# Physicochemical and Phytochemical Screening of *Trema orientalis* Leaves: In Vitro Assessment of Anti-Oxidant, Anti-Diabetic, and Anti-Inflammatory Properties

Submitted by

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# CERTIFICATE OF APPROVAL

This is to certify that the research work embodied in this thesis entitled—**"Physicochemical and Phytochemical Screening of *Trema orientalis* Leaves: In Vitro Assessment of Anti-Oxidant, Anti-Diabetic, and Anti-Inflammatory Properties"** was carried out by **Gulsana Yesmin**, (Exam Roll No. M4PHP24002, Registration No.163681 of 2022-2023) for the partial fulfillment of **Degree of Master of Pharmacy**, Jadavpur University, is absolutely based upon her own research project work under my supervision, in the Pharmacognosy & Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032.

She has incorporated her findings into this thesis. Her thesis has not been submitted before for any degree/diploma or any other academic award elsewhere. I am satisfied that she has carried out her thesis with proper care and confidence to my satisfaction.

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# DECLARATION

I hereby declare that the thesis contain literature survey and the original research work entitled- — **"Physicochemical and Phytochemical Screening of *Trema orientalis* Leaves: In Vitro Assessment of Anti-Oxidant, Anti-Diabetic, and Anti-Inflammatory Properties"** is carried out by me under the guidance of Prof. (Dr.) Subhash C. Mandal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032.The work is original and has not been submitted in part or in any form to any other university for the award of any Degree or Diploma or Fellowship. The results reported here are the findings of work carried out by me.

I also declare that, as required by the rules and conduct, I have fully cited and referenced all the materials and results that are not original to this work.

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Gulsana Yesmin

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# List of Abbreviations

| Sl No. | Abbreviation | Full Form |
|---|---|---|
| 1 | % | Percentage |
| 2 | µg | Microgram |
| 3 | ml | Microlitre |
| 4 | g | Gram |
| 5 | °C | Degree Celsius |
| 6 | Fig. | Figure |
| 7 | No. | Number |
| 8 | Sl.No. | Serial number |
| 9 | Abs. | Absorbance |
| 10 | ROS | Reactive oxygen species |
| 11 | RNS | Reactive nitrogen species |
| 12 | cm | Centimeter(s) |
| 13 | DPPH | 2,2-diphenyl-1-picryl hydrazyl |
| 14 | GAE | Gallic acid equivalent |
| 15 | $IC_{50}$ | Half maximal inhibitory concentration |
| 16 | kg | Kilogram |
| 17 | l | Litre(s) |
| 18 | m | Metre(s) |
| 19 | M | Molarity |
| 20 | mg | Milligram(s) |
| 21 | min | Minute(s) |
| 22 | mM | Millimolar |

| | | |
|---|---|---|
| **23** | no. | Number |
| **24** | QE | Quercetine quivalent |
| **25** | pH | Potential of hydrogen |
| **26** | SD | Standard deviation |
| **27** | SEM | Standard error of mean |
| **28** | UV-Vis | Ultra violet visible |
| **29** | v/v | Volume by volume |
| **30** | NaCl | Sodium Chloride |
| **31** | Conc. | Concentrated |
| **32** | $FeCl_3$ | Ferric chloride |
| **33** | $H_2SO_4$ | Sulfuric acid |
| **34** | DNS | 3,5-Dinitrosalicylic acid |
| **35** | WHO | World Health Organization |
| **36** | nm | Nanometer |
| **37** | µl | Micro liter |
| **38** | i.e. | That is |
| **39** | *et.al* | Et alia ( and the other contributors) |
| **40** | w/w | Weight/weight |
| **41** | TLC | Thin LayerChromatography |
| **42** | & | and |
| **43** | ppt | Precipitate |
| **44** | Etc. | Et cetera (and the other things) |
| **45** | TM | Traditional Medicine |

| | | |
|---|---|---|
| **46** | DM | Diabetes Mellitus |
| **47** | ADA | American Diabetes Association |
| **48** | dl | Decilitre |
| **49** | mmol | Millimolar |
| **50** | HbA1c | Glycated haemoglobin |
| **51** | Hb | Haemoglobin |
| **52** | L. | Linneaus |
| **53** | Linn. | Linnaeus |
| **54** | h | Hour |
| **55** | HCl | Hydrochloric Acid |
| **56** | Na | Sodium |
| **57** | NaOH | Sodium Hydroxide |
| **58** | FCR | Folin-Ciocalteu reagent |
| **59** | TPC | Total Phenolic Content |
| **60** | TFC | Total Flavonoid Content |
| **61** | w/v | Weight by volume |
| **62** | *T.orientalis* | *Trema.orientalis* |
| **63** | e.g | Exempli gratia (for example) |
| **64** | $R_f$ | Retention factor |
| **65** | α | alpha |
| **66** | b.w | Body Weight |
| **67** | OH | Hydroxyl |
| **68** | STD | Standard |
| **69** | EDTA | Ethylenediamine tetraacetic acid |

## 1. INTRODUCTION

For thousands of years, people have used medicinal and aromatic plants, particularly those with ethnopharmacological applications, as a natural source of healing and healthcare**[1].**The natural environment is a great apothecary of cures for all human diseases. In addition to providing humans with the three fundamental needs of food, clothing, and shelter, plants have given rise to a variety of natural compounds that have the power to cure all illnesses and alleviate human suffering due to our extreme susceptibility to infection and disease**[2].**Plants nowadays are used for the treatment of almost all diseases like gastrointestinal disease, Skin disease, skeletal disease, Bronchitis disease, Cardiovascular disease etc**[3].** Irrespective of the underlying philosophical basis, the use of plants in all major medical systems serves as an example of the universal significance that they serve in the treatment of sickness. Written records indicate that the Sumerians, who made clay tablets with listings of hundreds of medicinal plants, have been associated with starting the study of herbs more than 5,000 years ago. The wealth of information found in the numerous documents from around the globe has given man the understanding of healthcare that he possesses today**[4].**

Various chronic and degenerative diseases, including as atherosclerosis, ischemic heart disease, aging, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases, and others, have been shown to be initiated by oxidative stress**[5].**

A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems**[6].**

Antioxidative defense mechanisms are the most efficient way for inhibiting and reducing the activity of free radicals, which are the source of oxidative stress. Antioxidants are compounds with the ability to break the chain reaction of free radicals.
There has been a recent rise in interest in the medicinal plants' potential as antioxidants in reducing tissue damage brought about by oxidative stress**[7].**

Inflammation is typically characterized by four main symptoms: pain, swelling, heat, and redness. The body's natural reaction to damaging stimuli is inflammation, which is brought on by the flow of plasma and leukocytes into wounded tissues from the circulation. This specific immune response, which is categorized as acute inflammation, is necessary for the body to fight off dangerous microorganisms**[8].**
Leukocytes build up and intensify the reaction during inflammation. Under normal conditions, the immune system uses a number of processes. In order to stop the inflammatory reactions. In order to resolve inflammation and for the return of normal tissue function, pro-inflammatory signaling pathways must be cut off and inflammatory cells must be removed. Chronic inflammation and disease could result from these processes failing. A variety of cell types, including monocytes that locally develop into macrophages, are recruited during the inflammatory process. As a result, the production of pro- and anti-inflammatory mediators is regulated. These mediators include chemokines, inducible enzymes like cyclooxygenase.
(COX)-2, all of which play critical roles in controlling the inflammatory process. For its part, Nuclear Factor (NF)-κB regulate genes involved in many aspects the inflammatory response**[9].**

Diabetes mellitus is a group of metabolic dis orders with one common manifestation ñ hyper glycemia .Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart and blood vessels . It is caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced**[10].**
Two major types of Diabetes Mellitus are there –

Type 1 : Insulin Dependent Diabetes Mellitus (IDDM )/ Juvenile Onset Diabetes Mellitus : There is beta cell destruction in pancreatic islets , majority of cases are autoimmune ( type 1A ) - Antibodies that destroy beta cells are detectable in blood but some are idiopathic ( type 1B ) – No beta cell antibody is found . In all type 1 cases ,circulating immune levels are low or very low, and patients are more prone to ketosis. This type is less common and has a low degree of genetic predisposition.

Type 2:Non Insulin Dependent Diabetes Mellitus( NIDDM ) / Maturity Onset Diabetes Mellitus :There is no loss or only moderate reduction in beta cell mass , insulin in circulation is low , normal or even high, no anti-beta-cell antibody is demonstrable, has a high degree of genetic predisposition , generally has a late onset ( past middle age ). Over 90 % cases of diabetes are type 2 Diabetes Mellitus.

**Table 1. List of Plants Having Anti-oxidant Activity**

| Serial No. | Scientific Name | Family | Common Name | Parts used |
|---|---|---|---|---|
| 1. | *Camellia sinensis* **[11]** | Theaceae | Green Tea | Leaves |
| 2. | *Curcuma longa* **[12]** | Zingiberaceae | Turmeric | Rhizome |
| 3. | *Ginkgo biloba* **[13]** | Ginkgoaceae | Ginkgo | Leaves |
| 5. | Vitis vinifera **[14]** | Vitaceae | Grapes | Seeds,Skin |
| 6. | *Phyllanthus emblica* **[15]** | Phyllanthaceae | Amla | Fruit |
| 7. | *Allium sativum* **[16]** | Amaryllidaceae | Garlic | Bulb |
| 8. | *Vaccinium corymbosum* **[17]** | Ericaceae | Blueberries | Fruit |
| 9. | *Olea europaea* **[18]** | Oleaceae | Olive | Leaves, Fruit |
| 10. | *Moringa oleifera* **[19]** | Moringaceae | Moringa | Leaves, Seeds |

**Table 2. List of Plants Having Anti-inflammatory Activity**

| Serial No. | Scientific Name | Family | Common Name | Parts used |
|---|---|---|---|---|
| 1. | *Curcuma longa* **[20]** | Zingiberaceae | Turmeric | Rhizome |
| 2. | *Zingiber officinale* **[21]** | Zingiberaceae | Ginger | Rhizome |
| 3. | *Salix alba* **[22]** | Salicaceae | Willow Bark | Bark |
| 4. | *Boswellia serrate* **[23]** | Burseraceae | Boswellia | Resin |
| 5. | *Harpagophytum procumbent* **[24]** | Pedaliaceae | Devil's Claw | Root |
| 6. | *Oenothera biennis* **[25]** | Onagraceae | Evening Primrose | Seeds (Oil) |
| 7. | *Aloe barbadensis* **[26]** | Liliaceae | Aloe Vera | Leaves (Gel) |
| 8. | *Matricaria chamomilla* **[27]** | Asteraceae | Chamomile | Flowers |
| 9. | Glycyrrhiza glabra **[28]** | Fabaceae | Licorice | Root |
| 10. | *Tanacetum parthenium* **[29]** | Asteraceae | Feverfew | Leaves |

**Table 3. List of Plants Having Anti-diabetic Activity**

| Serial No. | Scientific Name | Family | Common Name | Parts used |
|---|---|---|---|---|
| 1. | *Momordica charantia* **[30]** | Cucurbitaceae | Bitter Melon | Seeds |
| 2. | *Trigonella foenum-graecum* **[31]** | Fabaceae | Fenugreek | Seeds |
| 3. | *Cinnamomum verum* **[32]** | Lauraceae | Cinnamomum | Bark |
| 4. | *Ocimum sanctum* **[33]** | Lamiaceae | Holy Basil | Leaves |
| 5. | *Gymnema sylvestre* **[34]** | Apocynaceae | Gymnema | Leaves |

| | | | | |
|---|---|---|---|---|
| 6. | *Allium sativum* **[35]** | Liliaceae | Garlic | Bulb |
| 7. | *Pterocarpus marsupium* **[36]** | Leguminosae | Indian Kino Tree | Bark |
| 8. | *Aloe barbadensis* **[37]** | Liliaceae | Aloe vera | Leaves (Gel) |
| 9. | *Syzygium cumini* **[38]** | Myrtaceae | Jamun | Seeds |
| 10. | *Panax ginseng* **[39]** | Araliaceae | Ginseng | Roots |

## 2.1: AIMS AND OBJECTIVES:

Aims and objectives of the study includes- **Pharmacognostic study of the plant *Trema orientalis* , Physico-chemical evaluation, Phytochemical screening of the plant, TLC studies, tincture preparation and characterization, In vitro anti-oxidant activity assay, In vitro anti-inflammatory activity and Evaluation of *in-vitro* anti-diabetic activity of the leaves extract.**

## 2.2: BASIS OF PLANT SELECTION

In West Bengal, this plant has been used to treat Diabetes, Inflammation, and Anti-oxidants for many years. The Ethnomedicinal evidence supports the use of these plant leaves as an anti-diabetic, anti-inflammatory, and anti-oxidant. However,The full extent of the antioxidant, anti-inflammatory, and anti-diabetic properties of *Trema orientalis* extracts in various solvents has not been fully known. Therefore, I chose this plant to evaluate which extract of *Trema orientalis* is more efficacious I conducted a series of systematic experiments comparing different extracts (e.g., petroleum ether, methanol,acetone) for their anti-diabetic, anti-inflammatory, and anti-oxidant activities.

