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# Phytochemical Screening, HPTLC Fingerprint Analysis and Antibacterial Activity of Diethyl Ether Extract of *Ambrosia Artemisiifolia* L. Leaves

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## ABSTRACT

*Ambrosia artemisiifolia L.* Commonly known as Nivali belongs to family *Asteraceae*. *Ambrosia artemisiifolia L.* is a plant of Indian origin having tremendous therapeutic potential but is not fully utilized. The present study, primarily aims to carry out a preliminary phytochemical screening so as to detect the major class of compounds present in *Ambrosia artemisiifolia L.* leaves and to perform thin layer chromatography (TLC) profiling of all sequential extracts. Phytochemical analysis was performed by various qualitative methods and TLC profiling was carried out using diethyl ether extracts. The solvent system of varying polarity ethyl acetate: n-hexane (1:1) and ethyl acetate: chloroform (1:1) respectively. Qualitative phytochemical analysis reflects the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins and terpenoids in the plant extract. TLC profiling of the *Ambrosia artemisiifolia L.* was constituted different colored phytochemical compounds with different Rf values. The present study provides evidence that solvent extract of *Ambrosia artemisiifolia L.* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. The present study was aimed to determine the antibacterial efficacy of *Ambrosia artemisiifolia Linn*. leaf extracts against secondary bacterial pathogens such as Escherichia coli and Staphylococcus aureus and to investigate the presence of phytocompounds through High Performance Thin Layer Chromatographic (HPTLC) method of the potential extract. The varying degree of extract concentrations has a greater influence in the inhibitory effect against test pathogens. The different Rf values, maximum percentage concentration, area percentage of polyvalent chemical constituents was recorded in HPTLC profiling of Diethyl ether leaf extract, where the maximum percentage concentration was found to be 14.07% at 0.09 Rf. The HPTLC studies has confirmed that the compounds present in the diethyl ether extract might be responsible for the inhibitory effect against the bacterial pathogens and are more soluble in semi-polar solvent. Therefore, the present investigation forms the basis as preliminary study of antibacterial efficacy of *Ambrosia artemisiifolia L.* leaf extracts and phytocompound HPTLC profiling of potential extract, which could be used for quality evaluation of compound and standardization of drug in future work. Keywords: *Ambrosia Artemisiifolia L.*, Phytochemical Analysis, Thin Layer Chromatography, Diethyl Ether Extract, Ethyl Acetate, N-Hexane, Chloroform, High Performance Thin Layer Chromatography, Antibacterial Activity.

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## 1. INTRODUCTION

The substantial proportion of the population of India have been using traditional medicines since many centuries. Medicinal herbs with high therapeutic value are used to treat multitude of ailments and diseases. Plants synthesize abundant chemical compounds (phytochemicals) that possess pharmacological actions with medicinal properties widely used in traditional medicine, since pre- historic times. Ambrosia artemisiifolia L. constituted one largest genus of tropical plants. It is much branched, and grows up to 70cm in height. The pinnately divided soft and hairy leaves are 3-7cm long. Its bloom period is July to October in tropical areas.[1] The leaves of these plants are traditionally used in the treatment of strangury and other urinary tract infections. The other reported pharmacological activities of Ambrosia artemisiifolia leaves include antibacterial and antimicrobial activity. [2,4] A reliable pharmacological or clinical study must employ well authenticated plant material. Thus, the present investigation was aimed to investigate the phytochemical analysis for identification and authentication of the plant.

**Fig. no 1: *Ambrosia artemisiifolia L.***

Plant The aim of present study was to investigate the presence of phytochemicals and to determine anti-bacterial efficacy against secondary bacterial pathogens such as Escherichia coli and Staphylococcus aureus of diethyl ether extract of *Ambrosia artemisiifolia* leaves. volume of analyte in HPTLC plate, which serves as a major advantage when compared to other analytical methods. [28-31] The varying degree of extract concentrations has a greater influence in the inhibitory effect against test pathogens.

