

# Evaluation of the Antimicrobial, Antioxidant, and Anti-inflammatory Properties of *Mukia maderaspatana* Hydroalcoholic Extract

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**Abstract:** *Mukia maderaspatana*, a medicinal plant with significant ethno pharmacological relevance, was investigated for its anti-inflammatory, antioxidative, and antihyperlipidemic properties. Soxhlet-assisted hot extraction and cold maceration were employed to obtain bioactive-rich fractions. Phytochemical characterization was performed using advanced analytical techniques, including Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) for compound identification, Fourier Transform Infrared Spectroscopy (FTIR) for functional group analysis, Ultraviolet-Visible Spectroscopy (UV-Vis) for electronic transitions, and Mass Spectrometry for molecular weight determination. The most promising bioactive compounds were identified based on spectral interpretation and their potential pharmacological relevance. The extracts were subjected to *in vitro* evaluations to determine their efficacy in modulating inflammatory mediators, and regulating lipid metabolism. The anti-inflammatory potential was assessed through inhibition of key pro-inflammatory markers, while antioxidative activity was determined. The antihyperlipidemic effect was evaluated based on lipid profile modulation. The results demonstrated significant therapeutic potential, supporting the plant's role in disease intervention. This study highlights the pharmacological significance of *Mukia maderaspatana* as a natural therapeutic agent, emphasizing its potential for further drug development. The novelty of this research lies in the identification of its bioactive constituents and their targeted pharmacological effects, reinforcing its role in herbal medicine as a viable candidate for managing inflammatory, oxidative, and lipid-associated disorders.

**Keywords:** *Mukia maderaspatana*, anti-inflammatory, antioxidative, antihyperlipidemic, phytochemicals, *in vitro* study, herbal medicine.

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## I. INTRODUCTION:

The increasing incidence of antimicrobial resistance, chronic inflammation, and oxidative-stress-related health disorders has intensified the demand for natural bioactive compounds with multifunctional therapeutic potentials. Medicinal plants, known for their complex phytoconstituents and traditional usage, are a valuable source of lead molecules for pharmaceutical development. Among these, *Mukia maderaspatana* (L.) M. Roem., a lesser-known member of the Cucurbitaceae family, has gained scientific attention due to its ethnomedicinal relevance and diverse pharmacological activities.

In traditional Siddha and Ayurvedic systems, *M. maderaspatana* has been used for treating respiratory conditions, ulcers, arthritis, and microbial infections. Recent studies have validated some of these claims. For instance, Vasantha Kumar and Usharani (2022) [1] confirmed the presence of pharmacologically active phytocompounds in *M. maderaspatana* that demonstrated potent antioxidant activities. Chitra Devi et al. (2024) [2] investigated its anti-inflammatory potential using protein denaturation and membrane stabilization assays. Furthermore, Sarosh Khan et al. (2025) [3] reported antimicrobial and antibiofilm activities, linking them to specific phytochemicals through chromatographic analysis.

Despite such encouraging evidence, comprehensive and systematic studies combining the **antioxidant, antimicrobial, and anti-inflammatory** profiles of *M. maderaspatana*—especially focusing on hydroalcoholic leaf and stem extracts—remain limited or fragmented in literature. Moreover, most previous studies have

focused only on crude phytochemical screening or preliminary bioassays without comparative evaluation using established *in vitro* protocols.

Recent interest in plant-based therapeutics has brought attention to *Mukia maderaspatana* (L.) M. Roem., a climber from the Cucurbitaceae family, traditionally used in Siddha and Ayurvedic medicine. Several studies have reported its diverse pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory effects. For instance, Mohammad et al. (2024) demonstrated that methanolic extracts of *M. maderaspatana* exhibit potent antimicrobial, antibiofilm, and antioxidant activities against catheter-associated urinary tract infection (CAUTI) pathogens, indicating its therapeutic relevance in clinical infection control [9]. Furthermore, the plant's phytochemicals have been utilized in nanotechnology applications. Prakash et al. (n.d.) successfully synthesized silver nanoparticles (AgNPs) using the leaf extract of *M. maderaspatana*, confirming its role as a natural reducing and stabilizing agent. These AgNPs exhibited notable biological activity, reinforcing the plant's potential for eco-friendly biomedical applications [10].