## 2.3: PLAN OF WORK

1. Collection, drying and grinding of leaves
2. Authentication of the plant specimen
3. Pharmacognostic study of the plant *Trema orientalis*
4. Physico-chemical evaluation of the leaves powder
5. Extraction of the plant leaves with suitable solvent
6. Phytochemical screening of the leaves extract
7. TLC studies
8. Preparation and characterization of tincture
9. To carry out *In-vitro* anti-oxidant study
    i. Determination of DPPH radical scavenging activity
    ii. Determination of Hydrogen peroxide radical scavenging activity
    iii. Determination of Total Phenolic content
    iv. Determination of Total Flavonoid content
10. To carry out *In-vitro* anti-diabetic study
    i.α-amylase inhibition assay
11. To carry out *In-vivo* anti-inflammatory study
    i. Human Red Blood Cell (HRBC) Membrane Stabilization Method

**3.1: Synonym**: *Celtis orientalis* L. **[40]**

**3.1.1: Vernacular Name**

English: Charcoal Tree, Indian Charcoal Tree, Gunpowder Tree

Bengali: Jibon, Nalita, Champa Gach

Assamese: Jiban

Tamil: Eriporul Maram

Hindi: Chatai, Akadu , Charcoal ka Ped **[41]**

## 3.2: TAXONOMICAL CLASSIFICATION

**Domain**: Eukaryota

**Kingdom**: Plantae

**Phylum**: Angiosperms

**Sub-phylum**: Dicots

**Class**: Eudicots

**Sub-class**: Rosids

**Order**: Rosales

**Family**: Cannabaceae

**Genus**: Trema

**Species**: *Trema orientalis*

**Botanical Name**: ***Trema orientalis*** **(L.) Blume [42]**

**Figure 1: Plant Photograph**

### 3.3: MORPHOLOGY

It is a medium-sized tree or shrub that can grow up to 15 meters tall. In certain areas, it may appear as a tiny shrub. The leaves are simple, alternating, and ovate to lanceolate in shape, measuring 10-20 cm in length and 5-10 cm in width. They are generally serrated along the edges and have a pointed tip. The leaves are normally green with a little hairy texture on the underside. The flowers are tiny, unisexual, and unremarkable. They are found in axillary panicles or terminal racemes. The blooms are tiny and greenish or yellowish, with male flowers standing out more than females. The fruit is a tiny, round, or ellipsoid drupe that measures about 1 cm in diameter. The fruit starts out green and matures to be reddish-brown to blackish. It contains one seed. The bark is smooth and ranges in color from light gray to brown. It may develop fissures with age. The wood is lightweight and commonly used to make charcoal**[ 43,44,45].**

### 3.4: DISTRIBUTION

*Trema orientalis*, commonly known as the pigeon wood or charcoal tree, is a tropical and subtropical tree found in various regions across Africa, Asia, and the Pacific. It is known for its wide ecological tolerance, often growing in disturbed sites, secondary forests, and along riverbanks.Trema orientalis is widely distributed across sub-Saharan Africa, including countries like Nigeria, Kenya, Uganda, and Tanzania.The tree is found in many parts of Asia, including India, Nepal, Thailand, Vietnam, and the Philippines.

### 3.5: GROWTH CONDITION

*Trema orientalis,* a rapidly growing pioneer species, thrives in a variety of habitats, including tropical and subtropical zones. It prefers well-drained soils and can survive a variety of soil types, including sandy and clayey. The plant is drought hardy, but it also thrives in locations with heavy rainfall. It is frequently found in disturbed environments, demonstrating its significance in ecological succession**[46,47].**

### 3.6: TRADITIONAL USES

*Trema orientalis*, also known as "Charcoal Tree," has been traditionally used for various medicinal purposes across different cultures. Its leaves, bark, and roots are utilized for treating ailments such as fever, dysentery, wounds, respiratory issues, and hypertension. The plant is also known for its role in ethnomedicine as an anti-inflammatory, antimicrobial, and antidiarrheal agent. Additionally, it has been used in traditional practices to relieve pain and to promote wound healing**[48,49]**

### 3.7: PHYTO - CHEMISTRY OF *Trema orientalis*

*Trema orientalis* contains various phytochemical constituents with notable percentages. Key compounds include flavonoids, with quercetin and kaempferol being prominent; their total content can be around 2-4%.The plant's essential oils contain sesquiterpenes and monoterpenes, making up about 0.5-1% of the extract. Additionally, the presence of saponins and tannins, although in lower concentrations, adds to the plant's medicinal properties**[50,51].**

### 3.8: PHARMACOLOGICAL ACTIVITIES OF *Trema orientalis*

Modern pharmacological studies provide scientific evidence that bitter vine possess outstanding therapeutic potencies i.e anti-microbial , anti-inflammatory , anti-diabetic , anti-oxidant**[52,53,54].**

## 4.1: LITERATURE REVIEW:

The goal of the literature review is to find out different activities reported on *Trema orientalis* plant and to check whether it possesses any anti-oxidant, anti-diabetic and anti-inflammatory activity or not.

1. **Adinortey and M. B, 2013** said that *Trema orientalis* has traditionally been employed to treat diabetes, respiratory disorders, oliguria, and malaria. This paper gives a thorough summary of its therapeutic properties, biology, and phytochemical profile, which includes tannins, saponins, flavonoids, triterpenes, phytosterols, and xanthones. Pharmacological investigations demonstrate its potential for hypoglycemia, pain relief, inflammation reduction, malaria treatment, diuretic effects, constipation relief, seizure control, anti-parasitic action, sickle cell anemia alleviation, and antibacterial activity. The review supports its therapeutic potential and recommends additional clinical study to corroborate the findings.

2. **Al-Robai,*et.al*2022** done the phytochemical investigation,Antioxidant,Anticancerv and Antimicrobial activity of methanol extract from aerial parts of *Trema orientalis* palnt. The extracts have high total polyphenol and flavonoid contents, with strong radical scavenging activity in ABTS and DPPH assays. The leaf and twig extracts are notably cytotoxic against the HCT116 cell line, and leaf and bark extracts show activity against the HT29 cell line. The study signifies that *T.orientalis* possesses , antioxidant and anticancer activity.

3. **Adjileye, *et.al* 2019** investigated the phytochemical study and antioxidant activity of *Trema orientalis* and *Dialium guineense.* The phytochemical screening was carried out using standard procedures. The phenolic and flavonoids contents were determined using Folin-Ciocalteu and aluminium chloride reagent. The antioxidant activity was evaluated using 2, 2-diphenyl-1-picryl-hydrazyl, Ferric reducing capacity, superoxide anion and hydrogen peroxide methods. . The results of the study signify that the plant extract of *Tremas orientalis* was the most active in the ferric reducing capacity with 6007.8 ± 175.57µmol AAE g-1. The superoxide anion and hydrogen peroxide scavenging activities ranged from 88.52±0.68 % to 91.33±4.01%. The DPPH scavenging activity was dose dependent. Dialium guineense was the most active with an inhibitory percentage of 96.06 ± 0.34 % comparable to ascorbic acid (99.46 ± 0.37 %).

4. **Uddin, S. N., *et.al* 2009** performed a study on a nti-inflammatory, anti-nociceptive and diuretic activities of *Trema orientali.* The crude methanolic leaves extract of T. orientalis was investigated for its possible anti-inflammatory activities using carrageenin induced rat paw edema model and cotton pellet implantation method in mice. Then the extract analyzed for its antinociceptive activities by acetic acid induced writhing model in mice. The extract showed significantly reduced the number of acetic acid-induced abdominal constriction in mice of 200 and 400 mg/kg body weight. The extract also showed positive diuretic activity in albino mice.

5. **Trono,*et.al* 2016** evaluated the In-vitro alpha-amylase and antioxidant activities o f bark extracts of *Trema orientalis.* The methanol extract demonstrated higher DPPH scavenging ability and α-amylase inhibitory activity, with IC50 values of 48.40 µg/ml and 127.56 µg/ml, respectively, and contained higher total phenolics. Preliminary phytochemical profiling and GC-MS analysis identified various bioactive compounds, including phenols, flavonoids, and tannins.The results from the study indicated that methanolic extract have weak cytotoxicity in a brine shrimp lethality assay, while the aqueous extract was non-toxic. The findings suggest potential antidiabetic properties of the plant, attributed to the synergistic effects of its active compounds.

6. **Azad, *et.al* 2016** studied the In-vitro antioxidant, and thrombolytic activity of Methanol and Ethylacetate extracts *Trema orientalis* flowers. The MeOH extract exhibited mild free radical scavenging activity with an IC50 of 131.2 µg/ml, compared to the standard ascorbic acid (IC50: 12.4 µg/ml). Both MeOH and EtOAc extracts showed moderate thrombolytic activity, resulting in 21.4% and 21.5% clot lysis, respectively, in contrast to the 51% clot lysis achieved by the positive control, streptokinase.

7. **Rout, J., *et.al* 2012** determined the antibacterial efficacy of bark extracts of an *Trema orientalis* using aqueous extracts against six bacterial strains, focusing on its traditional medicinal use in the Dima Hasao Hill district. Plant materials were processed using herbarium techniques, and antimicrobial activity was tested via the Kirby-Bauer Agar Disc Diffusion method. The extracts showed significant antibacterial activity, with inhibition zones ranging from 11 to 15 mm and a minimum inhibitory concentration (MIC) as low as 0.625 mg/ml. The results support the potential use of *T. orientalis* as an antibacterial agent in traditional medicine.

8. **Saleh, A., *et.al* 2020** studied the polyphenolic content and antioxidant activity of *Trema orientalis* extracts. Phytochemical analysis identified saponins, tannins, steroids, cardiac glycosides, alkaloids, triterpenes, flavonoids, and phenolic compounds. The ethanol extract contained higher total phenolic content (260.96 mg GAE/g) and flavonoid content (32.71 mg GAE/g) compared to the aqueous extract. The ethanol extract also demonstrated significant antioxidant activity, with an IC50 of 9.27 µg/mL. The study showed that the therapeutic potential of *T. orientalis*, especially due to its rich polyphenolic composition and antioxidant properties.

9. **Uddin and S. N., 2008** studied the antioxidant and antibacterial activities of methanol and aqueous leaf extracts of *Trema orientalis*, known for its traditional medicinal uses. The methanol extract exhibited strong antioxidant activity with an IC50 of 110.25 µg/ml, while the aqueous extract showed mild activity. Both extracts demonstrated antibacterial effects against Gram-positive and Gram-negative bacteria, with effectiveness observed at low concentrations. The results support the traditional use of *T. orientalis* and suggest further investigation into its pharmacological properties.