The HPTLC studies has confirmed that the compounds present in the diethyl ether extract might be responsible for the inhibitory effect against the bacterial pathogens and are more soluble in semi-polar solvent.[30] Therefore, the present investigation forms the basis as preliminary study of antibacterial efficacy of A. artemisiifolia leaf extracts andphytochemical HPTLC profiling of potential extract, which could be used for quality evaluation of compound and standardization of drug in future work. The substantial proportion of the population of India have been using traditional medicines since many centuries. *Ambrosia artemisiifolia L.* constituted one largest genera of tropical plants[1] .

It is much branched, and grows up to 70cm in height. The pinnately divided soft and hairy leaves are 3-7cm long. It's bloom period is July to October in tropical areas.

The plant leaves reported to contain several phytochemicals [2]. These leaves are traditionally used in the treatment of strangury and other urinary tract infections. The other reported pharmacological activities of *Ambrosia artemisiifolia* leaves include antibacterial and antimicrobial activity[4]. A reliable pharmacological or clinical study must employ well authenticated plant material. Thus the present investigation was aimed to investigate the phytochemical analysis for identification and authentication of the plant[5].

Origin: [1-4]

*Ambrosia artemisiifolia*, also known as common ragweed, is native to North America. Its exact origin is unclear, but it is believed to have evolved in the eastern and central regions of the continent.

- Distribution*: Ambrosia artemisiifolia* has become a widespread invasive species, with a global distribution that includes:

North America: Europe: Asia: South America: Australia: Morphological Characteristics:

1. Annual Herb: *Ambrosia artemisiifolia* is an annual herb that grows up to 1-2 meters tall.
2. Stem: The stem is erect, branched, and covered with fine hairs.
3. Leaves: The leaves are alternate, pinnate, and have a distinctive fern-like shape.
4. Flowers: The flowers are small, greenish-yellow, and arranged in spikes at the top of the stem.

Other Characteristics: 1. Allergenic Pollen: The plant produces highly allergenic pollen, which can cause significant respiratory issues.

Invasive Species: *Ambrosia artemisiifolia* is considered an invasive species in many parts of the world, outcompeting native vegetation and altering ecosystem dynamics.

General Characteristics: 1. Annual Herb: *Ambrosia artemisiifolia* is an annual herb. 2. Height: 1-2 meters (3-6 feet) tall. 3. Stem: Erect, branched, and covered with fine hairs.

Scientific classification:[1]

1. Kingdom: Plantae
2. Clade: Angiosperms
3. Clade: Eudicots
4. Clade: Asterids
5. Order: Asterales
6. Family: Asteraceae
7. Genus: *Ambrosia*
8. Species: A. artemisiifolia

Chemical composition and uses: This plant is addressed to contain various phytochemicals which are essential to show antimicrobial ant antioxidant activity. Thus, this plant is used to treat various infectionary diseases like urinary tract infections. [4]

***STRANGURY***

Strangury is a medical term referring to painful, frequent urination of small volumes, often accompanied by a sense of urgency and incomplete emptying. It's essentially painful, slow, and difficult urination, sometimes described as drop-by-drop urination. ❖ Symptoms:

- Pain and Urgency: The sensation of needing to urinate is intense and painful, even with a small amount of urine being passed. • Small Volumes: The urine produced is usually in very small amounts. • Slow and Difficult: Urine is expelled slowly, often requiring straining, and there can be a feeling of not completely emptying the bladder.
- Possible Causes: Strangury can be a symptom of various conditions, including urinary tract infections, bladder stones, or other issues affecting the urethra or bladder.

Distinguishing from Dysuria: While both strangury and dysuria involve painful urination, strangury specifically emphasizes the slow, drop-by-drop urination and the feeling of urgency, as opposed to dysuria, which can be more broadly described as painful urination.

This study is therefore essential to:

- Identify and characterize the phytochemicals present in the diethyl ether extract using HPTLC.
- Evaluate the antibacterial potential of the extract against clinically relevant bacterial strains.
- Contribute to the scientific validation of a traditionally known plant and explore its potential for natural drug development. The outcomes of this study may support the use of A. artemisiifolia as a source of new plant derived antibacterial agents, offering an alternative route in combating antimicrobial resistance and enriching the current pharmacopeia with natural products.

