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**Original Research Article**

# Phytochemical Estimation and *In Vitro* Antihyperglycemic and Antioxidant Study of Leaf Extract of *Wrightia Tinctoria* (Roxb) R.BR

Vijaya Baskaran M[1*], Muthupriya S[1], Manobala S[1], Hemanathi M[1], Ragul Raj T[1]

Department of Pharmaceutical Chemistry,J. K. K. Nattraja college of pharmacy, Kumarapalayam-638613, Tamil Nadu, India, Affiliated to The Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India.

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***Corresponding author:** Vijaya Baskaran M
Department of Pharmaceutical Chemistry,J. K. K. Nattraja college of pharmacy, Kumarapalayam-638613, Tamil Nadu, India, Affiliated to The Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India.

## Abstract

***Background*:** Medicinal plants have been widely used in traditional medicine for managing chronic diseases. Wrightia tinctoria (Roxb.) R. Br., traditionally utilized in Ayurveda and Siddha medicine, has been reported to possess antihyperglycemic and antioxidant properties. Given the increasing prevalence of diabetes and oxidative stress-related disorders, exploring plant-based therapeutic alternatives is crucial. ***Aim*:** This study aims to evaluate the phytochemical constituents, in vitro antihyperglycemic, and antioxidant activities of Wrightia tinctoria leaf extract using advanced analytical techniques. ***Materials and Methods*:** The hydroalcoholic extract of Wrightia tinctoria leaves was prepared and subjected to qualitative and quantitative phytochemical analysis using Thin thin-layer chromatography (TLC), UV-visible spectroscopy, and High-Performance Liquid Chromatography (HPLC). The antihyperglycemic activity was assessed using an α-amylase inhibitory assay, while the antioxidant potential was evaluated through DPPH, ABTS, and FRAP assays. ***Results and Discussion*:** Phytochemical screening confirmed the presence of flavonoids, phenolic acids, tannins, alkaloids, and glycosides. HPLC analysis identified gallic acid, quercetin, and rutin as major bioactive compounds. The extract exhibited significant α-amylase inhibition, indicating its antihyperglycemic potential. Additionally, strong free radical scavenging activity was observed, suggesting its role in oxidative stress management. ***Conclusion*:** The findings validate the therapeutic potential of Wrightia tinctoria in diabetes management and oxidative stress reduction, supporting its traditional use in herbal medicine. Future in vivo and clinical studies are recommended to further explore its pharmacological applications.

**Keywords:** Wrightia Tinctoria, Phytochemical Analysis, Antihyperglycemic, Antioxidant, HPLC, TLC, Diabetes Management.



## 1. INTRODUCTION

### Importance of Medicinal Plants in Healthcare

### 1.1 Role of Nature in Human Life

Nature has been a part of human life from the emergence of human civilization. It has achieved the essential cycle of food, shelter, and medicine to be harnessed by humans in some way or another. Among them, plants have played an important role in the development of healthcare systems across all cultures and traditions [1].

Humans used plants for therapeutic purposes long before written records were available. Medicinal plants date back 60,000 years. Archaeological findings in Shanidar caves, Iraq, prove the use of plants such as *Achillea millefolium* and *Ephedra sinica* for medicinal purposes [2]. Other ancient civilizations, including the Egyptians, Greeks, Chinese, and Indians, also recorded the use of plant-based medicine to treat various diseases. For example, a 3,500-year-old Egyptian document called the Ebers Papyrus details hundreds of medicinal plants used to treat ailments like inflammation and infections [3].

In today's world, plant medicines remain an essential source of medicines. This is beyond the direct medical benefits that plants provide but also due to the chemical diversity of compounds that plants produce to work as templates for the discovery of drugs. In developed countries, more than 25% of the prescribed medicines come from plants, thus emphasizing the significance of plants in healthcare [4].

### 1.2 Global Importance of Medicinal Plants

Medicinal plants continue to play a core role in healthcare, particularly within developing countries,

where 80% of the population depends on traditional medicine as their main source of healthcare [5]. The economic, cultural, and therapeutic availability of plant-derived drugs makes them very essential, especially within unprivileged settings characterized by a lack of pharmaceuticals.

**Usage statistics:**

- In China, it is estimated that traditional medicines contribute to about 40% of total medicinal consumption, with more than 13,000 herbal formulations registered [6].
- India, rich in heritage of Ayurveda, Siddha, and Unani systems, has about 8,000 plant species used in traditional healthcare practices [7].
- The global herbal medicine market size was estimated at USD 70.57 billion in 2023 and is projected to grow at a CAGR of 20.91% from 2024 to 2030 [8].

**Contributions to Healthcare Systems:**

The incorporation of medicinal plants in modern healthcare has greatly improved the management of chronic diseases. For instance, the use of *Artemisia annua* in developing artemisinin-based antimalarial drugs has significantly reduced mortality rates in malaria- endemic regions [9]. *Catharanthus roseus* (Madagascar periwinkle) has also provided vinblastine and vincristine, which are vital in treating Hodgkin's lymphoma and childhood leukemia. These examples emphasize the therapeutic potential of medicinal plants in addressing global health challenges [10].