10. **Uddin and S. N., 2008** studied the analgesic and anti-diarrheal activities of methanol and aqueous leaf extracts of *Trema orientalis* in mice. The aqueous extract showed significant analgesic effects in the acetic acid-induced writhing test at a dose of 500 mg/kg. Both extracts also demonstrated anti-diarrheal activity in the castor oil-induced diarrhea test, increasing the latent period and reducing stool frequency at the same dose, comparable to the standard drug loperamide.

11. **Niranjan and D.,2023** studied the in vitro antioxidant activity of methanolic extract of *Trema orientalis* . The extract showed minimal antioxidant activity at 200 µg/ml and 250 µg/ml doses, compared to ascorbic acid, a standard reference. The presence of phenolic compounds, such as tannins and flavonoids, likely contributed to METO's antioxidant properties. The relatively lower potency observed may be influenced by the solvent and plant materials used.

12. **Pramod and C., 2020** studied the hypolipidemic activity of an ethanolic extract of *Trema orientalis* leaves using hyperlipidemic rat models. The extract significantly reduced serum levels of total cholesterol, triglycerides, LDL, and VLDL while increasing HDL levels, comparable to the standard drug Simvastatin. EETO also improved the atherogenic index and exhibited antioxidant activity, indicated by elevated glutathione reductase and glutathione peroxidase levels. Histopathological liver examinations supported these biochemical findings. The hypolipidemic effects are attributed to active phytoconstituents like flavonoids, glycosides, and alkaloids, validating *T. orientalis* as a potential hypolipidemic agent.

## 5.1: COLLECTION AND AUTHENTICATION OF THE PLANT COMPONENT

The leaves of the plant *Trema orientalis* (Cannabaceae) were collected in November of 2023 from Siliguri, West Bengal. A specimen of the plant was deposited in the Central National Herbarium at the Botanical Survey of India, Shibpur, Howrah (CNH/Tech.II/2024/92) for identification and authentication. The leaves were collected, thoroughly washed with water, and then dried for a month under shade. The dried leaves were then mechanically ground into a powder and placed in an airtight container for later usage.

## 5.2: MORPHOLOGICAL AND ORGANOLEPTIC CHARACTERS

The fresh leaves of the *Trema orientalis* were collected and subjected for evaluation morphological characteristics like colour, size, shape, and other macroscopic characteristics. Other organoleptic characteristics including taste, odour and texture were also evaluated.

## 5.3: MICROSCOPIC CHARACTERISTICS

Microscopic characterization of the leaves were carried out by cutting thin transverse sections of the lamina of the fresh leaves. The sections were mounted on a glass slide with the help of glycerin and covered with cover slips. The sections were observed under compound microscope with 10X magnifications to study the internal anatomical characteristics of the leaves. In the next step, dried leaves were converted into coarse powder with help of a mortar and pestle and passed through a sieve to separate the coarse powder from larger fragments. Then a pinch of the powder was placed on a glass slide and mounted with glycerin. The glass slide was covered with cover slip and observed under compound microscope with 10X magnification for powder characteristics**[70].**

## 5.4: PHYSICOCHEMICAL ASSESSMENT[71]

### 5.4.1: Loss on Drying (LOD)

2g of the powdered leaves were placed in a shallow, dried weighing bottle. The sample bed's height was kept 10 mm or less. The sample was then dried in a hot air oven at 105º C for 30 minutes. Then, the shallow weighing bottle was taken out and cooled in a desiccator, and weighed at room temperature. The procedure was repeated until the difference between two successive weights was less than 0.5 mg. The LOD was determined as a percentage of w/w.

The LOD was determined as a percentage of w/w.

### 5.4.2: Ash Value

#### Total Ash Value

2g of the powdered leaf sample was weighed accurately and placed in a silica crucible which had been weighed previously. The crucible was placed in a muffle furnace and incinerated at 450ºC until the sample was free from carbon. After the incineration is complete, the crucible is taken out from the muffle furnace and cooled in a desiccator. Once cooled, the weight of the crucible was recorded at room temperature in a careful way, so that the sample does not absorb moisture from the surrounding air. The total ash was calculated as the percentage (w/w) of ash generated from the leaf sample after incineration.

#### Water Soluble Ash Value

After obtaining the total ash residue from the crucible, it was carefully placed in a beaker with 25 ml distilled water. The beaker was then placed on a water bath and the contents were boiled for 25 minutes. After 25 minutes, the mixture was cooled and filtered using an ashless filter paper (Whatman filter paper 42). Upon completion of the filtration, the residue on the filter paper was thoroughly washed with hot water and placed in a silica crucible which had been weighed previously. The crucible was placed in a muffle furnace and then incinerated at 450ºC till no carbon residue of the filter paper was left in the crucible. The weight of the residue in the crucible was carefully recorded and subtracted from the weight of the total ash to calculate the fraction of ash that had been dissolved in water. The value of water soluble ash was calculated as percentage (w/w) of total ash that got dissolved in distilled water upon boiling.

#### Acid Insoluble Ash

Total ash from the previous study was placed in a beaker and 25ml of 2N hydrochloric acid was added to the beaker. The contents of the beaker was then boiled on a hot plate for 5 minutes. After boiling, the contents of the beaker was cooled and filtered using ashless filter paper. The residue on the filter paper along with the filter paper was carefully taken in a previously weighed silica crucible and incinerated at 450ºC for 15 minutes. The incinerated residue was weighed carefully

to record the amount of ash that remained insoluble in the acid upon boiling. The calculations for the acid insoluble ash percentage (w/w) were made using dried sample data.

### 5.4.3: Extractive Value

#### Value of Water Soluble Extractive

In a closed conical flask, 5g of coarsely powdered leaves were macerated with 100 ml of water for 24 hours. The flask was shaken frequently for the first 6 hours before being left to stand for 18 hours. After 24 hours, the mixture was filtered and 25 ml of the filtrate was then measured, and evaporated in a small porcelain plate. The residue on the porcelain plate was weighed and recorded. With reference to the dried sample, the percentage(w/w) of water soluble extractive value was calculated.

#### Alcohol (Ethanol) Soluble Extractive Value

In a closed conical flask, 5g of coarsely powdered leaves were macerated with 100 ml of ethanol for 24 hours. Alcohol soluble extractive value was determined in the same way as water soluble extractive value.

### 5.4.4: Analysis of Crude Fiber

200ml of 1.25% (v/v) sulfuric acid and 2g of the dried leaf powder were heated together with constant stirring. After heating, the mixture was filtered using a filter paper and the filter paper was washed with hot water for few times. After that, 200ml of a solution containing 1.25% (w/v) sodium hydroxide was added to the filtrate, and it was heated for 30 minutes. The mixture was filtered after being rinsed in hot water, and the filtrate was then burned at $110^{o}$ C to a constant weight. With reference to the dried sample, the crude fiber percentage was computed.

### 5.4.5: Swelling Index

A 100 ml measuring cylinder was filled with 1g of dried powder and 25ml of water. For an hour, the cylinder was shaken every ten minutes. The measuring cylinder was then kept for 3 hours. Change in volume of the mixture was recorded to calculate swelling index of the leaves.

### 5.4.6: Foaming Index.

1g of the dried sample was added to a conical flask that held 500 ml of boiling water. It was then filtered into a volumetric flask after cooling. Water was added till the content reached 100 ml. Obtained and labelled ten test tubes with stoppers. The drug was added in successive sections of 1, 2ml up to 10 ml into different tubes, and the remaining volume was adjusted with water up to 10ml. The tubes were sealed with stoppers, shaken for 15 seconds, allowed to stand for 15 seconds, and then height of the foam from each tube was measured.

The foaming index is less than 100 if each tub's foam is less than 1cm in height.The dried sample solution decoction volume in this tube (a) is used to calculate the index if the height of foam of 1 cm is measured in the first through tenth tubes.To get a more accurate result, make an intermediate dilution if this tube is the first or second in a series.The foaming index exceeds 1000 if the height of the foam in the first through tenth tubes is greater than 1 cm.

Foaming index =1000/a

Where, a=the amount of decoction in milliliters that was used to prepare the dilution in the tube where foaming to a height of one centimeter is seen.

## 5.5: PREPARATION OF EXTRACTS

The following solvents, in order of increasing polarity, were used to extract the powdered leaves in a soxhlet extractor;petroleum ether (boiling point 60-80ºC) , acetone (boiling point 56.05ºC ) and methanol (boiling point 64ºC). Using petroleum ether, the powdered plant material (50g) was first extracted. The extraction procedure was carried out until all the contents had been removed, and this was verified by removing a sample from the soxhlet extractor's syphon tube. It was then evaluated by TLC using the appropriate solvent that had been used for the extraction procedure. The absence of a spot on the TLC plate in the iodine chamber served as a sign that the extraction process has been completed. The complete assembly of the soxhlet extractor has been depicted in Figure 2. The extract was then concentrated by vacuum distillation, dried in the open air, and stored in an airtight container. The marc was dried by air before undergoing a second methanol extraction process. The extracts that were produced as a result of each extraction procedure were kept in airtight containers.

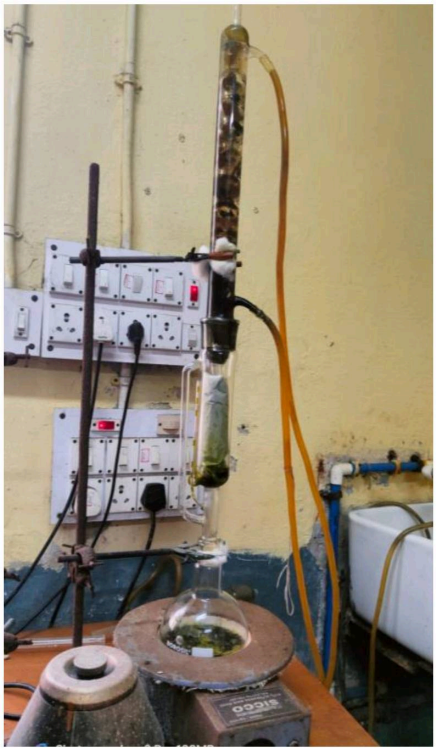


**Figure 2: Soxhlet extractor assembly**

## 5.6: SCREENING FOR PHYTO-CHEMICALS IN THE EXTRACT[72]

To identify the presence of various phyto components, different chemical analyses were run on the Three d rug extracts, petroleum ether extract ,methanolic extract and acetone extract.

**Test for Carbohydrates**

**Molisch Test(general)**

A few drops of the Molisch reagent were applied to 2–3 ml of the aqueous extract. Sulphuric acid in the amount of two ml was introduced from the test tube's side. Carbohydrates are present when a purple ring forms at the intersection of two liquids.

**Test for Reducing Sugars**

**Fehling's Test**

Boiling for one minute after combining 1 ml each of Fehling's solutions A and B. equal volume of the test solution was added. heated for five to ten minutes in a bath of boiling water. When reducing sugars are present, first yellow, then brick red ppt are seen.

**Benedict's Test**

In a test tube, combine benedict's reagent and test solution in an equal volume. 5 minutes of heating in a pot of boiling water. The colour of the solution depends on how much reducing sugar is present in the test solution; it might be green, yellow, or red.

**Test for Monosaccharide**

**Barfoed's Test**

Combine Barfoed's reagent and test solution in an equal volume. heated in a bath of boiling water for one to two minutes. Red ppt is formed if monosaccharides is present.

**For Non-Reducing Sugar**

Test solution does not show positive results for Fehling's and Benedict's test.

**Test for Proteins and Amino Acids**

**Biuret Test(general test)**

1 ml of biuret reagent were added to 2 ml of test solution (extract). Protein is present when the colour is violet.

