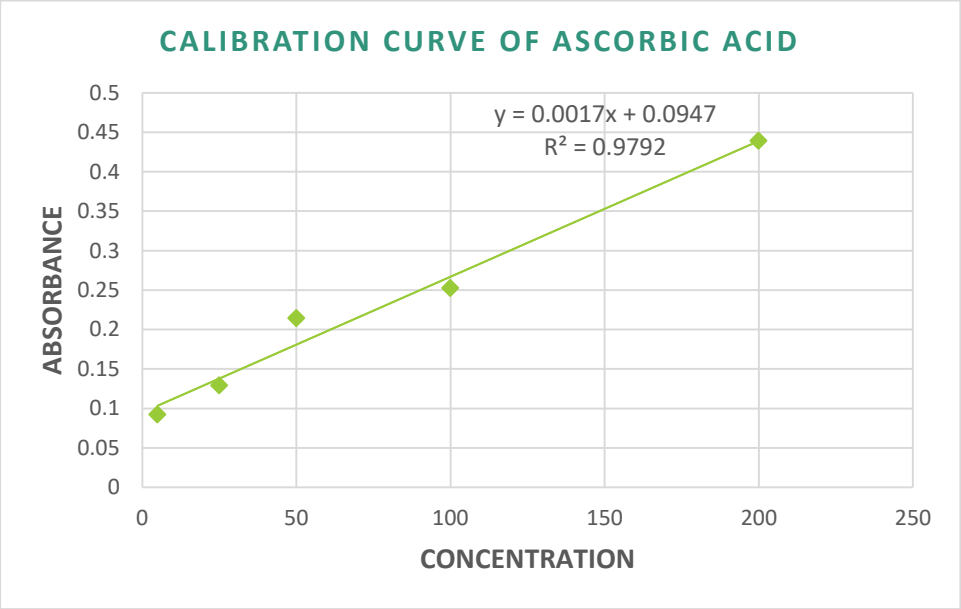


**Figure 4.** Calibration curve of ascorbic acid

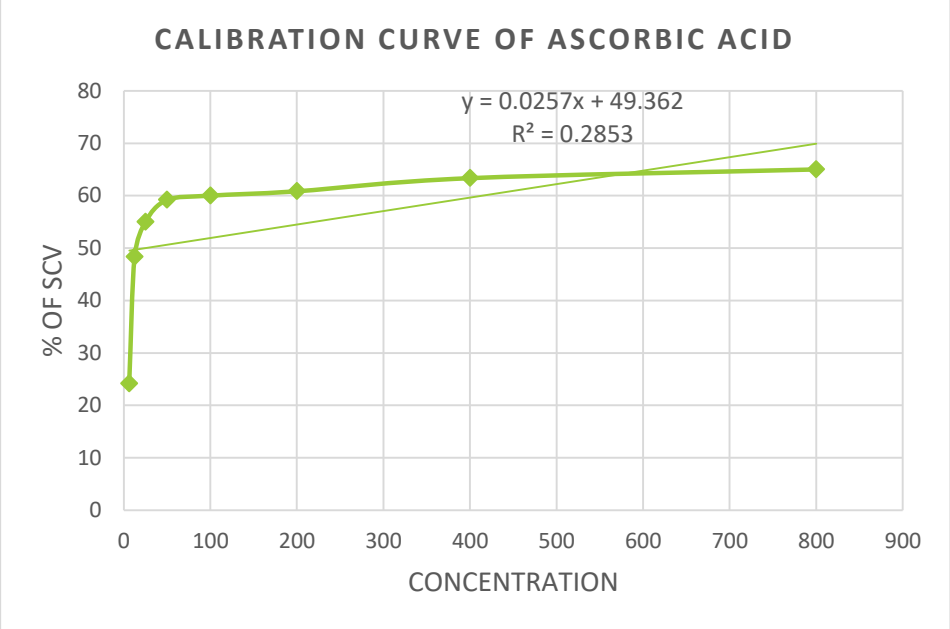


**Figure 5.** Calibration curve of Ascorbic acid

The methanolic extract of the stem of *Helicteres isora* showed remarkable amount of total antioxidant capacity but less than the amount of fruit (in term of ascorbic acid equivalent).

### 3.5. DPPH radical scavenging assay

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance at 517 nm. The IC50 values of the methanolic extract of the stem of *Helicteres isora* was presented in the scavenging of DPPH radical was found to rise with increasing concentration of the extracts with good scavenging displayed by the methanolic extract of the stem of *Helicteres isora* (Figure 6).