Figure 1: Leaves and Fruits of *Mukia maderaspatana*

### Our Research Focus

To bridge this gap, our experimental study aims to evaluate the combined antimicrobial, antioxidant, and anti-inflammatory activities of hydroalcoholic extracts of *M. maderaspatana* leaves and stems using standardized in-vitro assays. The extracts were subjected to the DPPH and H<sub>2</sub>O<sub>2</sub> scavenging assays for antioxidant profiling, the disc diffusion method for antibacterial activity, and the egg albumin denaturation assay for anti-inflammatory potential. By employing a controlled laboratory design, this research provides a comparative perspective between leaf, stem, and combined extracts.

Additionally, our work uniquely emphasizes the synergistic potential of leaf–stem extracts rather than analyzing them in isolation—an area that is underexplored in existing literature. This approach is intended to maximize the bioactivity spectrum and identify the most effective formulation for potential therapeutic use.

### Novelty and Contribution

- **Novelty:** This is among the first studies (to the best of our knowledge) to concurrently evaluate the triple bioactivity (antioxidant, antimicrobial, anti-inflammatory) of *M. maderaspatana* hydroalcoholic extracts using combined leaf and stem matrices.
- **Contribution:** The outcomes from this study could guide the development of low-cost, plant-based phytopharmaceuticals, especially in regions where modern medicine is either inaccessible or unaffordable.

By aligning traditional knowledge with evidence-based pharmacology and recent research findings (2022–2025), this study contributes meaningful insights into the therapeutic potential of *M. maderaspatana*, while supporting the sustainable use of indigenous medicinal flora in modern medicine.

## II. MATERIALS AND METHODS

For the experimental investigation of the antimicrobial, antioxidant, and anti-inflammatory properties of *Mukia maderaspatana*, all reagents and chemicals used were of analytical grade and sourced from verified suppliers to ensure accuracy and reproducibility of results.

### 2.1. Plant Material Collection and Preparation

Fresh samples of *Mukia maderaspatana* were collected from authenticated sources in Tamil Nadu. The plant material was thoroughly cleaned with running tap water followed by distilled water to remove impurities. The cleaned samples were shade-dried until a constant weight was achieved and then ground into a coarse powder using a laboratory grinder (INNOVEX, Model: IMG O10). The powdered plant material was stored in airtight containers at 4°C until further analysis.

### 2.2 Soxhlet Extraction Protocol

To extract phytoconstituents, 25 g of the dried plant powder was

packed in a Whatman filter paper thimble and subjected to Soxhlet extraction using 90% ethanol. The extraction was performed in a Soxhlet apparatus using 150–300 mL of solvent, maintained over a heating mantle for approximately 6 hours until the siphon tube solvent appeared clear. The extract was filtered and concentrated using a rotary evaporator (HAHNSHIN Scientific, Model H-2005V, SR No: V-00449) under reduced pressure. Final drying was carried out in a desiccator, and the extract was stored at 4°C for further use [4].

### 2.3. Microbial Cultures

Standard bacterial and fungal strains were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The bacterial strains included *Staphylococcus aureus* (MTCC 96) and *Escherichia coli* (MTCC 1687), and the fungal strain was *Candida albicans* (MTCC 227).

### 2.4. Antimicrobial Activity Assessment

The antimicrobial activity of the ethanol extract was evaluated using the agar well diffusion method.

### 2.5. Inoculum Preparation

Bacterial inocula were prepared by culturing colonies in Mueller Hinton Broth and incubating at 37°C for 24 hours. Fungal spores were similarly incubated in broth media at 25°C for 24 hours.