Ninhydrin Test(general test)

2 ml of test solution and 2 drops of 5% Ninhydrin solution were heated for 10 minutes in a boiling water bath. Thetest solution turns purple or bluish when amino acids are present.

**Millon's Test**

Combine 5 ml of Millon's reagent with 3 ml of test solution. White ppt is formed which turns brick
red after warming if protein is present.

**Test For Steroids**

**Salkowski's Test**

Add 2 ml of chloroform and 2 ml of conc. Sulphuric acid to 2 ml of extract and thoroughly shaken. The appearanceof a reddish-brown tint in the bottom layer is a sign of steroids.

**Liebermann-Burchard Test**

Acetic anhydride was usedto first treat the extract solution before a few drops of strong sulfuric acid were added along the test tube's side. Green coloration is a sign of the presence of steroids.

**Test for terpenoids**

**Noller's Test**

Noller's reagent, which contains 0.1% stannic chloride in thionyl chloride, was applied to the extract solution. Terpenoids are present when red hue appears, indicating their existence.

**Test For Glycosides**

**For Cardiac Glycosides**

**(For cardenolides) Legal's test**

1 ml pyridine and 1 ml sodium nitroprusside were added to the extract. Glycosides are indicated by the colour pink.

**Keller-Killiani test (for deoxy-sugars)**

Glacial acetic acid, one drop of 5% FeCl3, and sulfuric acid were added to the 2 ml of extract. If glycosides arepresent, a reddish brown colour occurs at the junction of the two liquid layers, while the upper layer appears bluish green.

**Test for anthraquinone**

**glycosides**

**Borntrager's test**

Diluted sulfuric acid was added to 3 ml of extract. filtered and boiled. Chloroform was added in an equal amount to the cold filtrate. firmly shaken. Make the organic solvent separate. Added ammonia. Anthraquinone glycosides cause the ammonical layer to turn pink or crimson.

**Modified Borntrager's test**

5% FeCl3 and 5 ml of diluted HCl were added to 5 ml of extract. heated in a bath of boiling water for five minutes. Chloroform was added after cooling. firmly shaken. poured an equal proportion of diluted ammonia to the separate organic layer. The coloration of the ammoniacal layer is pinkish red.

**Test for Saponins**

Shake the water and powdered extract vigorously. Saponin is detected when there is persistent froth.

**Test for Flavonoids**

**Shinoda test**

5 ml 95% ethanol and a few drops of conc HCl were added to the dried extract. 0.5 gm of magnesium turnings was added to it. Flavonoid's give off an orange, pink, red, or purple tint when they are present.

**Ferric ChlorideTest**

Added a few drops of 10% Fecl3 to the extract. It emits a green tint to show flavonoids are present.

**Sodium hydroxide Test**

Solution of sodium hydroxide was added to the extract. The presence of flavonoids is shown by the appearance of yellow hue, which is destroyed by the addition of acid.

**Test for Alkaloids**

**Mayer's test**

Mayer's reagent in a few drops with 2- 3 ml of filtrate results in a cream-colored ppt.

**Dragendorff 's Test**

Add a few drops of Dragendorff's reagent to 2 to 3 ml of filtrate. Orange and brown ppt. is created

**Hager's test**

With Hager's reagent, 2- 3 ml of filtrate produces yellow ppt.

**Wagner's test**

With Wagner's reagent, 2–3 ml of filtrate yields reddish brown ppt.

**Test for Tanins and Phenolics**

**FeCl3 test**

Dark green or deep blue colouring after treatment with 5% Fecl3 solution suggests the presence of tannin and phenolics.

**Gelatin Test**

10% sodium chloride and 1% gelatin solution were added to the test solution. Precipitate formation suggests the presence of tannins.

**10%NaOH test**

When 4 ml of 10% NaOH solution is added to the extract solution and it is thoroughly agitated, an emulsion forms that shows tannins and phenolics are present.

## 5.7: CHROMATOGRAPHIC ANALYSIS OF THE PREPARED EXTRACTS

Thin layer chromatographic (TLC) analysis was performed on both the extracts prepared from the powdered leaves of the plant.

**Preparation of a sample:** Dried petroleum ether ,methanol extracts and acetone extract each weighing 1g, were diluted in an adequate quantity of the extraction solvents, petroleum ether,methanol and acetone ,and then utilized as samples for TLC analysis.

**Stationary phase**

Pre-coated Silica gel G was utilized as stationary phase for performing TLC analyses of the extracts.

**Mobile phase**

For methanol extract, the mobile phase was used as hexane: chloroform: methanol (6:2.5:1.5) while for the petroleum ether extract, petroleum ether: acetone (8.5:1.5), was used as mobile phase and acetone extract, the mobli phase was used as

**Observation** - Retention factor ($R_f$) value for each of the spots was determined after the spots were observed in an UV chamber at wavelengths of 254 nm and 366 nm.

### 5.8: PREPARATION AND CHARACTERIZATION OF TINCTURE[73]

A total of 21 amber-colored bottles with 10 ml capacity were used. To these, 10 ml of alcohol with varied strengths—40, 50, 60, 70, 80, and 90 percent (V/V) and absolute alcohol were added. Each of the twenty-one bottles contained 1g of *Trema orientalis* leaves powder. Each batch was allowed to macerate for a specific number of days: 7, 14, and 21. The materials were filtered through a sintered glass crucible (G4) while under reduced pressure after the required amount of maceration time. Physical characteristics were investigated, including colour, pH, specific gravity, and total solid content.

**Figure 3**: **Tinctures of different alcohol concentrations after maceration for differing time period**

The word anti-oxidant simply means " against oxidation". An anti-oxidant is any substance that retards or prevents the deterioration, damage, or destruction of cells by oxidation **[74].** It is a classification of a number of organic compounds, including selenium, the carotenoids chemical family, vitamin A (which is made from beta carotene), and the vitamins C and E.

### 5.9.1: DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content of the sample was determined using the Folin-Ciocalteu in 96-well plate**[75].** with slight modifications. About 25µl of the extract solution was mixed with 100µl of Folin-Ciocalteau reagent (FCR). After 3 min, 75µl of sodium carbonate solution was added and the mixture was allowed to stand for 1 h at room temperature followed by absorbance was measured at 765 nm using a microplate reader. From the calibration curve of gallic acid prepared in different concentrations (200,400,600,800 and 1000µg/ml). The amount of phenolic compounds was determined and expressed as mg gallic acid equivalent (GAE) of dried or fresh extract exudates. The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

$$T=C \times V \angle M$$

Where, T- Total phenolic contents in mg/g plant extract, in Gallic Acid Equivalent(GAE)C-Concentration (mg/ml) of Gallic acid obtained from the calibration curve, V=Volume of extract (ml),
M= Weight (mg) of plant extract.

### 5.9.2: DETERMINATION OF TOTAL FLAVONOID CONTENT

Total soluble flavonoid content of the fractions was determined with aluminium chloride using quercetin as a standard in 96 well plate**[76]**. Free radicals concentration(200,400,600,800,1000µg/ml) of quercetin, 150µl of methanol was added. Then it was mixed with 10µl of aluminium chloride and 10µl Extract solution of different concentration was also prepared using the same method. All the samples were incubated for

30 minutes at room temperature and absorbance was measured at 415 nm using a microplate reader against control. The total flavonoid content in the fractions was determined as µg quercetin equivalent by using the standard quercetin graph and using the following formula:

**T= C X V/ M**

Where, T-Total flavonoid content in mg/g plant extract, in Quercetin Equivalent (QE), C=Concentration (mg/ml) of Quercetin obtained from calibration curve,

V= Volume of extract (ml),

M= Weight (mg) of plant extract.

### 5.9.3: DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

Minor adjustments were made to an earlier approach**[77]** for measuring DPPH activity while using Ascorbic acid as the Standard for comparison in 96 well plate. In order to create a series of sample solutions with various pre-set concentrations(200,400,600,800,1000 µg/ml )the extract was dissolved in methanol. 50 µl of the extract sample solution were combined with 150µl of a 0.3 mM DPPH solution in methanol, and the mixture was then incubated for 30 min at 37°C. Absorbance was measured at 517 nm using a microplate reader against control. The same method was applied to standard solutions with comparable concentrations. The ability of the sample/standard to scavenge the DPPH radical was determined as the free radical inhibition percentage using the following formula:

**% Inhibition of DPPH scavenging activity = Ac - At/ Ac X 100**

Where Ac is the absorbance of the control reaction and At is the absorbance of the test/standard. The extract's antioxidant activity was quantified as $IC_{50}$. The $IC_{50}$ values were computed using linear regression of plots, where the ordinate represents the average percentof scavenging capacity and the abscissa indicates the concentration of the studied plant extracts. The $IC_{50}$ value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

### 5.9.4: DETERMINATION OF HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY

The ability of three *T.orientalis* extracts to scavenge hydrogen peroxide was determined according to the method of Oktay Munir and others**[78].**In phosphate buffer with a pH of 7.4, hydrogen peroxide (2 mmol/L) was produced as a solution. Hydrogen peroxide concentration was determined microplate reader from absorption at 230 nm with molar absorbtivity81 mol/L-1 /cm. Extracts samples (200–1000 µg/ml) in distilled water were added to a hydrogen peroxide solution (30 µl ). After 10 minutes, the absorbance of hydrogen peroxide at 230 nm was measured in comparison to a blank solution made up of phosphate buffer and no hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both *T.orientalis* extract and standard compounds(Ascorbic acid).

**% $H_2O_2$ radical Scavenging activity = Ac At/Ac X 100**

Where Ac is the absorbance of the control reaction and At is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as $IC_{50}$. The $IC_{50}$ value is defined as the concentration (µg/ml) of dry extract that inhibits the formation of $H_2O_2$ radicals by 50%.

### 5.10: IN VITRO ANTI-DIABETIC ACTIVITY

#### 5.10.1: Inhibition *of α Amylase*

70µl of root extract and positive standard (acarbose) of concentration ranging from 200-1000µg/ml were taken. To each concentration 70µl of α-amylase solution dissolved in phosphate buffer was added and incubated at 30°C for 10 minutes in 96 well plate. Then 70µl of 1% starch solution was added, incubated for 3 minutes. Finally the reaction was stopped by adding 30µl 0f 3,5-dinitrosalicylic acid (DNSA), kept in boiling water bath for 10 minutes, cooled and the absorbance was measured at 540nm using a microplate reader**[79].**Control was also treated similarly without the extract. *a-amylase* inhibitory activity was measured using the formula:

**% Inhibition = Abs Control – Abs Test / Abs Control X 100**

The $IC_{50}$ values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate . All the measurements were done in triplicate and results are expressed in terms of Mean ± standard deviation and $IC_{50}$ values were calculated using MS-Excel software.

## 5.11: IN-VITRO ANTI- INFLAMMATORY STUDIES

### 5.11.1: HRBC (Human red blood cell) membrane stabilization

Blood was collected from healthy volunteers, mixed with equal volume of Alsever's solution and the HRBC suspension was made. To 200µl of suspension equal volume of extract (200- 1000 µg/ml) was added, incubated and centrifuged. Diclofenac sodium (Positive standard) and the control (without extract) was also treated similarly and simultaneously. The haemoglobin content was estimated at 560nm using a microplate reader**[80]**

**The percentage protection was calculated (Abs of blank -Abs of extract) / Abs of control x 100.**

## 6.1: AUTHENTICATION OF THE PLANT MATERIAL

A figure of the matured leaves of *Trema orientalis* has been provided in figure 4. The authentication certificate from the central national herbarium, Botanical Survey of India is presented in figure 5. The certificate from the CNH confirms the identity of the collected species as *Trema orientalis* of Cannabaceae family.