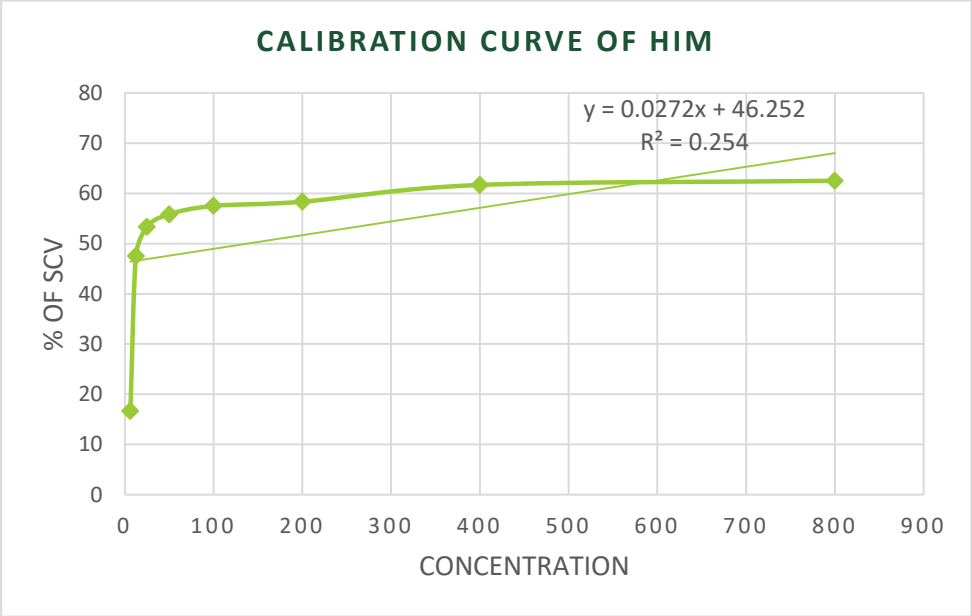


**Figure 6.** Calibration curve of HIM

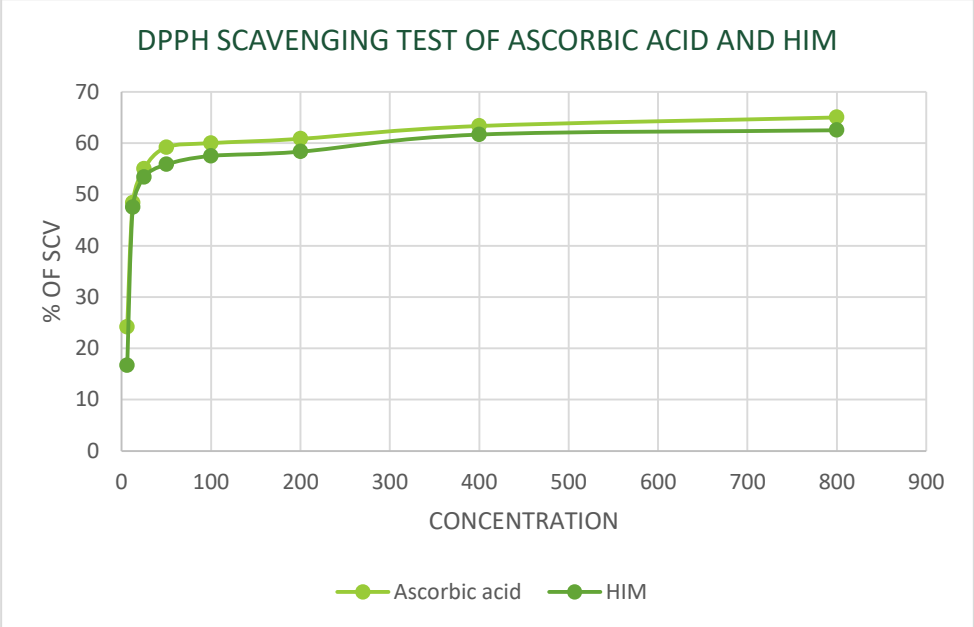


**Figure 7.** DPPH scavenging test of ascorbic acid and HIM

In DPPH radical scavenging assays, the methanolic extract of the stem of *Helicteres isora* showed dose dependent scavenging of DPPH radicals in a way similar to that of the reference antioxidant ascorbic acid.