### 1.3 Medicinal Plants: The Reservoir for New Drug Discovery

Medicinal plants have long inspired modern pharmacology. Secondary metabolites of these plants provide the foundation for new drug discoveries. The history of plant-derived drug discovery starts with the establishment of morphine from *Papaver somniferum* (opium poppy), first isolated by Friedrich Sertürner in 1804. Then, quinine was developed from the *Cinchona* bark and drastically changed the prognosis in the treatment of malaria. Aspirin, isolated from *Salix* species (willow bark), is another classic drug discovered from plant material that gained wide acceptance worldwide [11]

**Traditional Systems of Medicine History of Traditional Medicine**

Traditional medicine originated thousands of years ago from ancient people who relied heavily on plant- based medicines as a core part of healthcare. Many of these systems are so rooted in cultural and spiritual thought that they have become integral components of modern medical systems [12].

- **Ancient Mesopotamia:** Clay tablets from 2600 BCE detail the use of plants like *Commiphora* species (myrrh), *Cedrus* species (cedarwood), and *Papaver somniferum* (opium poppy) in the treatment of infections, wounds, and pain relief [13].
- **Ancient Egypt:** The Ebers Papyrus, dating back to around 1500 BCE, is one of the oldest known medical texts, including over 700 plant-based treatments such as *Aloe vera* for wound healing and *Allium sativum* (garlic) for respiratory diseases [14].
- **Ancient India:** India's Ayurvedic system, dating back to 3000 BCE, describes the use of plants like *Emblica officinalis* (Indian gooseberry) and *Terminalia chebula* for longevity and disease prevention.

**Traditional Usage of *Wrightia tinctoria* and Recent Research Traditional Uses of *Wrightia tinctoria***

*Wrightia tinctoria*, commonly known as the Pala indigo plant, has been widely used in traditional medicine systems such as Ayurveda and Siddha. The plant is known for its diverse medicinal properties, including:

- **Skin Disorders:** Used for treating psoriasis, eczema, and other dermatological conditions.
- **Anti-inflammatory Properties:** Used in treating joint pain and inflammatory conditions.
- **Digestive Health:** Employed in traditional medicine for managing diarrhea and dysentery.
- **Hair Care:** Traditionally used in hair oils to promote scalp health and reduce dandruff.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material Collection and Authentication

Leaves of Wrightia tinctoria (Roxb.) R.Br. were collected from Vatamalai, Komarapalayam, Namakkal District, Tamil Nadu, India. Authentication of the plant material was conducted by Dr. P. Radha, Research Officer (Botany), Siddha Medicinal Plants Garden, Central Council for Research in Siddha, Ministry of Ayush, Government of India, Mettur, Tamil Nadu. The leaves were thoroughly washed, shade-dried, and finely powdered for subsequent analysis

### 2.2 Preparation of Hydroalcoholic Extract

The dried leaf powder was subjected to defatting with petroleum ether (60–80°C), followed by extraction with a hydroalcoholic solvent (70% ethanol:30% water) using the maceration technique. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to yield a dark brown residue, which was stored at 4°C for further experimental studies

### 2.3 Phytochemical Screening [15]

A qualitative phytochemical investigation was performed using standard analytical procedures including:

- Alkaloids: Mayer's, Dragendorff's, Wagner's, and Hager's tests
- Carbohydrates: Molisch's, Fehling's, and Benedict's tests

- Tannins and Phenolics: Ferric chloride and Folin-Ciocalteu reagent tests
- Flavonoids: Shinoda's, Alkali, and Lead acetate tests
- Proteins and Amino Acids: Biuret, Ninhydrin, and Millon's tests
- Steroids and Terpenoids: Salkowski and Liebermann-Burchard tests
- Glycosides: Borntrager's test

### 2.4 Chromatographic Analysis

#### 2.4.1 Thin-Layer Chromatography (TLC)

TLC was performed on pre-coated silica gel plates, utilizing ethyl acetate:benzene (9:11) as the mobile phase. The developed chromatogram was visualized under UV light at 254 nm and 365 nm, and Rf values were determined [16].

#### 2.4.2 QUANTITATIVE ESTIMATION OF PHYTO CONSTITUENTS

**A. DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAEWT)[17]**

The total phenolic content of HAEWT was assessed using the Folin-Ciocalteu method, where phenolics reduce phosphomolybdic acid to form a blue complex measured at 760 nm. A calibration curve was prepared using gallic acid (5–25 µg/mL), and results were expressed as mg GAE/g of extract. The assay involved reacting HAEWT with Folin-Ciocalteu reagent and sodium carbonate, followed by spectrophotometric analysis. A UV-Visible spectrophotometer (Shimadzu 1800) was used for measurements. The reaction mixture without the sample served as the blank.