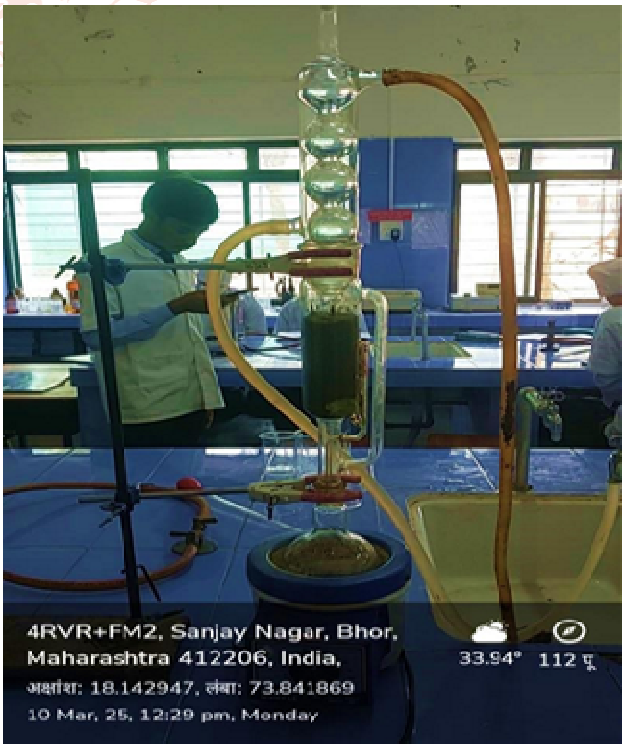
**Material and Methods Plant collection**

Plant was selected for this study is based on its traditional medicinal use. Fresh leaves of plant were collected from rural areas

**Preparation of plant extract**

After collection of plant material the fresh leaves were dried at room temperature until they were free from moisture. The leaves were grinded to get coarse powder and stored in clean and dry air tight container. The powder of leaves of *A. artemisiifolia L.* was subjected to Soxhlet extraction using diethyl ether as a solvent until the final output becomes colourless solvent. The extract was dried or evaporated at room temperature to get semisolid mass and stored in wide mouth air tight container for further use.


**Fig. no. 2: Extract of *Ambrosia artemisiifolia* Leaves**



Preliminary Phytochemical Screening of Extract of *A. artemisiifolia L.* Leaves: Qualitative phytochemical analysis of *Ambrosia artemisiifolia L.* leaves were carried out using standard procedures to identify the constituents such as alkaloids, flavonoids, tannins, phenolics and terpenoids as described. The colour intensity and appearance of solids in reaction mixture was observed.

Qualitative Phytochemical Screening [12-14]:

**Tests for Alkaloids**

**Tests for Carbohydrates**

| Sr. No. | Test | Procedure | Observations |
|---|---|---|---|
| 1 | Barfoed's test | 1mL filtrate +1mL Barfoed's reagent+ Heated for 2 min. | A red (monosaccharides) |

**Tests for Flavonoids**

| Sr. No. | Test | Procedure | Observations |
|---|---|---|---|
| 1 | Lead acetate test | 1mL plant extract + few drops of 10% lead acetate solution | A yellow precipitate |
| 2 | Ferric chloride test | Extract aqueous solution + few drops of 10% ferric chloride solution | green precipitate |
| 3 | Conc. H2SO4 test | Plant extract + conc. H2SO4 | orange colour |

**Tests for Tannins**

| Sr. No. | Test | Procedure | Observations |
|---|---|---|---|
| 1 | Gelatin test | Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl | A white precipitate |

**Tests for Terpenoids**

| Sr. No. | Test | Procedure | Observations |
|---|---|---|---|
| 1 | Conc. H2SO4 test | Filtrate + few drops of conc. H2SO4 (shaken well and allowed to stand). | Golden yellow layer is observed at the bottom o the test tube |
| 2 | Salkowski's test | 5 ml extract was dissolved in chloroform (2 ml) and then 3ml Concentrated sulphuric acid (1 ml) was added to the solution. | Formation of reddish brown coloured interface |

Chromatographic Purification: a) Thin Layer Chromatography (TLC) Study: [21-23] TLC was carried out to isolate the principal components that were presents in most effective extracts of plants the TLC was used by different solvent systems. • Solvent system: Ethyl acetate: n-Hexane (1:1) and Ethyl acetate: Chloroform (1:1).