The widely used and dependable technique of DPPH radical scavenging is utilized to evaluate the antioxidant or free radical scavenging potential of various chemicals or plant extracts [20]. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. Changes in absorbance can be used to quantify the decolorization of DPPH, which occurs when it takes an electron given by an antioxidant molecule. Thus, the *Helicteres isora* stem's methanolic extract demonstrated a remarkably strong capacity to give electrons.

### 3.6. Acute toxicity study

After the acute toxicity studies no mortality was observed up to dose as 6000 mg/kg for the extract. So no LC50 could be obtained and extracts were considered to be safer with broad therapeutic range. Therefore, two comparatively high doses (250 and 500 mg/kg) for the extract was taken for all in-vivo models.

**Table 3.** Dose and assigned code of extract

| Plant Extract | Dose (mg/kg) | Code |
|---|---|---|
| **Methanol extract of *Helicteres isora* stem** | 250 | HIM 250 |
| | 500 | HIM 500 |

### 3.7. Analgesic Activity Evaluation (Formalin-Induced Paw licking In Mice)

The extract was subjected to formalin-induced licking test and acetic acid writhing test to evaluate its analgesic activity.

**Table 4.** Effect of methanol extract of *Helicteres isora* in Formalin Induced Paw Licking test

| Group | Doses (mg/kg) | First 5 min | Inhibition (%) | Second 5 min | Inhibition (%) |
|---|---|---|---|---|---|
| **Control** | - | 56.9±5.98 | - | 6.89±4.46 | - |
| **Diclofenac Sodium** | 100 | 32.74±1.77** | 42.46 | 0 | 100 |
| **HIM** | 250 | 38.29±3.058* | 32.70 | 0 | 100 |
| | 500 | 52.78±12.04 | 7.24 | .3±.24 | 95.6 |

Values are presented in mean ± SEM (n= 6). Independent samples T test was performed to analyze this relationship. For *P<0.05=Significant, **P<0.01=Highly significant and ***P<0.001=Very highly significant, when compared against control.

The animal's response to moderate, ongoing pain caused by the wounded tissue is assessed using the formalin test. There are two stages to this test. The peripheral stimulus-induced C-fiber activation appears to be the cause of the early phase (shortly after injection), while the NO (nitric oxide) cascade in the peripheral tissue, anti-inflammatory response, activation of NMDA (N-methyl D-aspartate) and non-NMDA receptors, and functional alterations in the dorsal horn on the spinal cord appear to be responsible for the late phase (beginning about 20 minutes after formalin injection) [21].

The experimental result presented in the table shows that the methanol extract of *Helicteres isora* stem reduced the licking time of first phases (analgesic phase). In 250 mg/kg dose, HIM shows moderate percentage of inhibition

that is 32.7%. But in case of 500mg/kg, HIM shows lower percentage of inhibition that is 7.24%. In higher dose, toxicity may be show or receptor saturation occurs. So lower dose (250 mg/kg) is an effective dose. Where Diclofenac sodium at 100 mg/kg shows highest percentage of inhibition among others that is 42.46%. The methanol extract of *Helicteres isora* shows significant activity at 250mg/kg dose where Diclofenac sodium shows highly significant activity at 100 mg/kg dose. But in case of HIM of 500mg/kg dose, it shows no significant activity.