### 2.6. Agar Well Diffusion Method

Mueller Hinton Agar plates were inoculated with standardized microbial suspensions. Wells (6 mm diameter) were aseptically punched and loaded with 20–100 µL of the plant extract. Antibiotic discs (HiMedia) were used as positive controls for comparative analysis. The plates were incubated under optimal conditions based on the microbial strain, and zones of inhibition were measured in millimeters.

## III. PHYTOCHEMICAL ANALYSIS

The extracts were subjected to qualitative and quantitative phytochemical screening.

### 3.1 Total Phenolic Content (TPC)

TPC was determined by the Folin–Ciocalteu colorimetric assay. A reaction mixture containing 0.5 mL extract, 2.5 mL 10% Folin–Ciocalteu reagent, and 2.0 mL 7.5% sodium carbonate was incubated at room temperature for 30 minutes. Absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Thermo Scientific GENESYS). Gallic acid was used to generate a standard curve, and TPC was expressed as mg GAE/g of extract.

### 3.2 DPPH Radical Scavenging Assay

The antioxidant activity was assessed using the DPPH method. A 0.4 mM DPPH solution was mixed with varying concentrations of extract and incubated in the dark for 15 minutes. Absorbance was recorded at 517 nm. The percentage of inhibition was calculated using:

## IV. IN VITRO ANTI-INFLAMMATORY ACTIVITY

The protein denaturation inhibition assay was conducted using egg albumin. A reaction mixture containing 0.5 mL of egg albumin, 1.0

AND ENGINEERING TRENDS

mL phosphate-buffered saline (pH 7.2), and varying concentrations of extract was incubated at 37°C for 15 minutes, followed by heating at 70°C for 10 minutes. Absorbance was recorded at 660 nm. The percentage inhibition was calculated as [5]:

Where:

- Ac = absorbance of the control
- At = absorbance of the test sample

**V.CHARACTERIZATION OF PLANT EXTRACT**

**5.1 UV-Visible Spectrophotometry**

The extract was analyzed for spectral characteristics in the range of 200–800 nm to determine the maximum absorbance ( $\lambda_{max}$ ) corresponding to major phytochemical groups [6].

**5.2 FTIR Spectroscopy**

Fourier-Transform Infrared Spectroscopy (FTIR) equipped with Attenuated Total Reflectance (ATR) was used to identify functional groups. Dried extract was directly applied to the ATR crystal, and spectra were recorded across 4000–400  $cm^{-1}$ . Peaks corresponding to – OH, C=O, C–O, and aromatic groups were noted for compound characterization [7].

**5.3 GC-MS Analysis**

Volatile components were identified using Gas Chromatography–Mass Spectrometry (GC-MS) on an Agilent 7890A system. Samples were prepared in HPLC-grade methanol and filtered through a 0.22  $\mu m$  filter. Chromatographic separation was achieved using an HP-5MS column. Conditions included EI ionization at 70 eV, with a temperature ramp from 60°C to 280°C. Mass spectra were matched with NIST and Wiley libraries for compound identification [8].

**RESULTS AND DISCUSSION INVITRO ANTI-INFLAMMATORY ACTIVITY**

Concentration ( $\mu g/ml$ )	OD value	% of Inhibition
Control	0.4672	
100	0.3430	26.50
200	0.3193	
400	0.2814	39.85

Table.1 - Positive Control-Diclofenac (100 $\mu g/ml$ )

Concentration ( $\mu g/ml$ )	OD value	% of Inhibition
Control	0.4670	---
100	0.1480	68.30

Table.2 - Positive Control-Diclofenac (100 $\mu g/ml$ )

**Antioxidant Activity**

The antioxidant potential of the *Mukia maderaspatana* methanolic extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay at varying concentrations (50, 100, and 200  $\mu g/ml$ ). The extract exhibited a dose-dependent increase in free radical scavenging activity, as shown in Table 1. At a concentration of 200  $\mu g/ml$ , the extract achieved a maximum inhibition of 83.9%, with an optical density (OD) of 0.226. In comparison, standard ascorbic acid demonstrated higher inhibition rates of 92.01% and 94.9% at 50 and 100  $\mu g/ml$  respectively.