Formula :

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

High Performance Thin Layer Chromatography (HPTLC) Study: High- performance thin-layer chromatography is one of the sophisticated instrumental techniques based on the full capabilities of thin-layer chromatography. The advantages of HPTLC are automation, scanning, optimization & computerization, selective detection, and minimum sample preparation. High-performance thin-layer chromatography (HPTLC) is useful in detecting chemicals of forensic concern. Various advance techniques in reference to HPTLC like hyphenations in HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser have made HPTLC a power analytical tool in the field of analysis. Applications of HPTLC include phytochemical and biomedical analysis, herbal drug quantification, active ingredient quantification, fingerprinting of formulations, and check for adulterants in the formulations. HPTLC is useful in detecting chemicals of forensic concern.

High-performance thin-layer chromatography (HPTLC) is an improved method of TLC which is used for separation of mixtures and preparative, qualitative, and quantitative analysis of drug samples. [24,26]

Why HPTLC: 1.
This is an optimized analytical method which is suitable for automation and computerization. This is a highly useful method for qualitative and quantitative analysis. 2. This is more rapid and sensitive than other methods. 3. Sample cleanup is easy as the stationary phase is disposable and 100% sample is accountable. 4. HPTLC plates are generally coated with smaller,

narrow size distribution of silica gel particles which lead less diffusion and more compact and concentrated spots for better sensitivity. Since silica gel particles are smaller (5–12 um) size of particles cause smaller size of plates and requires small amount of sample solution (0.lul) for spotting gives shorter development distance which leads to utilize of less amount of mobile phase and shorter duration of time. [28,29]

➢ **HPTLC instrumentation typically includes:**

HPTLC plates: Specialized plates coated with a thin layer of stationary phase.

1. Sample applicator: Device used to apply samples to the HPTLC plate.
2. Chamber: Enclosure used to develop the chromatogram.
3. Scanner: Instrument used to detect and quantify the separated components.
4. Mobile phase: Tolune: Ethyl Acetate: Formic acid (4:2:0.1 v/v/v)

**Procedure:**

HPTLC fingerprinting analysis Chromatography was performed on Merck Silica gel 60 F254 TLC precoated aluminium plates. 20 µl of freshly prepared samples were applied on the plate as a band of 10 mm width with the help of LINOMAT V Automatic Sample Spotter at the distance of 10 mm from the edge of the plate and from each other. Mobile phase was used Tolune: Ethyl Acetate: Formic acid (4:2:0.1 v/v/v) demonstrated best separation. CAMAG twin trough chamber (10x 10 cm) was used for development of plates. Saturation time of 20 minutes was given. The mobile phase was allowed to run to a distance of 80 mm. The evaluation of developed plates was done at the wavelength of 254 nm with Win Cats Software (CAMAG) using TLC scanner.

Applications of HPTLC: HPTLC is widely used in:

1. Pharmaceutical industry.
2. Biomedical research.
3. Clinical analysis.
4. Cosmetic industry.
5. Environmental analysis.
6. Food industry.
7. To analyse the aflatoxins.
8. Composition of brain gangliosides.
9. Quantitative detection of prostaglandins in plasma.
10. Analysis of environmental contaminants.
11. Analysis of Hg in drinking water.
12. Analysis of human skin lipids.
13. Determination of sorbic acid in wine.
14. Characterization of industrial waste.

**Anti-bacterial activity of Diethyl ether extract of *Ambrosia artemisiifolia L.* leaves:**

The rise of antibiotic-resistant bacteria possesses a serious threat to global health, making the search for alternative antimicrobial agents a critical area of research. Plants have long been used in traditional medicine due to their rich diversity of bioactive compounds, many of which possess antimicrobial properties. Numerous studies have demonstrated that plant extracts can effectively inhibit the growth of both Gram-positive and Gram-negative bacteria, suggesting their potential as natural antibacterial agents.