**B. DETERMINATION OF RUTIN (FLAVONOID) EQUIVALENT IN (HAEWT)[18]**

Flavonoid content in HAEWT was determined using an aluminum chloride assay, where flavonoids form a pink complex with aluminum and potassium acetate in an alkaline medium, measured at 415 nm. A UV-Visible spectrophotometer (Shimadzu 1800) was used for analysis. The reaction involved mixing HAEWT extract with aluminum chloride, potassium acetate, and ethanol, followed by incubation for 20 minutes. Standard rutin was used for calibration. The absorbance was recorded, and results were expressed as rutin equivalent per gram of extract.

#### 2.3.4 High-Performance Liquid Chromatography (HPLC)

For the quantification of phenolic and flavonoid constituents, HPLC analysis was carried out using a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm) with acetonitrile and 0.1% orthophosphoric acid as the mobile phase at a flow rate of 1 mL/min. The detection was performed at 280 nm, using gallic acid, rutin, and quercetin as standard calibration compounds [19].

#### 2.4.4 PHARMACOLOGICAL STUDIES

Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. That leads to prolonged hyperglycemia with disturbances in most metabolic processes inside the human body [20]. Untreated cases show severe tissue and vascular damage leading to serious complications such as retinopathy [21], neuropathy [22], cardiovascular complications [23], nephropathy [24], and ulceration [25].

### *2.5 In Vitro* Antihyperglycemic Activity

#### 2.5.1 α-Amylase Inhibitory Assay

The antihyperglycemic activity of the extract was evaluated through the α-amylase inhibition assay. A reaction mixture containing 1% starch solution, phosphate buffer (pH 6.9), α-amylase enzyme (0.5 mg/mL), and varying extract concentrations (10–100 µg/mL) was incubated at 37°C for 10 minutes. The reaction was terminated by the addition of DNS reagent, and absorbance was measured at 540 nm.

Acarbose was used as the reference standard [27].

### *2.6 In Vitro* Antioxidant Activity

#### 2.6.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of the extract was determined using the DPPH assay. A methanolic DPPH solution (0.1 mM, 1 mL) was mixed with different extract concentrations and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm, and scavenging activity was calculated as:

**% inhibition = [(Control–Test) / Control] × 100**

where A control is the absorbance of DPPH without the extract, and A sample is the absorbance in the presence of the extract [28].

#### 2.6.2 ABTS Radical Scavenging Assay

ABTS radical cation decolorization assay was conducted by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing it to incubate in the dark for 12 hours. The solution was diluted to achieve an absorbance of 0.7 at 734 nm, following which extract samples were introduced.

Absorbance was recorded at 734 nm after 30 minutes [29].

#### 2.6.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent was prepared by combining acetate buffer (pH 3.6), TPTZ (10 mM), and $FeCl_3$ (20 mM). The extract was incubated for 30 minutes, and absorbance was measured at 593 nm to evaluate its ferric reducing potential [30].

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of significant bioactive constituents such as flavonoids, alkaloids, tannins, sterols, triterpenoids, glycosides, and carbohydrates. The absence of proteins and saponins indicates that the pharmacological activity is primarily attributed to polyphenolic compounds and alkaloids.

**Table 1: Preliminary phytochemical screening of hydro-alcoholic extract of *Wrightia tinctoria***

| S.No | Name of the Test | Haewt |
|---|---|---|
| 1 | Test for carbohydrate | |
| | A) Fehling's test | Positive |
| | B) Molisch test | Positive |
| | C) Benedict's Test | Positive |
| 2 | Test for proteins | |
| | A) Biuret test | Negative |
| 3 | Test for Flavonoids | |
| | A) Ferric chloride test | Positive |
| | B) Shinoda test | Positive |
| | C) Alkaline reagent test | Positive |
| 4 | Test for alkaloids | |
| | A) Mayer's test | Positive |
| | B) Wagner's test | Positive |
| | C) Dragendorff's test | Positive |
| 5 | Test for saponins | |
| | A) Foam test | Negative |
| 6 | Test for sterols | |
| | A) Salkowaski test | Positive |
| | B) Libbermann Burchard test | Positive |
| 7 | Test for triterpenoids | |
| | A) Salkowaski test | Positive |
| | B) Liebermann Burchard test | Positive |
| 8 | Test for Glycosides | |
| | A) Borntragers test | Negative |
| 9 | Test for Tannins | |
| | A) Ferric-chloride test | Positive |

The hydroalcoholic extract of *Wrightia tinctoria* (HAEWT) tested positive for carbohydrates, flavonoids, alkaloids, sterols, triterpenoids, and tannins, while proteins, saponins, and glycosides were absent.

### 3.2 Chromatographic Analysis

TLC and HPLC analyses identified major phytochemicals, including flavonoids and phenolic acids. HPLC confirmed the presence of gallic acid, rutin, and quercetin, known for their antioxidant and antihyperglycemic activities. The Rf values in TLC were consistent with standard references, further validating the presence of these bioactive compounds.

#### 3.2.1 THIN LAYER CHROMATOGRAPHY PROFILE

The TLC analysis of the hydroalcoholic extract of *Wrightia tinctoria* identified phenolic compounds with Rf values of 0.72, 0.73, and 0.96 when using a solvent system of ethyl acetate:benzene (9:11), which was confirmed by the blue coloration with Folin reagent. In the second system (ethyl acetate:formic acid:acetic acid:water, 10:11:11:26), the extract showed Rf values of 0.42 (Rutin standard) and 0.45, indicating the presence of flavonoids, as evidenced by an orange band under UV ligh.