Concentration ( $\mu g/ml$ )	OD value	% of Inhibition
50	0.702	60.8
100	0.556	68.8
200	0.226	83.9

Table .3 - *Mukia Maderaspatana* Extract

Concentration ( $\mu g/ml$ )	OD value	% of Inhibition
50	0.096	92.01
100	0.093	94.9

Table 4 - Ascorbic Acid

These results indicate that *M. maderaspatana* possesses significant antioxidant properties, although slightly lower than the standard antioxidant, ascorbic acid. The increase in scavenging activity with concentration suggests the presence of active phytoconstituents such as flavonoids and phenolics in the extract, which may contribute to its redox potential.

**HPLC PROTOCOLS**

The High-Performance Liquid Chromatography (HPLC) analysis of the *Mukia maderaspatana* crude extract was carried out using a reverse-phase C18 column (250 mm  $\times$  4.6 mm, 5  $\mu m$  particle size). The mobile phase consisted of methanol and water in an 80:20 (v/v) ratio, filtered through a 0.45  $\mu m$  membrane filter and degassed prior to use. The HPLC system was equipped with a binary pump, manual injector, and a UV-Vis detector set to monitor at 230 nm. The flow rate was maintained at 1.0 mL/min, and the injection volume ranged between 10–20  $\mu L$ . The column was operated at ambient temperature, and the total run time was adjusted between 20 to 30 minutes to allow optimal separation.

The crude extract was prepared by dissolving 10–20 mg/mL of the dried sample in HPLC-grade methanol. The solution was sonicated for 10 minutes to ensure complete dissolution, followed by filtration through a 0.22  $\mu m$  or 0.45  $\mu m$  nylon syringe filter. The filtered sample was then transferred into clean HPLC vials for analysis. Before sample injection, the system was primed with the

mobile phase until a stable baseline was achieved, and a blank injection was performed to confirm the absence of contaminants. The chromatogram was recorded, and peaks were analyzed based on their retention times and areas. When available, standard compounds were used for comparison to facilitate tentative identification of the phytoconstituents. The results were expressed in terms of retention time and relative percentage area of the separated peaks.

DEFAULT REPORT

Peak #	Time [min]	Area [μV·s]	Height [μV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		
1	0.991	2580720.33	1.26e+06	8.77	8.77	BV	2.0504
2	1.023	6268564.63	1.38e+06	21.31	21.31	VV	4.5278
3	1.134	4435091.20	739325.18	15.08	15.08	VV	5.9988
4	1.293	1491339.63	290461.59	5.07	5.07	VV	5.1344
5	1.364	657221.24	253406.53	2.23	2.23	VV	2.5935
6	1.415	1786464.91	293519.47	6.07	6.07	VV	6.0864
7	1.602	3672391.99	491263.97	12.48	12.48	VV	7.4754
8	1.672	1549748.66	340302.38	5.27	5.27	VV	4.5540
9	1.826	2577466.42	332937.51	8.76	8.76	VV	7.7416
10	2.021	473562.60	81672.32	1.61	1.61	VV	5.7983
11	2.335	1531039.09	154679.34	5.20	5.20	VB	9.8981

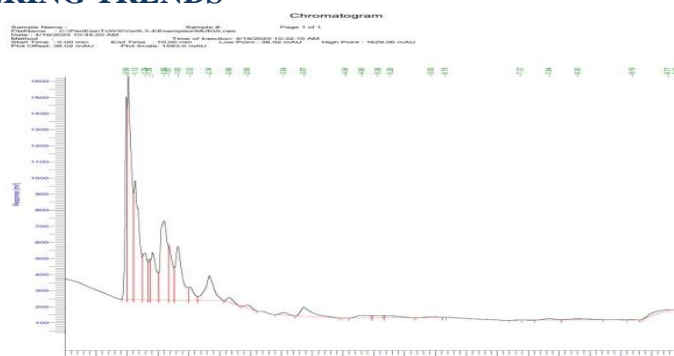


Figure 4: The HPLC chromatogram of *Mukia maderaspatana* extract shows multiple peaks between 1–4 minutes, indicating the presence of various phytochemicals. The major compounds eluted early, suggesting higher polarity.