➢ **Phytochemicals with Antibacterial Properties:**

Plant extracts contain various secondary metabolites that contribute to their antibacterial effects. These include:

- ➢ Alkaloids
- ➢ Flavonoids
- ➢ Tannins
- ➢ Saponins
- ➢ Terpenoids

These compounds may act by disrupting bacterial cell walls, interfering with protein synthesis, or inhibiting nucleic acid replication.

➢ **Evaluation of Antibacterial Activity:**

Antibacterial activity is typically assessed using:

- Agar well diffusion and disc diffusion methods (to measure zones of inhibition)
- Broth dilution methods (to determine the Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal Concentration [MBC])
- Time-kill assays

➢ **Procedure:**

The potential of diethyl ether extract of *A. atremisiifolia* leaves to inhibit Gram-positive bacteria like Staphylococcus aureus and Gram- negative bacteria like Escherichia coli was determined by Agar well diffusion method. In this method, the test bacteria were aseptically inoculated into sterile nutrient broth tubes and incubated at 37°C for 24 h. The broth cultures were swab inoculated on sterile nutrient agar plates, with the help of a sterile gel borer, wells of 6 mm diameter were punched in the inoculated plates. Different concentrations (5mg/mL and 10mg/mL) of extract were prepared by dissolving crude extract in 10% dimethyl sulfoxide (DMSO), standard antibiotic [Streptomycin, 1mg/mL of sterile distilled water (positive control)] and 10% DMSO (negative control) were also prepared. 100 µL of different concentration extract along with positive and negative control, were transferred aseptically in labelled wells. The plates were left undisturbed for 30 min and then incubated in upright position for 24 hours at 37°C for bacteria. Using a zone scale or ruler, zone of inhibition formed around the wells was measured. The presence of zone of inhibition around the wells is the indication of antibacterial activity of extract.

**Colour and yield of the extract:**

The colour of the obtained crude diethyl ether extract of *Ambrosia artemisiifolia* was 'Greenish yellow' colour shown in Fig. 4.

Yield of the crude extract: 8.16%

Weight of the empty Petri plate (W1) = 44.34g
Weight of Petri plate with extract (W2) = 51.32g and
Weight of the crude extract (W2-W1) = 5.31g

Yield (%) of crude extract (CE)=

$$\frac{\text{Weight of crude extract} \quad x \quad 100}{\text{Weight of powdered sample taken}}$$

$$= \frac{5.31 \times 100}{65}$$

= 8.16%

Therefore, the yield of the obtained diethyl ether extract of *Ambrosia artemisiifolia* was 8.16%

Chromatographic Purification: a) Thin Layer Chromatography (TLC): 1. Ethyl acetate: n-Hexane (1:1 v/v) TLC of diethyl ether extract of Ambrosia artemisiifolia L. leaves revealed the presence of 2 bands having Rf values 0.87 and 0.22 respectively.

Ethyl acetate: Chloroform (1:1 v/v) TLC of Diethyl ether extract of Ambrosia artemisiifolia L. leaves revealed 2 bands having the Rf values of 0.74 and 0.38 respectively.

**Fig no.3: TLC study**

HPTLC fingerprinting analysis: In the current study HPTLC analysis of

*A. artemisiifolia* diethyl ether extract was performed using quercetin as reference standards, as plant was found to be rich in tannins and flavonoids. The mobile phase Tolune: Ethyl Acetate: Formic acid (4:2:0.1 v/v/v) showed best separation of chemical constituents. The fingerprinting analysis of diethyl ether extract by HPTLC chromatograms revealed the presence of one peak at Rf value as presented in Table 10. Peak 1 in leaf extract (Rf value= 0.653) was found comparable with that of standard Quercetin at wavelength 254 nm.