**Table 2: TLC of The Hydroalcoholic Extract of *Wrightia tinctoria***

| S. No | Mobile phase | Rf value | | Detection | Colour of spot | Report |
|---|---|---|---|---|---|---|
| | | Standard | HAEWT | | | |
| 1 | Ethyl acetate: Benzene (9:11) | Gallic acid 0.72 | HAEWT 0.73 | Folin reagent | Blue colour | May indicate the presence of Phenolic compound |
| 2 | Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:11:11:26) | Rutin 0.42 | HAEWT 0.45 | isible light | Orange colour | May indicate the presence of Flavonoids. |

TLC analysis of *Wrightia tinctoria* (HAEWT) confirmed the presence of phenolic compounds and flavonoids with Rf values of 0.73 and 0.45, matching gallic acid and rutin standards.

### 3.2.2 Determination of Gallic Acid Equivalent in HAEWT

The hydro-alcoholic extract of *Wrightia tinctoria* (HAEWT) contained 254 mg/g of gallic acid equivalent (GAE), indicating that it had a high concentration of phenolic compounds, which were essential for antioxidant and antimicrobial activities.

**Table 3: Determination of Gallic Acid Equivalent in HAEWT**

| S. No | Concentration | | Absorbance | Absorbance |
|---|---|---|---|---|
| | Gallic Acid (µg/ml) | HAEWT (µg/ml) | Gallic Acid | HAEWT |
| 1 | 10 | 10 | 0.0578 | 0.0602 |
| 2 | 20 | 20 | 0.094 | 0.0690 |
| 3 | 30 | 30 | 0.1675 | 0.0980 |
| 4 | 40 | 40 | 0.1753 | 0.105 |
| 5 | 50 | 50 | 0.3023 | 0.122 |

The table shows the absorbance of Gallic Acid and HAEWT at concentrations from 10 to 50 µg/mL. Gallic Acid absorbance increased with concentration, while HAEWT absorbance increased with increased in concentration.

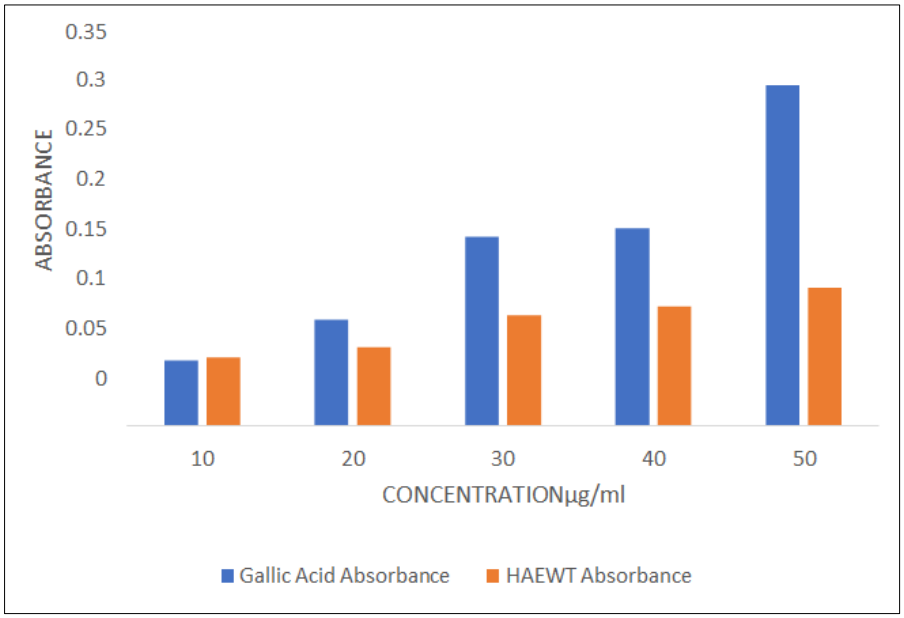


**Figure 1: Graph of Concentartion Vs Absorbance**

### 3.2.3 Determination of Rutin Equivalent in HAEWT

Hydroalcoholic extract of *Wrightia tinctoria* (Roxb.) was found to contain 399mg/g of RE and the standard curve of rutin.

**Table 4: Determination of Rutin Equivalent in HAEWT**

| S. No | Concentration | | Absorbance | Absorbance |
|---|---|---|---|---|
| | Gallic Acid (µg/Ml) | HAEWT (µg/Ml) | Rutin | HAEWT |
| 1 | 10 | 10 | 0.096 | 0.323 |
| 2 | 20 | 20 | 0.166 | 0.0730 |
| 3 | 30 | 30 | 0.249 | 0.115 |
| 4 | 40 | 40 | 0.326 | 0.185 |
| 5 | 50 | 50 | 0.399 | 0.210 |

The table presents the absorbance of Rutin and HAEWT at concentrations from 10 to 50 µg/mL. Rutin's absorbance increased with concentration, while HAEWT showed absorbance increased with increased in concentration.