**Figure 4**: ***Trema orientalis*** **leaves**

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
फैक्स/ Fax: (033)26686226
दूरभाष/ Phone: (033)26683235/3364
इमेल/ E-mail: calherbarium@yahoo.co.in

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या / No.: CNH/Tech.II/2024/92 दिनांक / Date: 05.07.2024

To,
Ms. Gulsana Yesmin
M. Pharm
Department of Pharmaceutical Technology
Jadavpur University
Kolkata- 700032

**Sub.: Identification of one plant specimen – reg.**

Dear Ms. Yesmin,

Please refer to your letter dated 26th May 2024 along with a plant specimen for identification. It is to inform you that the specimen has been identified by the concerned expert as:

| Sl. No. | Specimen No. | Scientific Name | Family |
|---|---|---|---|
| 1. | JU/GY–01 | **Trema orientale** (L.) Blume | Cannabaceae |

The receipt of ₹ 250/- (Rupees Two thousand fifty only) Receipt No. 2806240032884 dated 28.06.2024 payment made via bharatkosh.gov.in is enclosed herewith.

Your specimen is returned herewith.

Yours sincerely

11.7.2024

(R.K GUPTA)
**Scientist –'E' & Head of Office**
वैज्ञानिक "ई" व कार्यालय अध्यक्ष
Scientist "E" & Head of Office
केन्द्रीय राष्ट्रीय पादपालय
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावड़ा / Howrah-711103

**Figure 5: Authentication certificate from Botanical Survey of India**

## 6.2: MORPHOLOGICAL CHARACTERISTICS OF THE SPECIES

The morphological characters from the matured leaves of the plant is represented in Table 3. Upon studying the leaves, the colour was found to be dark green with faint aromatic odour. The ovate or elliptic,astringent , leaves had rough texture with a length of 6-12 cm according to their maturity.

**Table 4: Macroscopic Characteristics of Leaves**

| Sl No. | Characteristics | Leaves part |
|---|---|---|
| 1 | Color | Dark green |
| 2 | Odour | Faintly aromatic |
| 3 | Taste | Astringent , bland. |
| 4 | Texture | Rough |
| 5 | Shape | Ovate or elliptic |
| 6 | Size | 6 to 12 cm long |

## 6.3: MICROSCOPICAL CHARACTERISTICS OF THE SPECIES

The microscopic characters from the transverse section of the leaves of the plant is represented in Figure 6. The transverse section of the leaf reveals a dorsiventral lamina with distinct palisade and spongy parenchyma. The upper and lower epidermis features unicellular, multiseriate covering and glandular trichomes, with the upper epidermis being a single polygonal layer covered with a cuticle. Palisade cells extend up to the midrib in 2-3 layers, containing rosette-type calcium oxalate crystals. Beneath these, 2-3 layers of spongy parenchymatous cells with xylem vessels and more calcium oxalate crystals are present. The midrib showcases well-developed collenchyma beneath the upper epidermis and above the lower epidermis, with a central vascular bundle and surrounding parenchymatous cells also containing rosette-shaped calcium oxalate crystals. In the surface view of Trema orientalis leaf, the lower epidermal cells Figure 7. show the presence of irregular epidermal cells, anomocytic stomata and simple, unicellular covering trichomes and rosettes of calcium oxalate crystals. In the surface view of upper epidermis Figure 8. characteristics noted were absence of stomata (abaxial) and simple covering unicellular trichomes with only epidermalcells.Figure 9. Powder characterization of the leaves revealed presence Mesophyll

region which contain palisade and spongy parenchyma with epidermis. Lignified xylem vessel, Rosette andprism shape calcium oxalate crystals Anomocytic stomata. Figure 10. Annular thickening and spiral vessel Unicellular, multiseriate covering trichomes and glandular trichomes

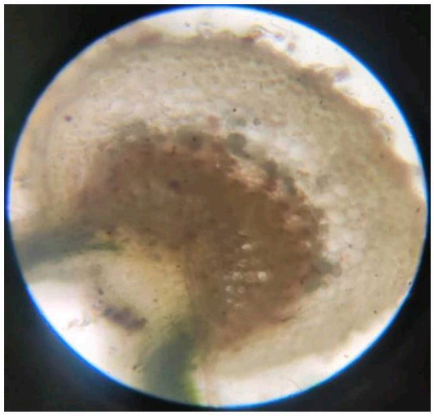


**Figure 6. The transverse section of the leaf**

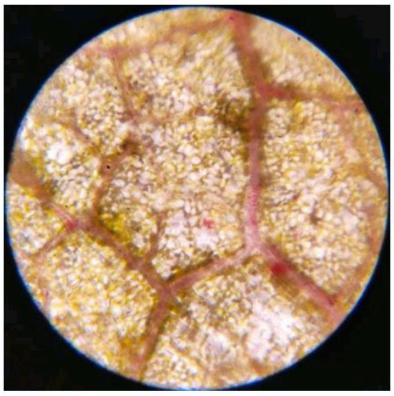


**Figure 7. Upper epidermis**

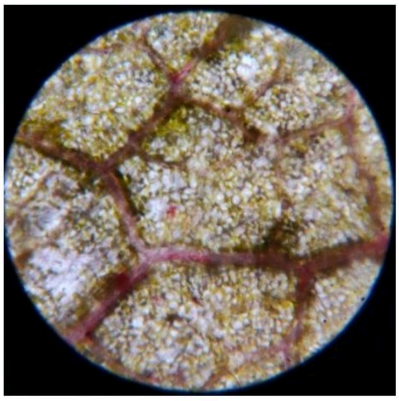


**Figure 8. Lower epidermis**

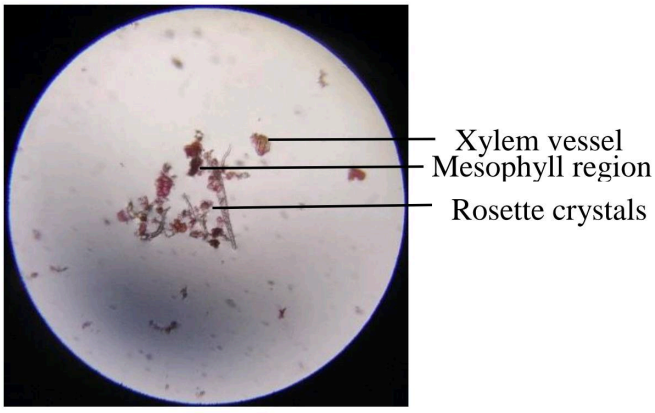



**Figure 9. Xylem vessel, Mesophyll region, Rosette crystals**

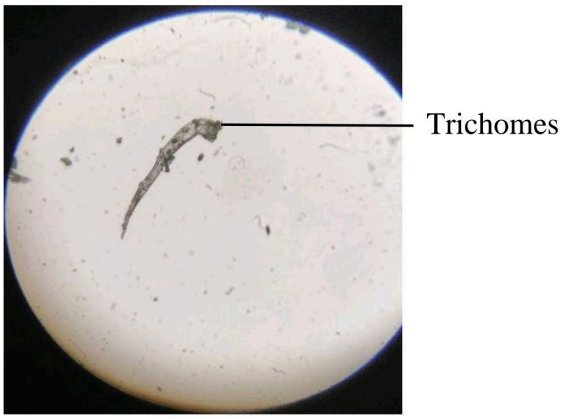



**Figure 10. Trichomes**

## 6.4: PHYSICOCHEMICAL CHARACTERISTICS OF LEAVES POWDER

Results of different physicochemical properties of leaves powder are shown in Table 5.

**Table 5: Physico-Chemical Characteristics of Leaves Powder**

| Sl No. | Physico-Chemical Parameters | Results |
|---|---|---|
| 1 | Loss On Drying | 8.33% w/w |
| 2 | Total Ash | 14.75% w/w |
| 3 | Acid Insoluble Ash | 8.35% w/w |
| 4 | Water Soluble Ash | 2.55% w/w |
| 5 | Water Soluble Extractive | 5% w/w |
| 6 | Alcohol(Ethanol) soluble extractive | 12% w/w |
| 7 | Crude fiber Content | 40% w/w |
| 8 | Swelling Index | 2.5 ml/g |
| 9 | Foaming Index | 100 |

## 6.5: PHYTOCHEMICAL SCREENING OF THE EXTRACTS

The results of phytochemical screening of both petroleum ether, methanol and acetone extracts of leaves were represented in table 6. The phytochemical studies have revealed the presence of carbohydrates, steroids, terpenoids,flavonoids and phenolics in the petroleum ether extract. Methanol extract has revealed the occurrence of carbohydrates, reducing sugar, terpenoids and phenolic. Acetone extract on the other hand has revealed the occurrence of flavonoids, tannins and phenolic through phytochemical analysis.

**Table 6: Phytochemical Screening of the petroleum ether , methanol and acetone extracts of the leaves**

| Chemical Constituents | Chemical Test | Petroleum ether extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| Carbohydrates | Molisch Test | + | + | - |
| Reducing Sugars | Fehling's Test | + | + | - |
| | Benedict's Test | + | + | - |
| Monosaccharides | Barfoed's Test | - | - | - |
| Proteins and Amino Acids | Biuret Test | - | - | - |
| | Ninhydrin Test | - | - | - |
| | Millon's Test | _ | - | - |
| Steroids | Salkowski reaction | + | - | - |
| | Liebermann-Burchard Test | + | - | - |
| Terpenoids | Noller's Test | + | - | - |
| Glycosides(Cardia Glycosides) | Legal's test | - | - | - |
| | Keller–killiani test | - | - | - |
| Anthraquinone glycosides | Borntrager's test | - | - | - |
| | Modified Borntrager's test | - | - | - |
| Saponins | Foam Test | - | - | - |

| | | | | |
|---|---|---|---|---|
| Flavonoids | Shinoda Test | - | - | + |
| | Ferric Chloride Test | + | - | + |
| | Sodium hydroxide Test | - | - | - |
| Alkaloids | Mayer's test | - | - | - |
| | Dragendorff'stest | - | - | - |
| | Hager's test | - | - | - |
| | Wagner's test | - | - | - |
| Tannins and Phenolics | Ferric Chloride Test | + | + | + |
| | Gelatin Test | + | + | - |
| | 10% NaOH test | + | + | + |

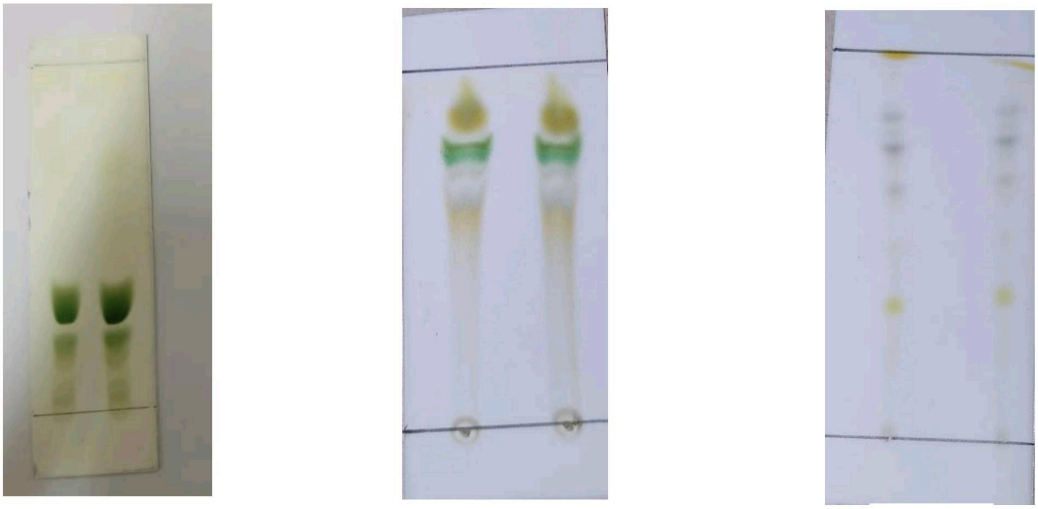
**6.6: TLC PROFILING**

For the methanol extract (a) chloroform : ethyl acetate (5 : 4) was used as mobile phase ,for the petroleum ether extract (b) hexane : ethyl acetate (3: 4) was used as mobile phase for acetone extract(c) methanol : ethyl acetate ( 3 : 2).