Test: HPTLC • Stationary Phase: Silica Gel 60 F254 • Mobile Phase: Tolune: Ethyl Acetate: Formic acid (4:2:0.1 v/v/v)

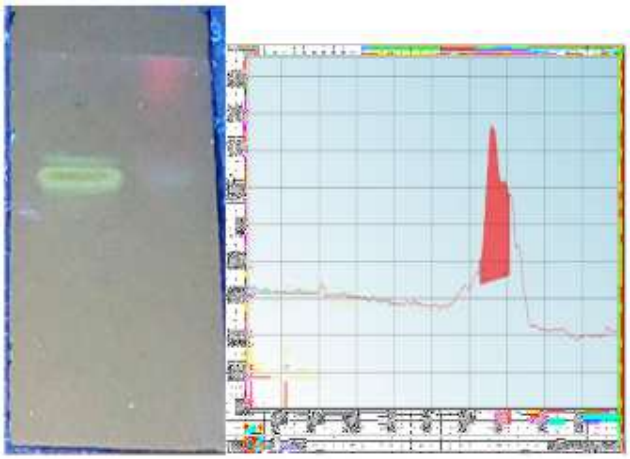


**Fig No. 4: HPTLC Study**

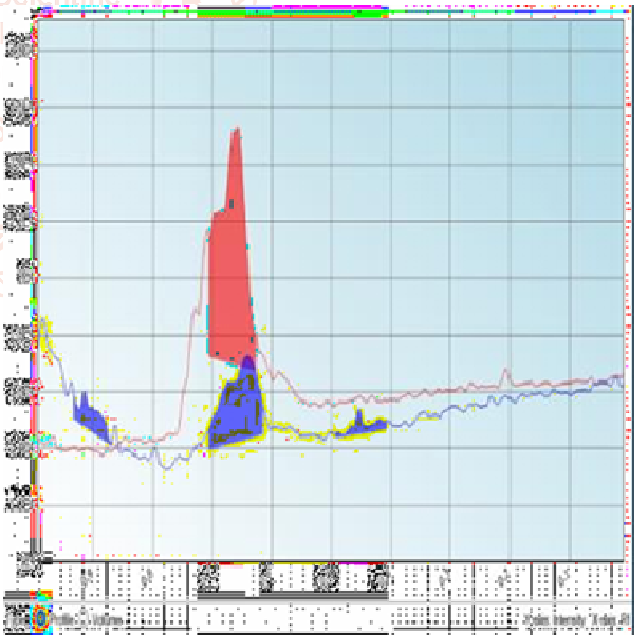


**Fig. No 8: Chromatogram of Quercetin and Extract**

**Table : Peak list and Rf values of chromatograms of *Ambrosia artemisiifolia* Leaf extracts at 254nm**

| Peak No | Maximum Rf | Peak Area | % Area |
|---|---|---|---|
| 1 | 0.653 | 43.71 | 20.02 |

**Lanes and Bands:**

| ID | Width | Bands | Rf | Area |
|---|---|---|---|---|
| Quercetin | 118 | 1 | 0.675 | 3540 |
| Extract | 78 | 1 | 0.653 | 2184 |

- **Anti-bacterial activity of diethyl ether extract of *Ambrosia artemisiifolia L.* leaves:**

Screened the efficacy of diethyl ether extract of *A. artemisiifolia* by agar well diffusion assay. The result of the inhibitory activity of extracts against Gram-positive and Gram-negative bacteria is shown in (Table 11). The presence of inhibition zone around the wells was considered positive for antibacterial activity. Extract of different concentrations were shown to inhibit all test bacteria, marked inhibitory effect was shown against *Staphylococcus aureus* shown in Fig. 8.

- **Observations:**
- standard: 1mg/ml
- Compound: 5mg/ml and 10mg/ml
- Control -DMSO

