RESEARCH PAPER

Integrated GC–MS Profiling, HPTLC Fingerprinting, Antimicrobial Evaluation, and Molecular Docking Analysis of Bioactive Constituents from Mukia maderaspatana (L.) M. Roem. Aerial Parts

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ABSTRACT

Mukia maderaspatana (L.) M. Roem. (Cucurbitaceae) is a traditionally important medicinal plant; however, comprehensive studies correlating its phytochemical composition with biological activity remain limited. The present study integrates GC–MS profiling, HPTLC fingerprinting, antimicrobial evaluation, and molecular docking to investigate the bioactive potential of aerial parts. GC–MS analysis of the methanolic extract revealed the presence of fatty acid esters, phenolic compounds, and bioactive amides. HPTLC fingerprinting confirmed gallic acid (21.86 µg/mL) and rutin (30.23 µg/mL) as major phenolic markers. The methanolic extract exhibited significant antimicrobial activity against Escherichia coli (MIC 32 µg/mL), Staphylococcus aureus (MIC 64 µg/mL), Klebsiella pneumoniae (MIC 64 µg/mL), and Candida albicans (MIC 8 µg/mL). Molecular docking demonstrated stable binding of gallic acid with DNA gyrase B (PDB ID: 1AM6), with a binding energy of −6.2 kcal/mol, involving key residues ASP73, ARG76, and GLY77. The integration of chemical profiling, biological evaluation, and in-silico analysis suggests a potential multi-targeted antimicrobial mechanism. The study supports the traditional use of M. maderaspatana and proposes gallic acid and rutin as potential quality-control markers for standardization and future drug development.

Keywords: Mukia maderaspatana; GC–MS; HPTLC; Antimicrobial activity; Molecular docking; Gallic acid; Rutin; DNA gyrase B

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Introduction: *Mukia maderaspatana* (L.) M. Roem. (Cucurbitaceae) is a climbing plant widely distributed in the tropics and subtropics, traditionally consumed as a leafy vegetable. It has significant phytochemicals and its uses against respiratory disorders, gastrointestinal disturbances, inflammatory diseases and microbial infections are described in ethnomedicinal practices throughout India and south-east-Asia. that a spectrum of secondary metabolites such as phenolics, flavonoids and terpenoids which adds to its therapeutic actions has been reported on *Mukia maderaspatana* investigations.[1]

Bioactive compounds of plant extracts have been used for the green synthesis of metallic nanoparticles with substantial antibacterial activity, thereby enabling the identification of bioactive molecules.[2] As for more recent reports, it is worth mentioning that hydroalcoholic leaf extract exhibited considerable cytotoxic activity against hepatocellular carcinoma cells supporting the wide pharmacological importance of this plant bioactives.[3]

Still, limited research has focused on the systematic examination of aerial parts (leaf, stem and petiole). In comparison, chemical characterization has been primarily qualitative or restricted to GC–MS analyses and very few studies have used advanced chromatographic fingerprinting techniques like HPTLC. Furthermore, the correlations of these identified phytochemicals and biological activities, particularly using in-silico molecular docking have not been studied systematically. As such, standardized quality-control markers for this species have not been well established at the moment.

To bridge these gulfs, the current research work integrates GC–MS profiling, HPTLC fingerprinting of phenolic signatures (e.g., gallic acid and rutin)

alongside in-vitro antimicrobial and antioxidant assessment, as well as molecular docking analysis of major components like gallic acid against DNA gyrase B subunit (PDB: 1AM6). A thorough chemical–biological profile of *Mukia maderaspatana* aerial parts could be provided to favour its validation and quality standardization with this holistic approach.

The novelty of this study lies in the integrated evaluation of aerial parts (leaf, stem, and petiole) of *Mukia maderaspatana* using combined GC–MS profiling, HPTLC-based quantification of phenolic markers, antimicrobial activity, and molecular docking analysis. To the best of our knowledge, this is the first study establishing a correlation between phenolic markers and antimicrobial mechanism through docking studies in this species.