The methanolic extract showed 5 distinct spots while the petroleum extract showed 5 spots as and for acetone extract showed 6 spots presented in Figure 11. The TLC characteristics in the form of retention factor ($R_f$) of both petroleum ether and methanol extract of *Trema orientalis* leaves powder were shown in table 7

**Table 7**:**TLC Profiling of extracts**

| Sl. No. | $R_f$ Value | | |
|---|---|---|---|
| | **Methanol extract** | **Petroleum Ether Extract** | **Acetone extract** |
| 1 | 0.16 | 0.14 | 0.2 |
| 2 | 0.31 | 0.25 | 0.25 |
| 3 | 0.59 | 0.26 | 0.31 |
| 4 | 0.65 | 0.31 | 0.38 |
| 5 | 0.71 | 0.45 | 0.45 |
| 6 | | | 0.7 |



**A** **B** **C**

**Figure 11. A: TLC of Pet. Ether Extract, B:TLC of Methanol Extract , C:TLC of Acetone Extract**

## 6.7: CHARACTERIZATION OF TINCTURE

The different characteristics of the prepared tincture were shown in table 8, and 9. The pH of the prepared tincture at different strengths of alcohol is represented in table 8. The study shows that greater alcohol concentration in the tincture increases acidity of the tincture while storage for longer period of time also slightly increases acidic property. Solid content of the tincture also decreases with time as seen from table 9, while greater alcohol concentration also imparts diminished solid content.

**Table 8: pH of the tincture**

| Alcohol Strength | p H | | |
|---|---|---|---|
| | **7 Days** | **14 Days** | **21 Days** |
| 40% | 6.05 | 5.85 | 5.73 |
| 50% | 5.93 | 5.81 | 5.64 |
| 60% | 5.64 | 5.63 | 5.61 |
| 70% | 5.73 | 5.59 | 5.54 |
| 80% | 5.71 | 5.63 | 5.59 |
| 90% | 5.39 | 5.56 | 5.58 |
| Absolute(100%) | 5.34 | 5.24 | 5.07 |

**Table 9:Total Solid Content of Tincture**

| Alcohol Strength | 7 Days | 14 Days | 21 Days |
|---|---|---|---|
| 40% | 19.09 | 17.2 | 14 |
| 50% | 17.18 | 15.47 | 12.83 |
| 60% | 16.95 | 15.38 | 12.5 |
| 70% | 16.69 | 14.81 | 10.33 |
| 80% | 15.96 | 13.57 | 10.71 |
| 90% | 12.13 | 13.28 | 10.16 |
| Absolute(100%) | 9.93 | 7.17 | 5.53 |

## 6.8: ANTIOXIDANT ASSAY

### 6.8.1: TOTAL PHENOLIC CONTENT ASSAY

The absorbnce of the Standard and test solution were represented in table no.13 The total phenolic content in terms of mg Gallic Acid Equivalent (GAE) of Petroleum ether extract was found to be 36.5mg/g , methanolic extract was found to be 20.6mg/g and acetone extract was found to be 7.4mg/g . These results suggest that higher the presence of phenolic components was responsible for the levels of antioxidant activity. The Standard curve of Gallic acid was shown in figure 12.

**Table 10. Observation of absorbance in Total phenolic estimation**

| Sr.No. | Concentration (µg/ml) | Absorbance | Phenolic content of Petroleum ether extract | Phenolic content of Methanolic extract | Phenolic content of Acetone extract |
|---|---|---|---|---|---|
| 1. | 200 | 0.349 | 20.6mg/g | 36.5mg/g | 7.4mg/g |
| 2. | 400 | 0.434 | | | |
| 3. | 600 | 0.505 | | | |
| 4. | 800 | 0.716 | | | |
| 5. | 1000 | 0.869 | | | |
| 6. | Equation of line | Y = 0.0007x + 0.178 | | | |
| 7. | Coefficient ($R^2$) | $R^2$ = 0.9594 | | | |

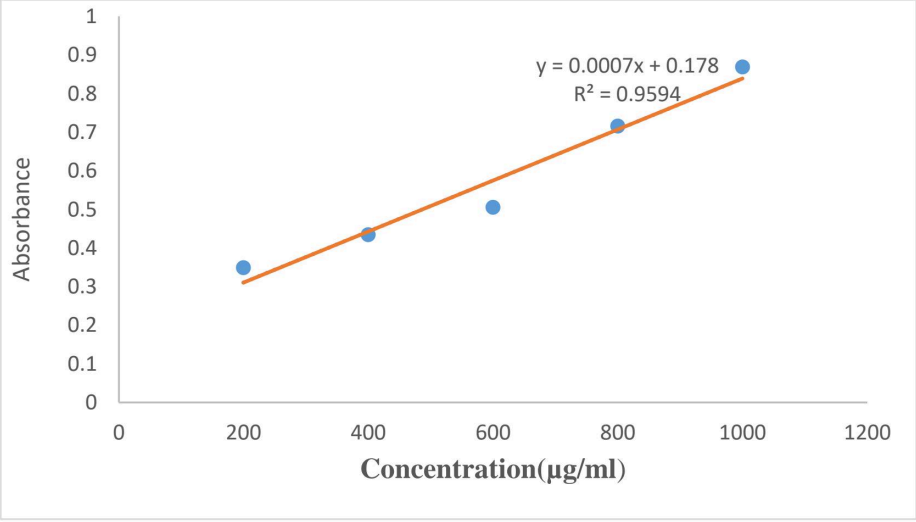



**Figure 12. Standard curve of Gallic acid**

### 6.8.2: TOTAL FLAVONOID CONTENT ASSAY

The content of flavonoid compound in Petroleum ether ,Methanolic extract and Acetone extract of *Trema orientalis* leaves was measured by aluminum chloride reagent in terms of quercetin equivalent and was found to be 38.81mg/g ,18.11 mg/g and 8.11mg/g . The absorbnce of the Standard and test solution were represented in table no. 11. It is well known that flavonoids have significant antioxidant activity and have a positive impact on human nutrition and health. The Petroleum extract of *Trema orientalis* leaves contains a substantial quantity of flavonoids which may contribute considerable function to the anti-oxidant activity of the plant. The Standard curve of Quercetin is shown in figure 13.

**Table 11: Observation of absorbance in total flavonoid estimation**

| Sr.No. | Concentration (µg/ml) | Absorbance | Flavonoid content of Petroleum ether extract | Flavonoid content of Methanolic extract | Flavonoid content of Acetone extract |
|---|---|---|---|---|---|
| 1. | 200 | 0.138 | 38.81mg/g | 18.11mg/g | 8.11mg/g |
| 2. | 400 | 0.250 | | | |
| 3. | 600 | 0.378 | | | |
| 4. | 800 | 0.454 | | | |
| 5. | 1000 | 0.547 | | | |
| 6. | Equation of line | Y = 0.0005x + 0.0468 | | | |
| 7. | Coefficient ($R^2$) | $R^2$ = 0.9594 | | | |

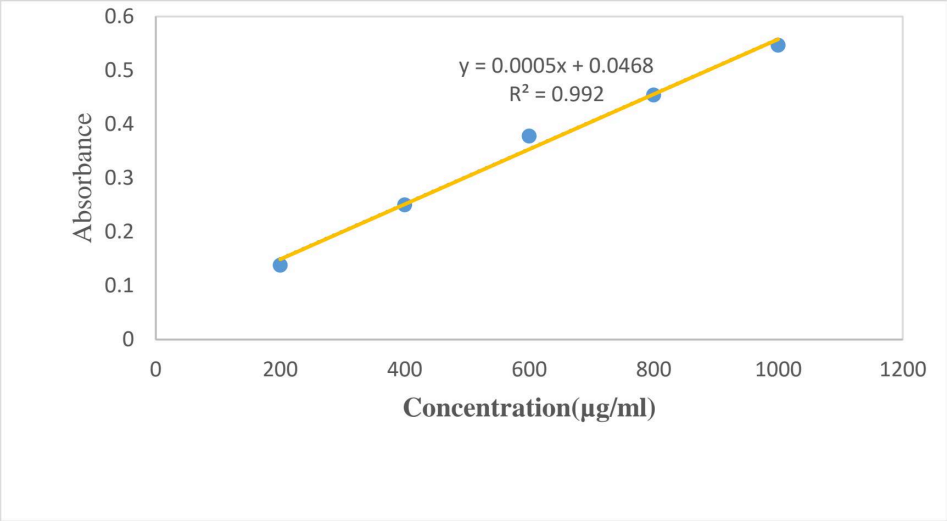



**Figure 13. Standard curve of Quercetin**

### 6.8.3: INHIBITION OF DPPH RADICALS

The drop in DPPH's absorbance at 517 nm, which is brought on by antioxidants, was used to assess its capacity for reduction. The extract showed maximum hydrogen donating ability in the presence of DPPH free radicals at high concentrations. The radical scavenging activity of each extract was compared with ascorbic acid and the IC50 values were found to be 5.32µg/ml, 3.95µg/ml, 2.35µg/ml and 0.11µg/ml for Ascorbic acid, Petroleum ether extract, Methanol extract, Acetone extract respectively in table no.12. Comparative IC 50 Values of the samples and standard in DPPH Model shown in Figure 15.

**Table 12 : DPPH Radical Scavenging Assay of Standard sample and Test Sample**

| Sr No. | Concentration (µg/ml) | Percentage Inhibition | | | |
|---|---|---|---|---|---|
| | | Standard( Ascorbic acid) | Petroleum extract | Methanol extract | Acetone extract |
| 1 | 200 | 15.49 | 35.45 | 41.99 | 15.11 |
| 2 | 400 | 25.94 | 37.66 | 47.37 | 20.61 |
| 3 | 600 | 30.07 | 38.45 | 60.36 | 24.01 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 800 | 40.92 | 40.68 | 72.57 | 30.56 |
| 5 | 1000 | 45.07 | 46.58 | 74.04 | 35.90 |
| **6** | **IC 50 Value** | **5.32434018** | **3.9596419** | **2.35967** | **0.118978** |