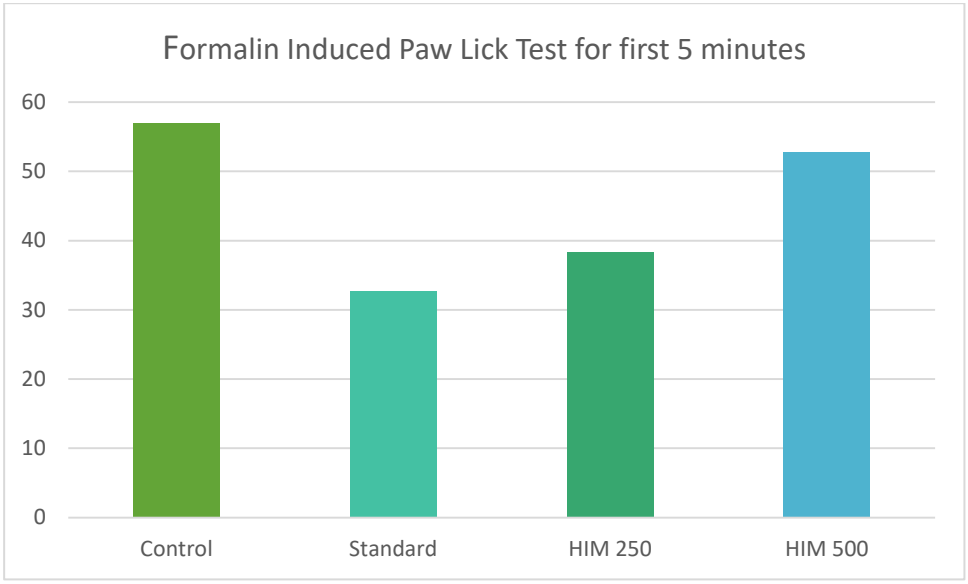


**Figure 8**. Formalin Induced Paw Lick Test for first phase 5 minutes

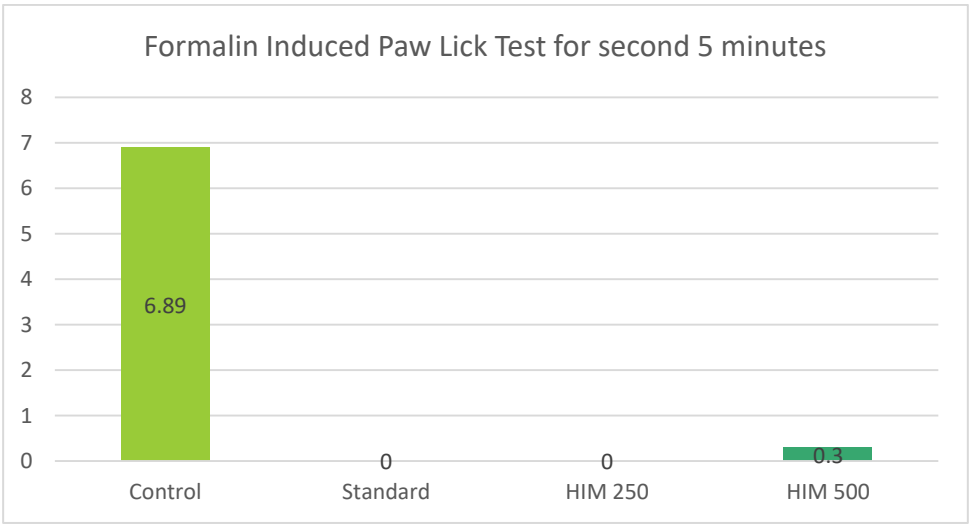


**Figure 9.** Formalin Induced Paw Lick test for second phase 5 minutes

In case of second phase, the methanol extract of *Helicteres isora* stem exhibited greater effects on the second phase of the nociceptive response. The NSAIDs (diclofenac sodium) which inhibit cyclooxygenase (COX) activity, proved to be effective against the early-phase and second phase of the formalin test. But this claim must be substantiated by more sophisticate experiments. Diclofenac sodium (100 mg/kg) and HIM (250 mg/kg) both show 100% inhibition where HIM (500 mg/kg) shows 95.6% inhibition.

### 3.8. Acetic Acid-Induced Writhing Test

**Table 5.** Effect of methanol extract of *Helicteres isora* in acetic acid induced writhing test

| Group | Dosage (mg/kg) | No. of Writhing | Inhibition (%) |
|---|---|---|---|
| **Control** | - | 20.83± 0.79 | - |
| **Diclofenac Sodium** | 100 | 16.50±.99 ** | 20.79 |
| **HIM** | 250 | 11.33±1.43 *** | 45.60 |
| | 500 | 19.17±1.25 | 7.97 |

Values are presented in mean ± SEM (n= 6). Independent samples T test was performed to analyze this relationship. For * P<0.05=significant, ** P<0.01=highly significant and *** P<0.001=very highly significant, when compared against control.