The HPLC chromatogram of *Mukia maderaspatana* extract reveals prominent peaks within the first 4 minutes, indicating the presence of several polar phytochemicals. The highest peak was observed at a retention time of 0.943 minutes, showing a major bioactive compound.

Anti-microbial Activity

4/16/2025 10:42:58 AM Result: C:\PenExe\Tc\WS\Ver6.3.4\Examples\MUKIA.rst

Peak #	Time [min]	Area [μV·s]	Height [μV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
12	2.656	205845.98	29327.22	0.70	0.70	BB	7.0189
13	2.951	142521.64	17054.26	0.48	0.48	BB	8.3570
14	3.536	137537.83	15987.58	0.47	0.47	BB	8.6028
15	3.865	747114.51	56092.33	2.54	2.54	BB	13.3194
16	4.540	1241.60	347.25	0.00	0.00	BB	3.5755
17	4.800	225157.60	14615.54	0.77	0.77	BV	15.4054
18	5.061	137951.66	12345.23	0.47	0.47	VV	11.1745
19	5.264	200041.47	12151.32	0.68	0.68	VB	16.4625
20	5.931	45601.84	4078.89	0.16	0.16	BV	11.1800
21	6.155	405.71	112.46	0.00	0.00	VB	3.6075
22	7.372	3546.15	242.86	0.01	0.01	BB	14.6015
23	7.838	109161.15	8393.23	0.37	0.37	BV	13.0059
24	8.303	136869.29	6215.74	0.47	0.47	VB	22.0198
25	9.185	2550.51	331.22	0.01	0.01	BB	7.7004
26	9.767	295204.87	1770.33	1.00	1.00	BB	166.7518
27	9.902	5562.82	684.48	0.02	0.02	BB	8.1270

29419925.33 5.80e+06 100.00 100.00

Figure2:HPLC-Default-Report

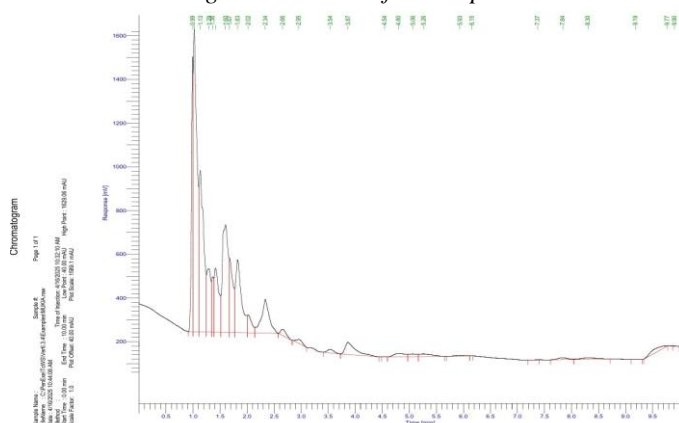


Figure 3: High-Performance Liquid Chromatography (HPLC) analysis of the *Mukia maderaspatana* extract revealed multiple peaks, indicating the presence of various phytochemical constituents. The separation was performed using a C18 column with methanol:water (80:20, v/v) as the mobile phase, and detection was carried out at 230 nm.