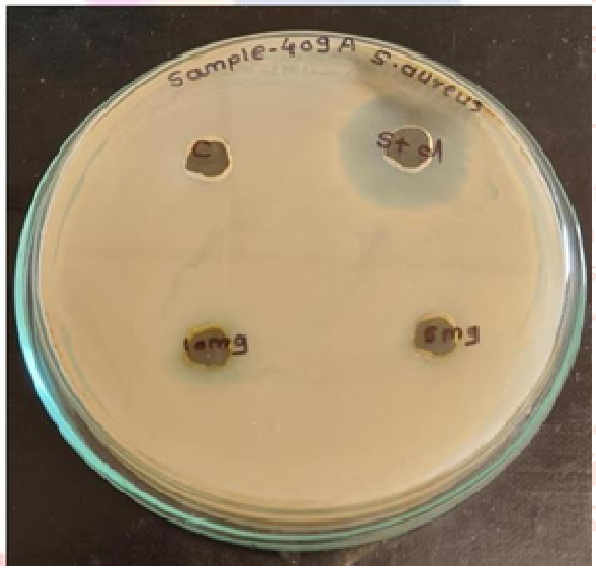



**Fig no.5: Staphylococcus aureus ATCC no.6538**

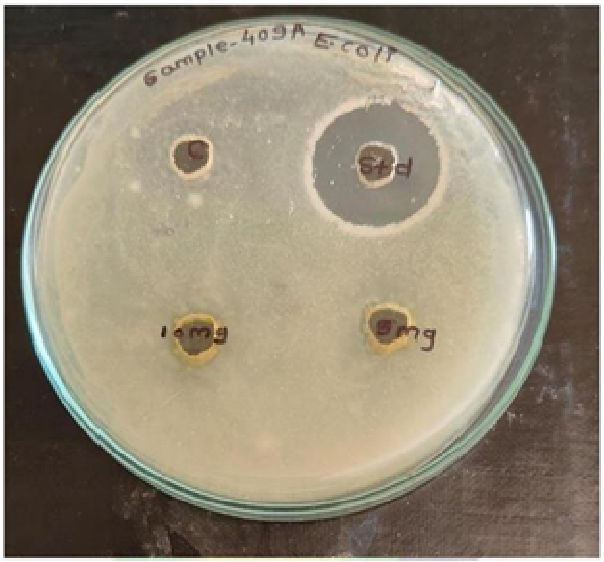



**Fig no.6: Escherichia Coli ATCC no- 8739**

**Table: Antibacterial activity of diethyl ether extract of Ambrosia artemisiifolia.**

| Sr. No. | Sample | Concentration | Zone of Inhibition (mm) |
|---|---|---|---|
| 1. | Control | | - |
| 2. | Standard (Streptomycin) | 1mg/ml | 22 |
| 3. | Sample Ambrosia artemisiifolia. | 5mg/ml | 03 |
| | | 10mg/ml | 08 |

**Discussion**

Qualitative phytochemical screening of plant extracts can help discover pharmaceutical agents. In this study, the diethyl ether extract of *A. atremisiifolia L.* leaves showed significant indications of metabolites, including alkaloids, phenols, tannins, terpenoids, and flavonoids. TLC profiling revealed the presence of various phytochemicals, with different Rf values indicating their polarity. HPTLC fingerprint analysis of the extract of *Ambrosia artemisiifolia* leaves revealed the presence of phytocompounds. The extract's antibacterial activity was tested against *E. coli* and *S. aureus* at concentrations of 5 mg/ml and 10 mg/ml. Results showed a concentration-dependent increase in the zone of inhibition, with weaker activity at 5 mg/ml and improved at 10 mg/ml. However, the extract demonstrated significantly lower antibacterial activity compared to standard antibiotic Streptomycin

**Conclusion**

The study investigated the phytochemical constituents and antibacterial potential of the diethyl ether extract from *Ambrosia artemisiifolia* leaves. The extract, rich in non-polar bioactive compounds, was subjected to HPTLC profiling and antibacterial evaluation. The results showed a concentration-dependent increase in the zone of inhibition against *E. coli* and *S. aureus* at different concentrations. At 5 mg/ml, the zones were small, indicating weak antibacterial activity. At 10 mg/ml, the inhibition zones improved, especially against *Staphylococcus aureus*. However, compared to the standard antibiotic Streptomycin, *Ambrosia artemisiifolia L.* extract demonstrated significantly lower antibacterial activity. The findings support the therapeutic potential of *Ambrosia artemisiifolia* and justify its traditional usage. The observed antibacterial effects suggest that the plant could serve as a source for the development of natural antimicrobial agents. Further studies are recommended to validate and expand upon these preliminary results.

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