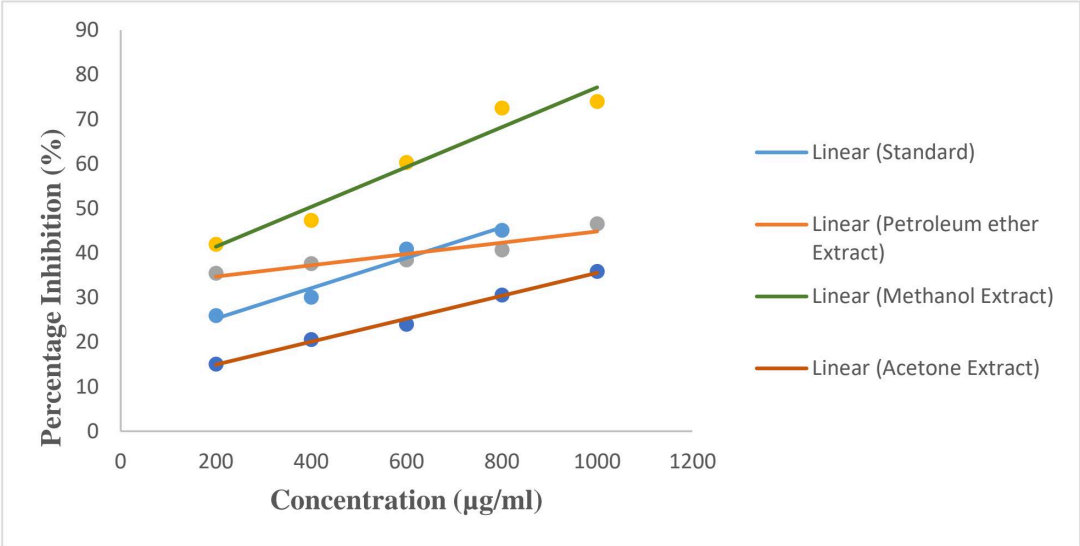



**Figure 14. DPPH Radical Scavenging Assay**

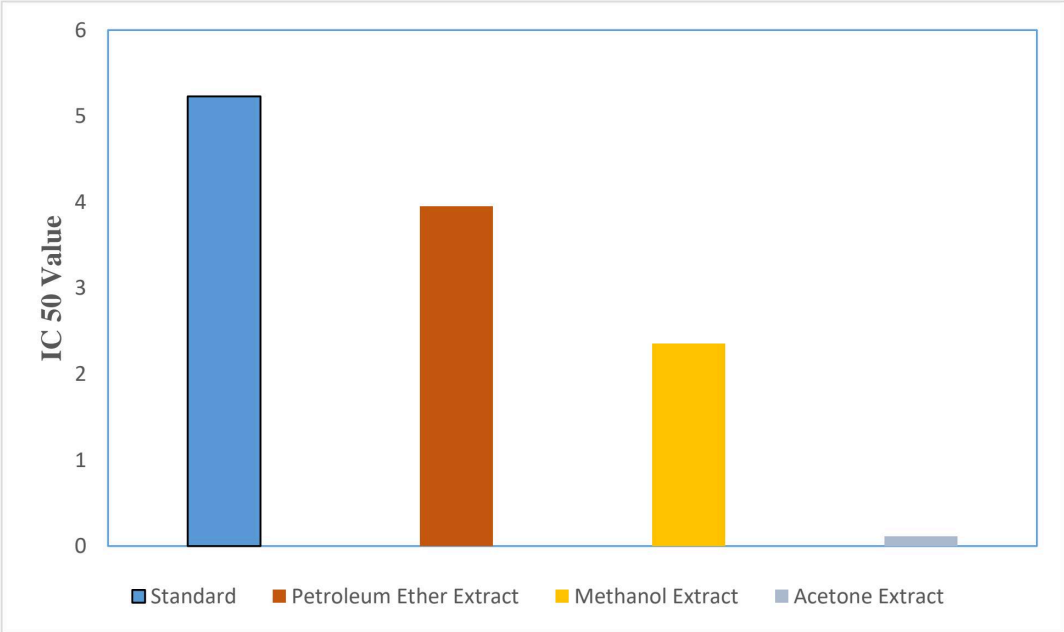



**Figure 15. Comparative IC 50 Values of the samples and standard in DPPH Model**

### 6.8.4: INHIBITION OF HYDROGEN PEROXIDE RADICALS

In addition to being highly reactive, hydrogen peroxide itself can occasionally be hazardous to cells due to the hydroxyl radical it produces inside them. Thus removing $H_2O_2$, as well as $O_2$ is very important for the protection of food systems. Here hydrogen peroxide scavenging activity of the extract is compared with ascorbic acid. The table below displays the outcomes. The $IC_{50}$ value of ascorbic acid and each extract were 4.34µg/, 3.76µg/ml , 2.38µg/ml and 1.678µg/ml respectively. The results showed that the test sample was effective in scavenging hydrogen peroxide in a dose- dependent manner. The outcome is comparable to that of the ascorbic acid reference standard. Figure 16.represents hydrogen peroxide radical scavenging assay of standard and test sample. Comparative IC 50 Values of the samples and standard in %Hydrogen Peroxide scavenging activity in Figure 17.

**Table 13: H2O2 radical scavenging activity of Extract and Standard**

| **Sr No.** | **Concentration (µg/ml)** | **Percentage Inhibition** | | | |
|---|---|---|---|---|---|
| | | **Standard( Ascorbic acid)** | **Petroleum extract** | **Methanol extract** | **Acetone extract** |
| 1 | 100 | 50.07 | 40.23 | 45.20 | 30.42 |
| 2 | 200 | 55.44 | 56.75 | 51.35 | 40.36 |
| 3 | 300 | 59.74 | 61.88 | 56.14 | 45.88 |
| 4 | 400 | 65.11 | 67.63 | 60.66 | 50.50 |
| **5** | **IC 50 Value** | **4.34568756** | **3.76459812** | **2.38765423** | **1.67890945** |

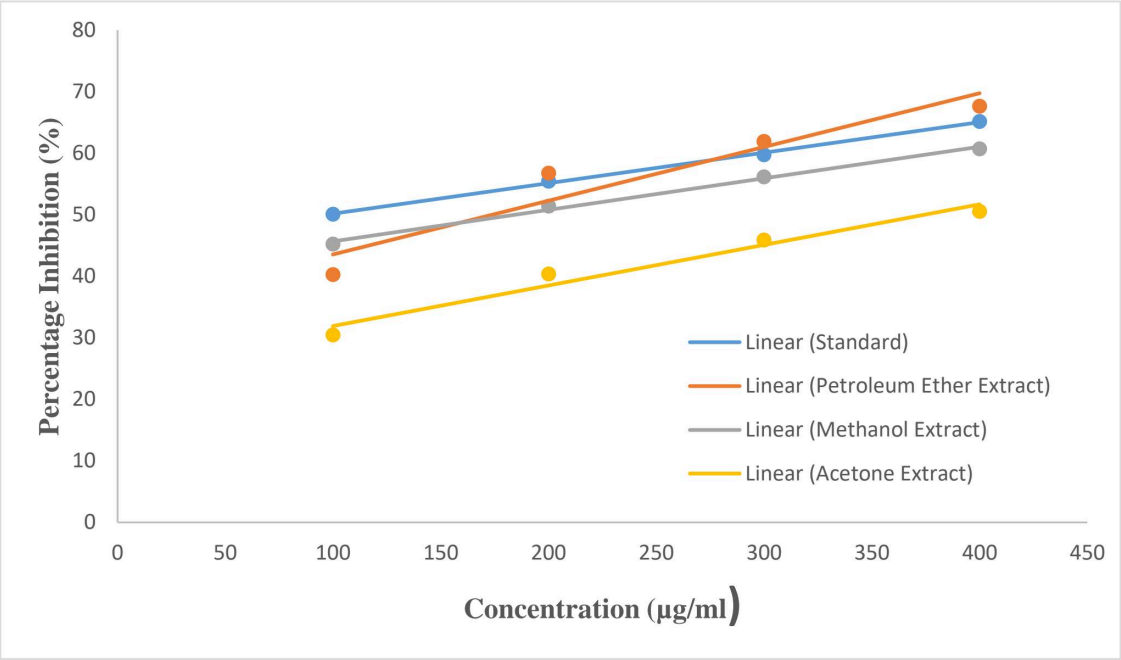



**Figure 16:%Hydrogen Peroxide scavenging activity**

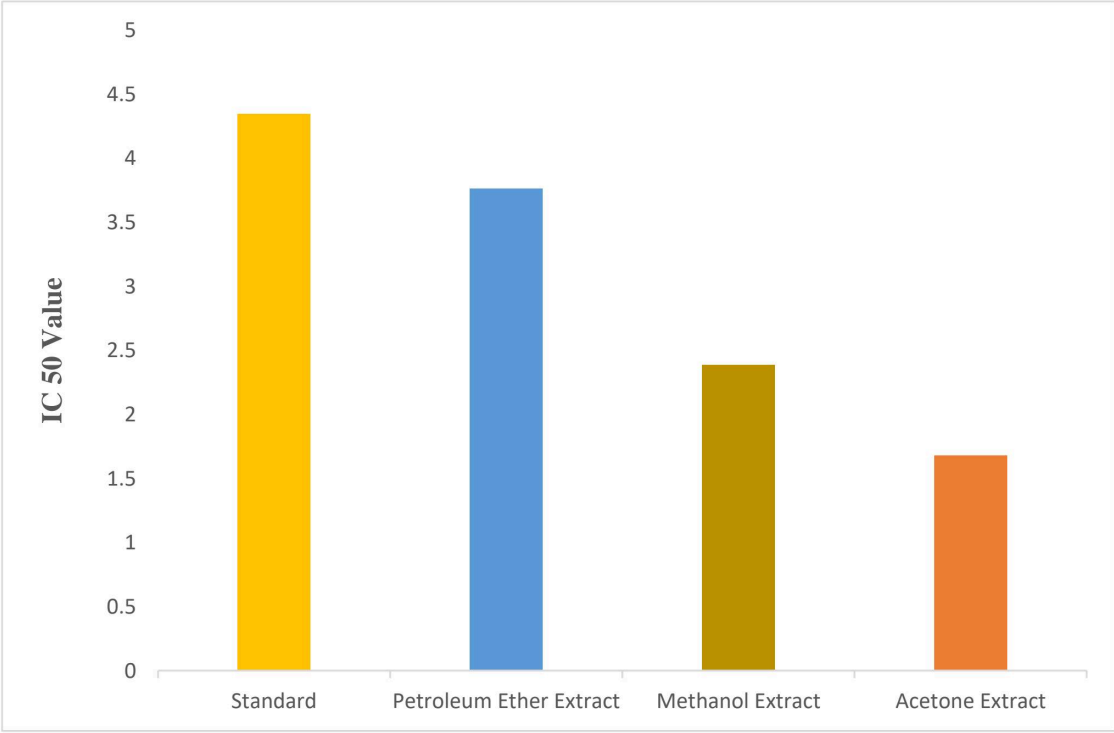



**Figure 17:Comparative IC 50 Values of the samples %Hydrogen Peroxide scavenging activity**

## 6.9: DETERMINATION OF *IN-VITRO* ANTIDIABETIC ACTIVITY

### 6.9.1:α-AMYLASE INHIBITION ASSAY

α-amylase is one of the enzymes that hydrolyses the α-1,4 glycosidic linkages in starch to produce glucose and maltose. α-amylase hydrolyses complex polysaccharides into oligosaccharides and disaccharides and then hydrolyses by α-glucosidase to monosaccharides. The in vitro antidiabetic activity was evaluated by using α amylase inhibitory assay. The Petroleum extract ,Methanolic extract and Acetone extract of *Trema orientalis* produces some inhibitory effects on this enzyme. Acarbose was used as a standard which shows marked inhibitory effect of the enzyme . The $IC_{50}$ of Acarbose , Petroleum extract, Methanolic extract and Acetone extract was calculated and found to be 225.50 µg/ml ,118.88µg/ml ,140.01 µg/ml and 96.56 µg/ml respectively. Table 14 shows the α-amylase inhibitory activity of Standard and test sample. Figure 18 represents the graph of α-amylase inhibition assay of Acarbose and test sample.