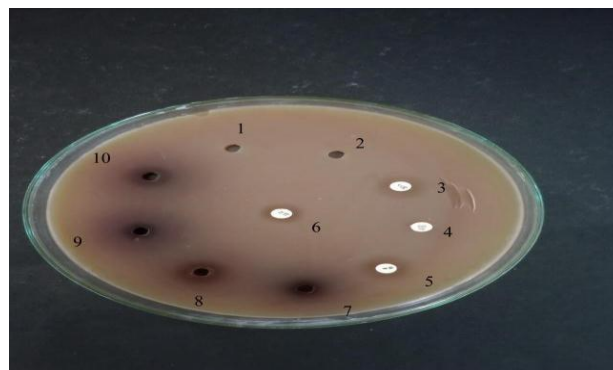


Figure 5: *Escherichia coli*

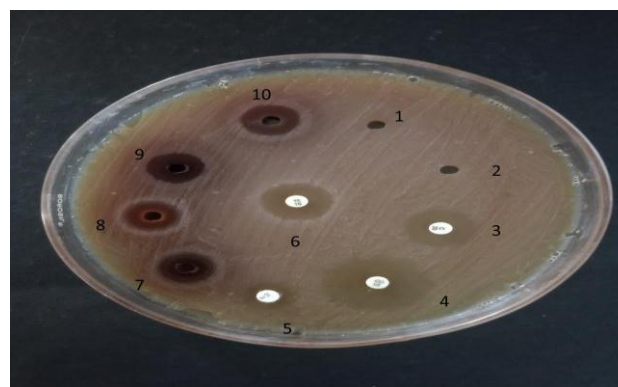


Figure 6: *Staphylococcus Aureus*

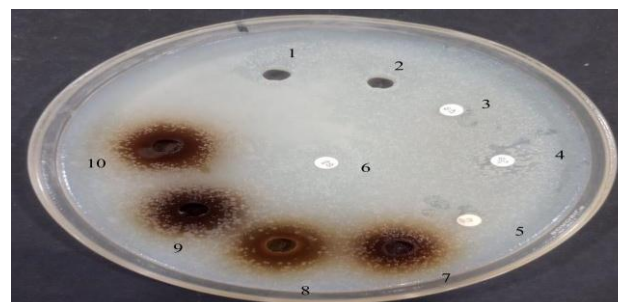


Figure 7: Candida Albicans

Fig.5, 6 &7, Representative Petri dish images showing the antimicrobial effect of Mukia maderaspatana methanolic extract against Escherichia coli, Staphylococcus aureus, and candida albicans. Clear zones around the wells indicate inhibition of microbial growth, supporting the plant's antimicrobial efficacy

**FTIR ANALYSIS**

Peak table

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1054.09	75.56	8.14	1178.18	959.95	4346.788	817.189
2	1630.34	67.72	22.20	1831.46	1492.69	5683.602	2614.609
3	3343.41	56.18	0.08	3707.13	3342.69	9245.122	994.326