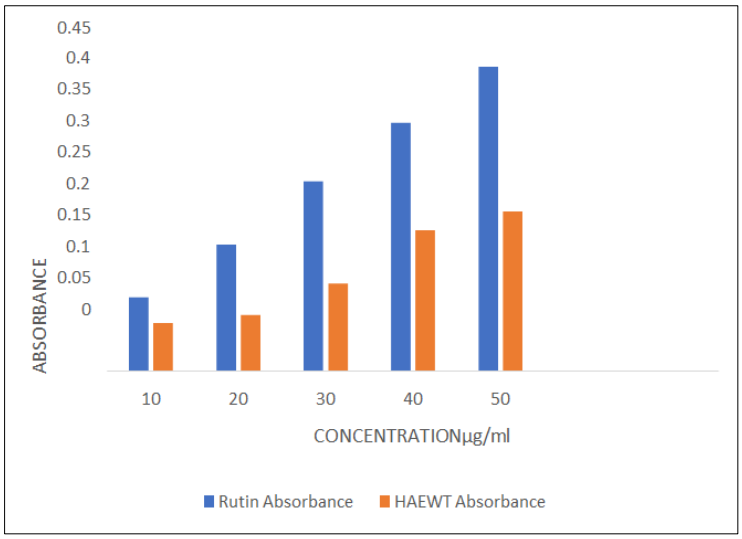


**Figure 2: Graph of Concentartion Vs Absorbance**

### 3.2.4. High-Performance Liquid Chromatography (HPLC) Analysis of *Wrightia tinctoria* (Roxb.) R. BR.

Gallic acid (254.6 µg/ml) was the key phenolic compound, while quercetin (303.4 µg/ml) was the most abundant flavonoid. Rutin, kaempferol, and unidentified bioactive molecules (34.5% peak area) enhanced the extract's profile. The findings highlight *Wrightia tinctoria*'s potential in managing oxidative stress and inflammation, reinforcing its medicinal and pharmacological value.

**Table 5: HPLC Data of *Wrightia tinctoria* (Roxb.) R. BR**

| Derivative | Retention Time (Min) | Peak Area (% Total) | Concentration (µg/Ml) |
|---|---|---|---|
| Gallic Acid | 3.45 | 15.2 | 254.6 |
| Catechin | 4.12 | 8.7 | 146.2 |
| Rutin | 6.32 | 12.5 | 207.3 |
| Quercetin | 8.78 | 18.3 | 303.4 |
| Kaempferol | 9.65 | 10.8 | 178.1 |
| Unknown Peaks | - | 34.5 | - |

The chromatographic analysis identified gallic acid, catechin, rutin, quercetin, and kaempferol as key bioactive compounds, with quercetin being the most abundant, while unidentified peaks accounted for 34.5% of the total peak area, indicating additional bioactive constituents.

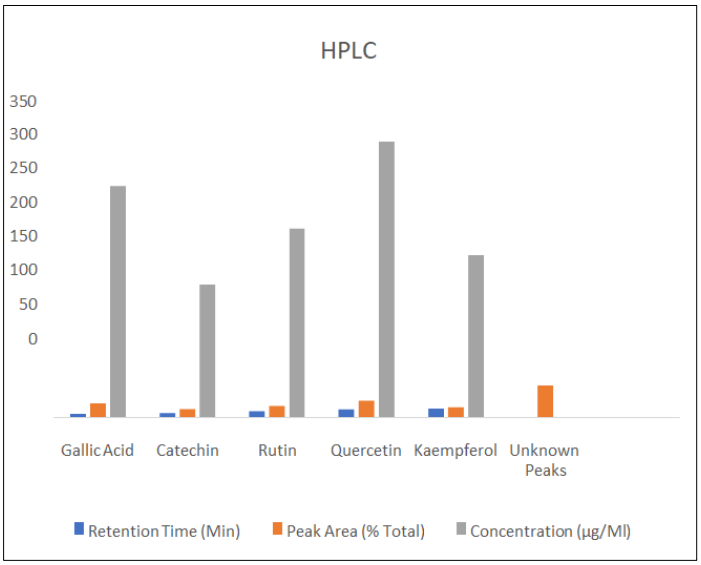


**Figure 3: Hplc Analysis of Hydroalcoholic Extract**

### 3.3 Antihyperglycemic Activity

#### *3.3.1 IN VITRO* Alpha Amylase Inhibitory Assay of HAEWT

The α-amylase inhibition assay demonstrated dose-dependent inhibitory activity, suggesting the extract's potential in diabetes management. Compared to standard acarbose, the plant extract exhibited moderate inhibition, indicating its potential as a natural alternative for controlling postprandial glucose levels. The inhibition values suggest that flavonoids and tannins play a crucial role in enzyme inhibition.