**Table 14: α -amylase inhibition activity of Extract and Standard**

| **Sr No.** | **Concentration (µg/ml)** | **Percentage Inhibition** | | | |
|---|---|---|---|---|---|
| | | **Standard(Acarbose)** | **Petroleum extract** | **Methanol extract** | **Acetone extract** |
| 1 | 200 | 33.75 | 32.65 | 10.25 | 12.75 |
| 2 | 400 | 49.22 | 40.01 | 13.32 | 39.60 |
| 3 | 600 | 59.20 | 48.89 | 20 | 41.24 |
| 4 | 800 | 67.22 | 52.34 | 32.51 | 45.15 |
| 5 | 1000 | 73.97 | 60.46 | 35.40 | 49.50 |
| **6** | **IC 50 Value** | **225.50** | **118.88** | **140.01** | **96.56** |

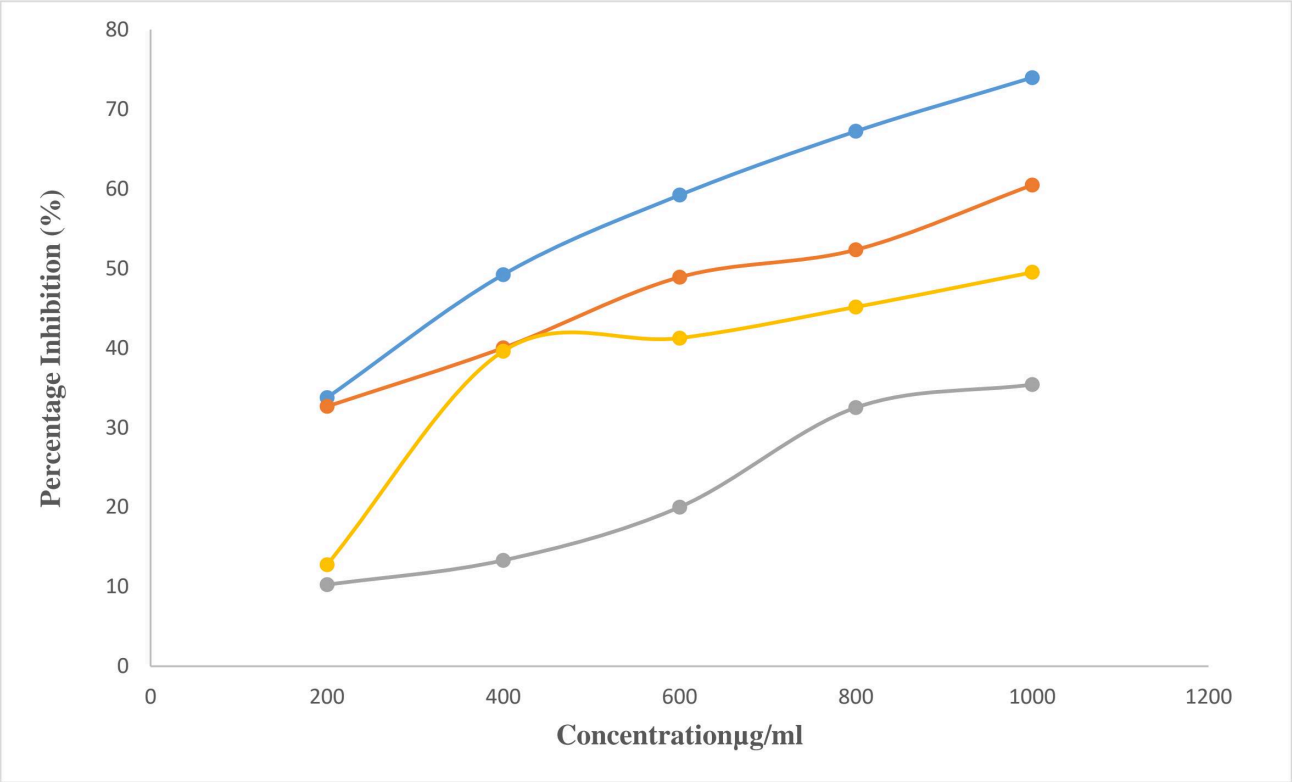



**Figure 18: Graph of α-amylase inhibition assay of Acarbose and test sample.**

## 6.10: DETERMINATION OF *IN-VITRO ANTI-INFLAMMATORY*

### 6.10.1:HUMAN RED BLOOD CELL (HRBC) MEMBRANE STABILIZATION METHOD

The Human Red Blood Cell (HRBC) membrane stabilization method is widely used to evaluate the anti-inflammatory properties of plant extracts, compounds, or drugs. This method is based on the principle that certain substances can stabilize the membrane of red blood cells, preventing lysis (rupture) when exposed to hypotonic or heat-induced stress. The in-vitro anti-Inflammatory activity was evaluated by using Human Red Blood Cell (HRBC) membrane stabilization method. The Petroleum extract ,Methanolic extract and Acetone extract of *Trema Orientalis* produces some inhibitory effects on this enzyme. Diclofenac sodium was used as a standard which shows marked inhibitory effect of the enzyme . The $IC_{50}$ of Diclofenac , Petroleum extract, Methanolic extract and Acetone extract was calculated and found to be 21.674 µg/ml ,13.461µg/ml ,12.477 µg/ml and

8.254 µg/ml respectively. Table 15 shows the anti-inflammatory inhibitory activity of Standard and test sample. Figure 19 represents the graph of anti-inflammatory inhibition assay of diclofenac and test sample.

**Table 15: Anti-inflammatory Activity of Standard and Extract By HRBC membrane stabilization**

| Sr. No. | Concentration(µg/ml) | Percentage Inhibition | | | |
|---|---|---|---|---|---|
| | | Standard(Diclofenac sodium) | Petroleum extract | Methanol extract | Acetone extract |
| 1 | 200 | 13.69 | 16.80 | 12.80 | 10.12 |
| 2 | 400 | 24.02 | 29.20 | 20.20 | 15.34 |
| 3 | 600 | 30.16 | 44.10 | 25.10 | 20.2 |
| 4 | 800 | 43.14 | 50.19 | 30.19 | 25.56 |
| 5 | 1000 | 61.16 | 60 | 40 | 31.34 |
| **6** | **IC 50 Value** | **21.674** | **13.461** | **12.477** | **8.254** |

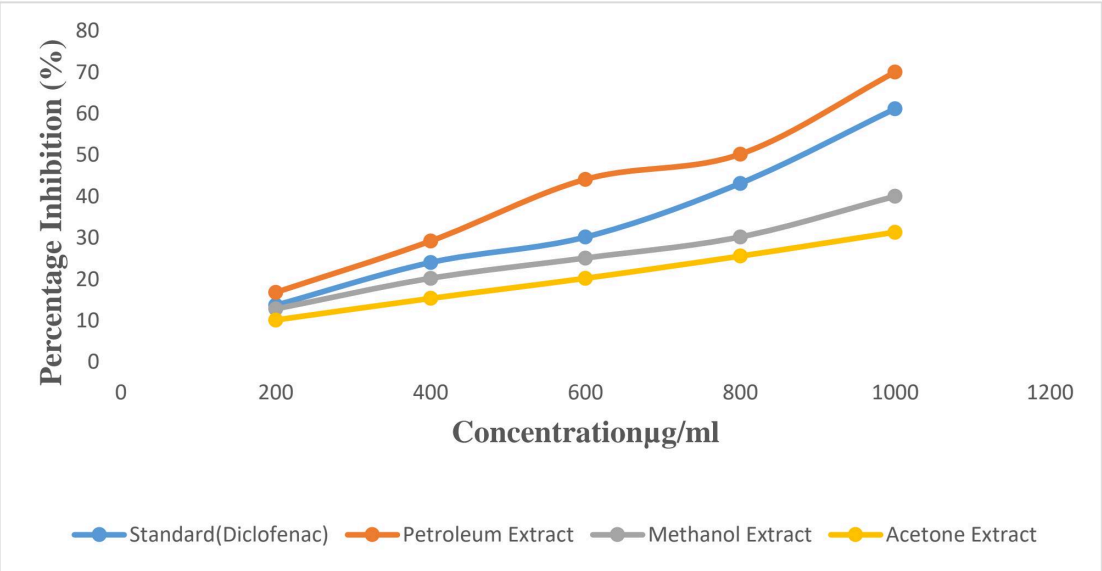



**Figure 19: Graph of anti-inflammatory activity of Standard and test sample**

## 7.DISCUSSION

The present study provides evidence of the antioxidant, antidiabetic, and anti-inflammatory activities of the extracts of *Trema orientalis*. The effectiveness of botanicals in lowering sugar levels has been validated using various Indian plant species. This thesis pioneers scientific investigations into the petroleum ether, methanolic, and acetone extracts of the leaves of *Trema orientalis* in the research field. The mature leaves of *T. orientalis* were collected from Siliguri, West Bengal, India. After collection, the leaves were cleaned, shade-dried, and ground into a coarse powder. The powdered plant material was extracted using petroleum ether, methanol, and acetone through Soxhlet extraction. The extracts were then filtered and evaporated to dryness to obtain the dry extracts used in the study.

Qualitative phytochemical analysis of the petroleum ether extract revealed the presence of carbohydrates, terpenoids, steroids, tannins, and phenolic compounds, while the methanolic extract revealed the presence of carbohydrates, reducing sugars, terpenoids, and phenolics. The acetone extract, on the other hand, revealed the presence of flavonoids, tannins, and phenolics through phytochemical analysis.

According to the TLC analysis, the acetone extract of *Trema orientalis* showed six spots, while the methanol and petroleum ether extracts each showed five spots.

The study also demonstrates that a higher alcohol concentration in the tincture increases its acidity, while storage over a longer period also slightly increases the acidic properties. The solid content of the tincture increases over time, while greater alcohol concentration results in diminished solid content. Additionally, the specific gravity of the prepared tinctures does not vary substantially over time.

The total phenolic content, measured in milligrams of Gallic Acid Equivalent (GAE), was found to be 36.5 mg/g for the petroleum ether extract, 20.6 mg/g for the methanolic extract, and 7.4 mg/g for the acetone extract.

The flavonoid content, measured using aluminum chloride reagent in terms of quercetin equivalent, was found to be 38.81 mg/g for the petroleum ether extract, 18.11 mg/g for the methanolic extract, and 8.11 mg/g for the acetone extract.

The anti-oxidant capacity was assessed by measuring the decrease in DPPH absorbance at 517 nm, indicating hydrogen donation by anti-oxidants. The extract showed maximum hydrogen-donating ability in the presence of DPPH free radicals at high concentrations. The radical scavenging activity of each extract was compared with that of ascorbic acid, with IC50 values of 5.32 µg/ml, 3.95 µg/ml, 2.35 µg/ml, and 0.11 µg/ml for ascorbic acid, petroleum ether extract, methanolic extract, and acetone extract, respectively.

The study also evaluated the hydrogen peroxide scavenging activity of the extracts, as $H_2O_2$ can be hazardous to cells due to the hydroxyl radicals it generates. The IC50 values for ascorbic acid and the extracts were 4.34 µg/ml, 3.76 µg/ml, 2.38 µg/ml, and 1.678 µg/ml, respectively.

The in vitro anti-diabetic activity was evaluated using an α-amylase inhibitory assay. The petroleum ether, methanolic, and acetone extracts of *Trema orientalis* exhibited inhibitory effects on this enzyme, with IC50 values of 225.50 µg/ml, 118.88 µg/ml, 140.01 µg/ml, and 96.56 µg/ml, respectively, when compared with acarbose, a standard inhibitor.

Finally, the anti-inflammatory activity was evaluated using the Human Red Blood Cell (HRBC) membrane stabilization method. The petroleum ether, methanolic, and acetone extracts of *Trema orientalis* showed inhibitory effects, with IC50 values of 21.674 µg/ml, 13.461 µg/ml, 12.477 µg/ml, and 8.254 µg/ml, respectively, compared to diclofenac sodium, which was used as the standard.

## 8. CONCLUSION

The study confirms the presence of significant anti-oxidant, anti-diabetic, and anti-inflammatory properties in the extracts of *Trema orientalis*. The petroleum ether extract, in particular, showed the highest potency across various assays. The total phenolic content in the petroleum ether extract was 36.5 mg/g, and the flavonoid content was 38.81 mg/g. The DPPH radical scavenging activity of the petroleum ether extract was observed at 3.95 µg/ml, and its hydrogen peroxide scavenging activity was 3.76 µg/ml. The in vitro anti-diabetic activity, evaluated using the α-amylase inhibitory assay, showed a high concentration of 118.88 µg/ml in the petroleum ether extract. The anti-inflammatory activity, assessed using the Human Red Blood Cell (HRBC) membrane stabilization method, yielded an IC50 value of 21.674 µg/ml for the petroleum ether extract. These findings contribute valuable insights into the potential therapeutic applications of *Trema orientalis* and lay the groundwork for future research into its bioactive compounds and mechanisms of action, particularly through in vivo studies of the extracts.

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