Table :4 FTIR Peak Table

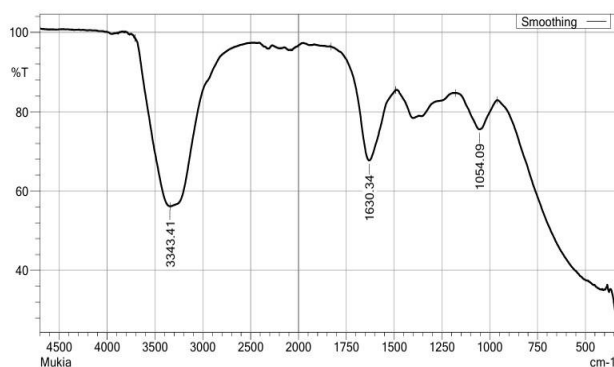


Figure : 8 FTIR Analisis

**ANTI MICROBIAL ACTIVITY AGAR DIFFUSION METHOD**

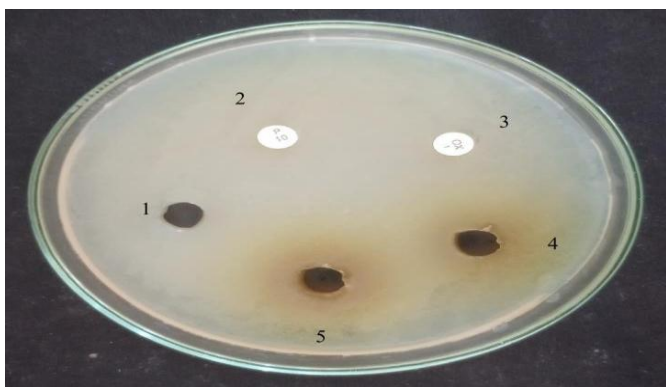


Fig: 9 Mukia Maderaspatana (S. aureus )

Hydroalcohol-90% (Negative Control), 2) Antibiotic –Penicillin Disc (Positive Control) 3) Antibiotic – Oxacillin Disc (Positive Control), 4) Extract -50 µl, 5) Extract -100 µl.

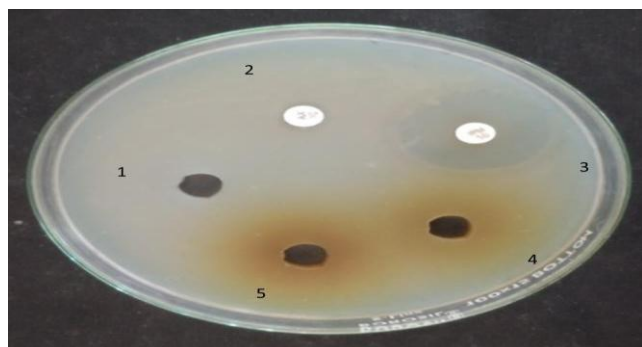


Fig: 10 Mukia Maderaspatana (E. coli)

1) Hydroalcohol-90% (Negative Control), 2) Antibiotic – Ampicillin Disc (Positive Control), 3) Antibiotic –Imipenem Disc (Positive Control) 4)Extract - 50ul, 5) Extract -100ul.

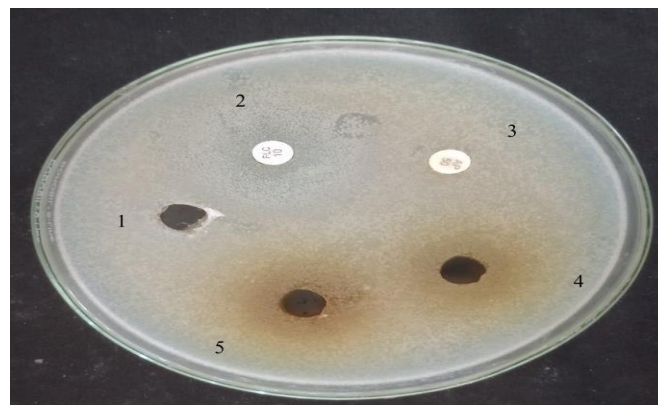


Fig: 11 Mukia Maderaspatana (C. albicans)

Hydroalcohol-90% (Negative Control), 2) Antibiotic - Flucanazole Disc (Positive Control), 3) Antibiotic - Amphotericin B Disc (Positive Control), 4) Extract - 50ul, 5) Extract -100ul.

<i>Mukia Maderaspatana (S. aureus )</i>		
S.No	Samples Name	Zone Measurement (mm)
1	Negative Control (Hydroalcohol-90%)	No Zone
2	Antibiotic Disc (Penicillin)	No Zone
3	Antibiotic Disc (Oxacillin)	No Zone
4	Extract(50ul)	No Zone
5	Extract(100ul)	No Zone

Table : 5 Staphylococcus Aureus

<i>Mukia Maderaspatana (E. coli)</i>		
S.No	Samples Name	Zone Measurement (mm)
1	Negative Control (Hydroalcohol-90%)	No Zone