**Table 6: Determination of Alpha Amylase Inhibitory Assay of HAEWT**

| S.NO | CONCENTRATION | | PERCENTAGE INHIBITION OF Acarbose(µg/ml) | PERCENTAGE INHIBITION OF *Wrightia tinctoria* (µg/ml) |
|---|---|---|---|---|
| | Acarbose (µg/ml) | *Wrightia tinctoria* (µg/ml) | | |
| 1 | 10 | 10 | 40.33 | 13.94 |
| 2 | 25 | 25 | 56.20 | 28.09 |
| 3 | 50 | 50 | 69.07 | 54.30 |
| $IC_{50}$ | | | 12.18 µg/ml | 16.41 µg/ml |

***IC₅₀* FORMULA**:

$$IC_{50} = C_1 + \left(\frac{50 - I_1}{I_2 - I_1}\right) \times (C_2 - C_1)$$

**Where:**

$C_1$ = Lower concentration where inhibition is just below 50% $C_2$ = Higher concentration where inhibition is just above 50 $I_1$ = Percentage inhibition at $C_1$

$I_2$ = Percentage inhibition at $C_2$

**Percentage Inhibition Fromula**:

$$\text{Percentage Inhibition}(\%) = \frac{(\text{Control Absorbance} - \text{Sample Absorbance}) \times 100}{\text{Absorbance}}$$

**Where:**

**Control Absorbance** = Absorbance of the blank or control (without inhibitor)

**Sample Absorbance** = Absorbance of the test sample (Acarbose or Wrightia tinctoria)

The IC50 values indicate that *Wrightia tinctoria* (16.41 µg/ml) exhibits inhibitory activity against αamylase, though less potent than acarbose (12.18 µg/ml), suggesting its potential as a natural antidiabetic agent.

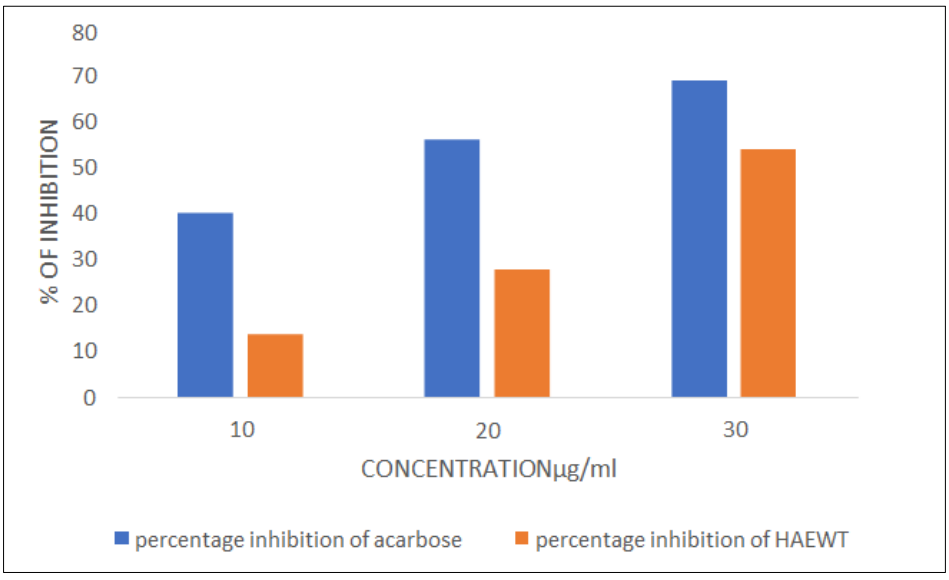


**Figure 4: Graph of Concentartion Vs Absorbance**

### 3.4 Antioxidant Activity

The antioxidant assays (DPPH, ABTS, and FRAP) revealed significant free radical scavenging potential. The IC50 value for DPPH inhibition was comparable to ascorbic acid, indicating strong antioxidant capacity. The FRAP assay results demonstrated a substantial reduction potential, further supporting its role in oxidative stress management.

#### *3.4.1 IN VITRO* ANTIOXIDANT STUDIES

**A) Determination of Hydrogen Peroxide Scavenging activity of HAEWT Table 7:** Determination of Hydrogen Peroxide Scavenging activity of HAEWT

| S.NO | CONCENTRATION | | Percentage Inhibition of Ascorbic Acid | Percentage Inhibition of *Wrightia Tinctoria* |
|---|---|---|---|---|
| | Ascorbic acid (µg/ml)) | *Wrightia tinctoria* (µg/ml) | | |
| 1 | 10 | 10 | 8.84 | 4.14 |
| 2 | 25 | 25 | 18.24 | 15.13 |
| 3 | 50 | 50 | 32.66 | 27.33 |
| 4 | 75 | 75 | 50.67 | 42.47 |
| 5 | 100 | 100 | 69.32 | 56.73 |
| IC50 | | | 73.08 µg/ml | 88.36 µg/ml |

The IC50 values reveal that *Wrightia tinctoria* (88.36 µg/ml) exhibits antioxidant activity, though less effective than ascorbic acid (73.08 µg/ml), highlighting its potential as a natural antioxidant source.