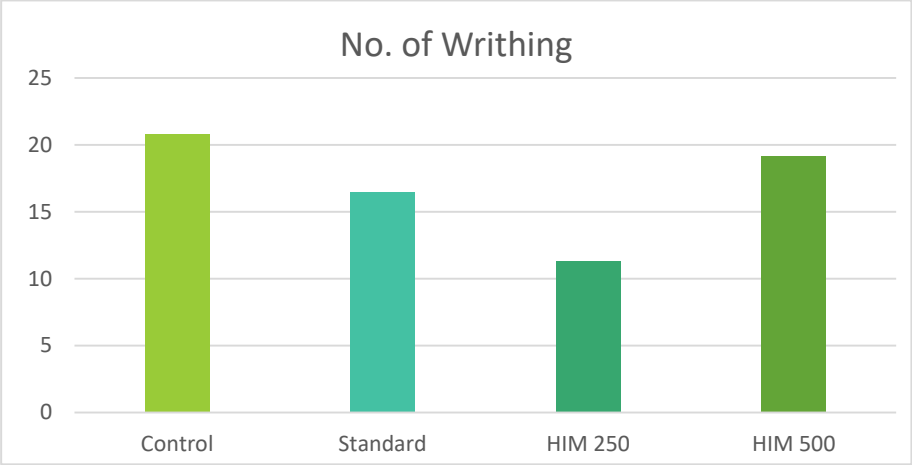


**Figure 10.** No. of writhing observed in acetic acid induced writhing test

The experimental result presented in the table shows that the methanol extract of *Helicteres isora* stem does not inhibited writhes in a dose dependent manner. In case of 250 mg/kg, HIM shows higher percentage of inhibition that is 45.6% where Diclofenac Sodium shows 20.79%. But in case of 500 mg/kg, HIM shows lower percentage of inhibition that is 7.97%. In higher dose, toxicity may be show or receptor saturation occurs. So lower dose (250 mg/kg)) is an effective dose. The methanol extract of *Helicteres isora* shows very highly significant at 250mg/kg dose where Diclofenac sodium shows highly significant activity at 100 mg/kg dose. But in case of HIM of 500 mg/kg dose, it shows no significant activity.

## 4. Future Suggestions

Medicinal plants available in nature possess different therapeutic activity that is highly potential for clinical use [22]. The experiments carried out in this study are all based on crude extract and are regarded as preliminary; further investigation is required to draw firm conclusions regarding the study's findings. It is necessary to set up a thorough phytochemical inquiry that could result in the identification and isolation of the chemical components

found in the crude extracts. To prove that a certain chemical ingredient is the cause of a given biological activity, separated phytoconstituents must then undergo all of the current pharmacological testing in addition to a few more sophisticated in vitro and in vivo studies. In order to ultimately identify novel lead compounds, more chemical and pharmacological research using *Helicteres isora* Linn. extract may be necessary in order to isolate new bioactive chemicals and assess their precise mechanism of action and chronic toxicity profile. To fully understand the precise mechanism of action of these compounds—which would enable their development as pharmaceuticals or chemical leads—more research is necessary. In fact, plant-derived chemicals have uses beyond just being pharmaceuticals or drug templates; they also aid in the identification and discovery of intricate and unusual biochemical pathways and targets related to the ailment.

## 5. Summary

From preliminary phytochemical screening we noticed the presence of different phytochemical constituents including the Alkaloids, glycoside, flavonoids, carbohydrates, tannins, fats & fixed oils. Where Alkaloids and fats & oils were moderately present. But steroids, saponins and Triterpenoids were absent in methanolic extract of *Helicteres isora* stem. Antioxidant potential was evaluated by using Total phenol content, Total flavonoid content, Total antioxidant capacity determination, DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay and reducing power capacity assessment assays. The Methanol extract of the stem of *Helicteres isora* showed remarkable potency in phenolic content determination assay. In DPPH assay the methanolic extract of stem of *Helicteres isora* showed the scavenging activity that was IC50 value 137.79 µg/ml whereas ascorbic acid was found to exhibit highly significant IC50 value of 24.83 µg/ml. Analgesic potential of the methanolic extract of stem of *Helicteres isora* was evaluated using acetic acid induced writhing and formalin induced paw licking test in mice. In case of formalin induced paw licking test, the methanol extract of *Helicteres isora* stem (250 mg/kg dose) shows moderate percentage of inhibition that is 32.7% with value $P<0.05$ which shows significant activity. Where Diclofenac sodium (100 mg/kg dose) shows highest percentage of inhibition. In case of acetic acid induced writhing test, the methanol extract of *Helicteres isora* stem (250 mg/kg dose) shows higher percentage of inhibition.

**Declarations**

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The study has not received any funds from any organization.

**Competing Interests Statement**

The authors have declared no competing interests.

**Consent for Publication**

The authors declare that they consented to the publication of this study.

**Authors' Contributions**

All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data.

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