2	Antibiotic Disc (Amphicillin)	No Zone
3	Antibiotic Disc (Imipenem)	18 mm
4	Extract(50ul)	No Zone
5	Extract(100ul)	No Zone

Table 6: E.coli

Mukia Maderaspatana (C. albicans)		
S.No	Samples Name	Zone Measurement (mm)
1	Negative Control (Hydroalcohol-90%)	No Zone
2	Antibiotic Disc (Flucanazole)	10 mm
3	Antibiotic Disc (Amphotericin B)	No Zone
4	Extract(50ul)	10 mm
5	Extract(100ul)	10 mm

Table :7 Candida Albicans

2	4H-Pyran-4-one, 2,3-dihydro	6.52	144.1	2.03
3.	1,2-Benzenediol	6.931	110.1	2.23
4.	1,2-Benzenediol, 3-methoxy	7.65	140.13	0.42
3	Thiophene, 2-propyl,	7.29	126.2	3.00
4	Thymol	7.93	150.22	18.97
5	Butanoic acid, 3-methyl-	8.22	102.13	1.38
6	2,3-Benzenetriol,	8.69	126.11	27.59
7	. N-Phenylmaleimide	9.94	173.171	1.82
8	Ethanone, 1-(2-hydroxy-4-methoxy	10.23	166.17	1.71
9	n-Hexadecanoic acid,	13.17	256.43	0.95
10	n-Decanoic acid,	14.46	172.26	0.55
11	Octadecanoic acid	17.56	284.48	0.60

Table : 8 GC-MS Analysis Phytochemical Compound

**VI.CONCLUSION**

The present study comprehensively investigated the pharmacological potential of Mukia maderaspatana hydroalcoholic extract. The extract exhibited significant antimicrobial activity against Escherichia coli, Staphylococcus aureus, and Candida albicans, alongside dose-dependent antioxidant and anti-inflammatory effects. Phytochemical and HPLC profiling revealed the presence of several bioactive compounds such as flavonoids (e.g., quercetin), alkaloids, phenolic acids (e.g., gallic acid), and terpenoids, which are likely contributors to its medicinal properties. The chromatographic analysis highlighted a major peak at 0.943 min, indicating the abundance of a dominant polar phytoconstituent. These findings validate the traditional use of Mukia maderaspatana and suggest its potential for further development into natural therapeutic agents. Future studies should focus on the isolation, structural characterization, and in vivo validation of individual compounds to explore their specific pharmacological roles.

**GC-MS Analysis**

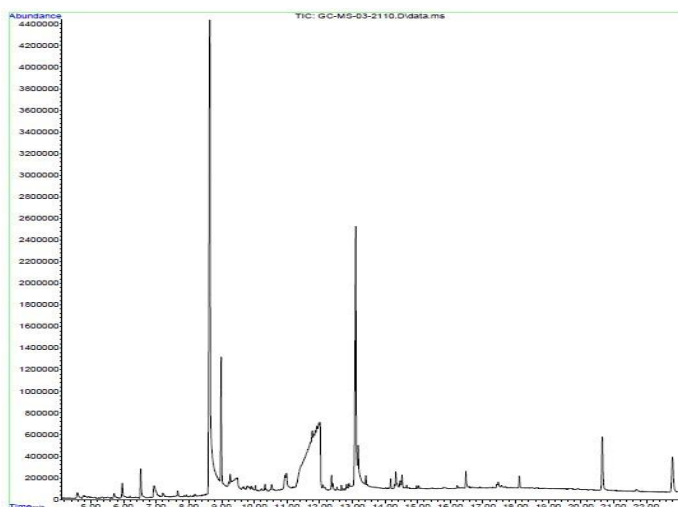


Figure :12 GC-MS Analysis Graph

S.No	Name of the Phytochemical compound	RT (min)	Molecular weight g/mol	Area %
				Untreated Tammiethanol extract
1	1-Butanol, 3-methyl,	5.96	88.15	1.05

## VII. ACKNOWLEDGEMENT

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