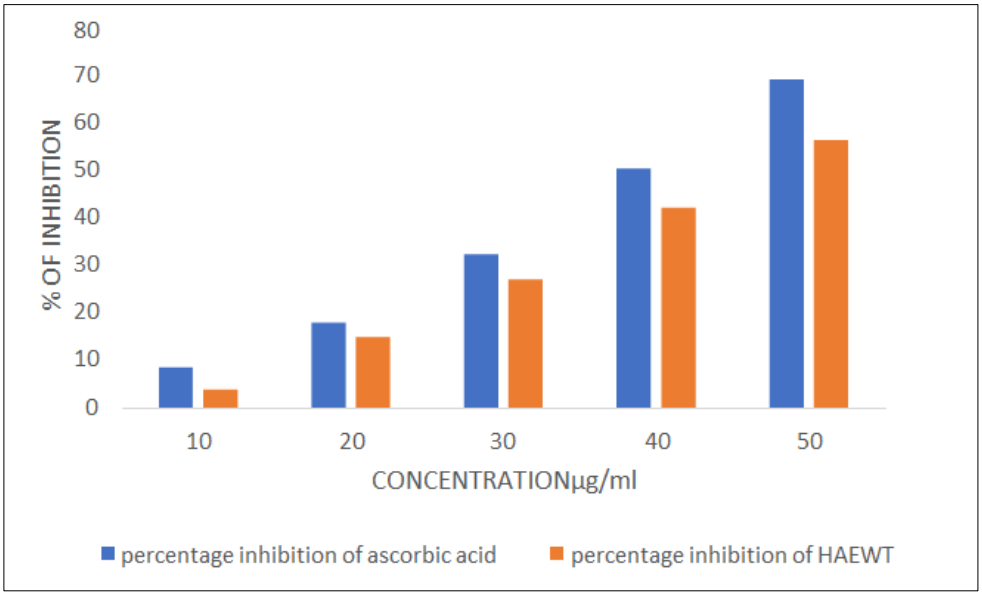


**Figure 5: Graph of Concentartion Vs % Of Inhibition**

**B) Determination of reducing power assay of *Wrightia tinctoria (Roxb)***

**Table 8: Determination of reducing power assay of HAEWT**

| S.NO | CONCENTRATION | | Percentage Inhibition Of Ascorbic Acid | Percentage Inhibition Of *Wrightia Tinctoria* |
|---|---|---|---|---|
| | Ascorbic acid (µg/ml)) | *Wrightia tinctoria* (µg/ml) | | |
| 1 | 10 | 10 | 30.94 | 22.07 |
| 2 | 20 | 20 | 55.1 | 35.79 |
| 3 | 30 | 30 | 58.3 | 49.14 |
| $IC_{50}$ | | | 17.73 µg/ml | 29.43 µg/ml |

The IC50 values indicate that *Wrightia tinctoria* (29.43 µg/ml) possesses antioxidant activity, though it is less potent than ascorbic acid (17.73 µg/ml), demonstrating its potential as a natural antioxidant.

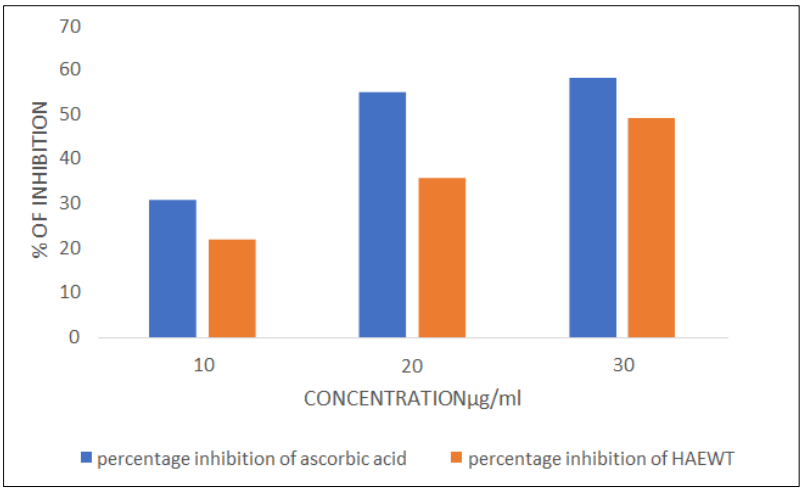


**Figure 6: Graph of Concentartion Vs % of Inhibition**

**C). Determination of total antioxidant activity of *Wrightia tinctoria (Roxb) (HAEWT)***

**Table 9: Determination of total Antioxidant Capacity of HAEWT**

| S.NO | CONCENTRATION | | Percentage Inhibition Of Ascorbic Acid | Percentage Inhibition Of *Wrightia Tinctoria* |
|---|---|---|---|---|
| | Ascorbic acid (µg/ml)) | *Wrightia tinctoria* (µg/ml) | | |
| 1 | 10 | 10 | 28.2 | 20.5 |
| 2 | 25 | 25 | 46.5 | 38.7 |
| 3 | 50 | 50 | 63.4 | 55.3 |
| 4 | 75 | 75 | 78.2 | 69.1 |
| 5 | 100 | 100 | 89.5 | 80.7 |
| $IC_{50}$ | | | 32.7 µg/ml | 41.2 µg/ml |

The IC50 values show that *Wrightia tinctoria* (41.2 µg/ml) exhibits antioxidant activity, though less effective than ascorbic acid (32.7 µg/ml), indicating its potential as a natural antioxidant source.

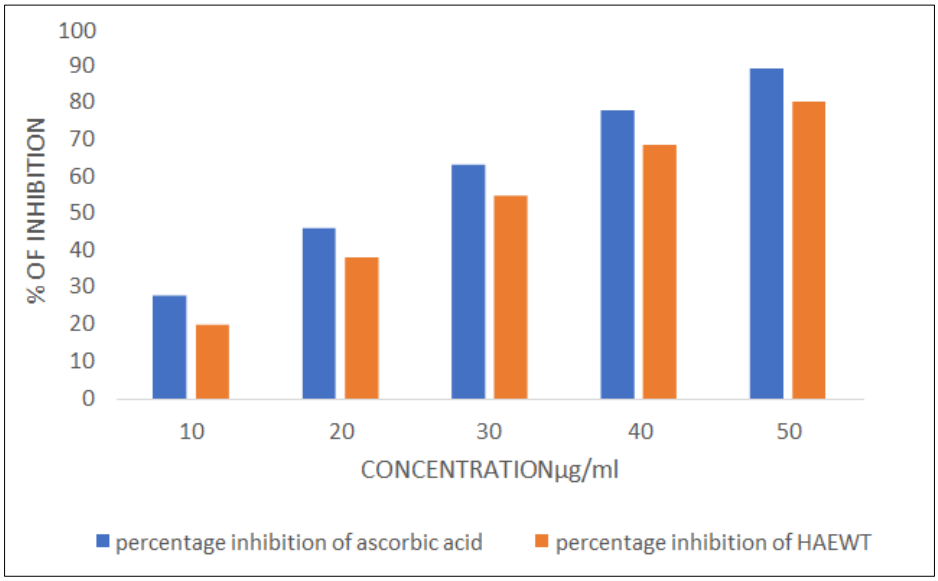


**Figure 7: Graph of Concentartion Vs % of Inhibition**

## 4. DISCUSSION

The results of this study align with previous findings on the pharmacological properties of *Wrightia tinctoria*. The presence of flavonoids and phenolic acids correlates with its strong antioxidant and antihyperglycemic effects. These findings justify the traditional use of this plant in herbal medicine and provide a scientific basis for its potential application in diabetes and oxidative stress-related disorders.

Furthermore, hydroalcoholic extraction proves to be a superior method compared to other solvents such as chloroform, petroleum ether, ethyl acetate, and pure alcohol. This is due to several key factors:

1. Higher Yield of Bioactive Compounds – Hydroalcoholic solvents efficiently extract both polar (phenolic acids, flavonoids) and moderately non-polar (alkaloids, glycosides) compounds, ensuring a higher extraction yield compared to non-polar solvents like petroleum ether or chloroform.
2. Environmentally Friendly and Safe – Unlike toxic organic solvents (e.g., chloroform, petroleum ether), hydroalcoholic extraction is eco-friendly, biodegradable, and safe for human use, making it preferable for pharmaceutical and nutraceutical applications.
3. Reduced Side Effects – Residual organic solvents can introduce toxicity in extracts, whereas hydroalcoholic extraction minimizes chemical contamination, ensuring a safer phytochemical profile for therapeutic applications.

Thus, the hydroalcoholic extraction of *Wrightia tinctoria* not only enhances the extraction efficiency of pharmacologically active compounds but also aligns with sustainable and safer extraction practices.

This further supports its potential therapeutic role in managing diabetes and oxidative stress-related conditions.

## 5. CONCLUSION

The present study successfully explored the phytochemical composition and pharmacological properties of *Wrightia tinctoria* leaf extracts. Various analytical and experimental approaches confirmed the presence of key bioactive compounds contributing to its medicinal potential.

Phytochemical screening using Thin Layer Chromatography (TLC), UV-Visible Spectroscopy, and High-Performance Liquid Chromatography (HPLC) identified essential secondary metabolites such as flavonoids, phenolic acids, alkaloids, tannins, and glycosides. Notably, HPLC analysis revealed the presence of significant bioactive compounds, including gallic acid, quercetin, rutin, and kaempferol, which have been associated with diverse pharmacological activities.

The antioxidant potential of *Wrightia tinctoria* was evaluated using DPPH radical scavenging, reducing power, and total antioxidant capacity assays. The results demonstrated a strong free radical scavenging capacity, reinforcing its role in oxidative stress management. Additionally, the α-amylase inhibitory assay provided evidence of its antihyperglycemic activity, suggesting its possible application in diabetes management.

Overall, this research supports the traditional medicinal use of *Wrightia tinctoria* and provides a scientific foundation for its pharmacological applications. However, further studies, including in vivo and clinical trials, are necessary to validate its therapeutic potential and establish its efficacy in metabolic disorders and oxidative stress-related diseases.

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