Materials and methods:

Plant collection and authentication:

Fresh aerial parts of *Mukia maderaspatana* were collected from National Highway 753F, Gangapur, Chh. Sambhajinagar (Aurangabad), Maharashtra, India, in July 2024. The plant was authenticated by a botanist at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University. A voucher specimen (No. 01387) was deposited in the herbarium for future reference.

Preparation of extract: The collected aerial parts were washed, shade-dried for 14-15 days, and powdered using a mechanical grinder to obtain a coarse powder.

Approximately 10 g of dried plant powder was packed in a cellulose thimble and subjected to Soxhlet extraction using 100 mL methanol as the solvent. The extraction was carried out for 6 hours at the solvent's boiling temperature, with 10–12 cycles completed until the siphon solvent became colorless. The extract was concentrated under reduced pressure using a rotary evaporator at 45°C, dried to obtain a semi-solid mass, weighed, and stored at 4°C for further analysis. The percentage yield of the extract was calculated and recorded.[4]

Statistical analysis:

All experiments were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism software. Differences were considered statistically significant at $p < 0.05$.

GC-MS Analysis:

GC–MS analysis of the extract was performed using a Shimadzu GC–MS QP2010 system equipped with SH-I-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm). The sample was dissolved in methanol and 2 µL was injected in splitless mode. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was set as initial temperature 45°C (held for 2 min), ramped to 140°C at 5°C/min, further increased to 280°C and held isothermally for 10 min. The mass spectrometric analysis was carried out using electron ionization at an energy level of 70 eV. The total run time ranged from 9.10 to 52.0 min. Identification of compounds was achieved by comparing the mass spectra with those in the NIST14.L (2020) mass spectral library.[5]Only compounds with high similarity index values and matching retention times were considered for identification.

HPTLC Fingerprinting:

HPTLC analysis was performed on pre-coated silica gel 60 F_{254} plates (50 × 100 mm; Merck, Darmstadt, Germany), which served as the stationary phase. Samples of the test substance (labelled KA1) were prepared in ethanol and applied to the plate in varying volumes (5.0, 6.0, 8.0, and 9.0 µL) using a CAMAG Linomat 5 automatic applicator (Serial No. 100632, CAMAG, Muttenz, Switzerland). Sample bands were applied 8.0 mm from the lower edge of the plate, with a band length of 8.0 mm and an inter-track distance of 10.4 mm. The application rate was maintained at 100 nL/s, with a pre-dosage volume of 0.20 µL to ensure precise sample delivery.

Chromatographic development was performed in a twin-trough glass chamber (20 × 10 cm) pre-saturated for 20 minutes with a mobile phase Ethyl acetate: Formic acid: Acetic acid: Water (v/v/v/v) (100:11:11:26). Saturation was carried out using appropriate filter paper liners to ensure consistent vapor phase conditions. The chromatographic plates were developed to a solvent front distance of 70 mm and subsequently dried at ambient temperature for 5 minutes.

Densitometric evaluation of the plates was conducted using a CAMAG TLC Scanner 3 (Serial No. 140507), operated under vision CATS software version 3.2.23095.1 (CAMAG, Switzerland). Scanning was carried out in absorbance mode at multiple wavelengths (254 and 366 nm) using deuterium and tungsten light sources. The scanner was operated in automatic detection mode with a scanning speed of 20 mm/s, a resolution of 100 µm per step, and a slit dimension of 5 × 0.45 µm.

baseline correction using the lowest slope method, and peak detection using a Gaussian legacy Data acquisition and processing included Savitzky–Golay smoothing (window size 7), algorithm with sensitivity set to 0.1, with the peak separation configured at 1 and the threshold fixed at 0.1.

Peak integration was performed across an Rf range of 0.00–1.00 Each sample volume yielded consistent peak patterns with reproducible RF values, supporting the robustness of the method. All operations were executed in accordance with CAMAG standard practices and analytical guidelines for HPTLC as described in the literature.[6-8]

Antimicrobial assay:

Table 1. Microorganisms used in the present study along with their strain numbers

Microorganism Type	Name	Strain/Reference Number
Gram-positive bacteria	*Staphylococcus aureus*	NCIM 2079
	Bacillus subtilis	NCIM 2250
Gram-negative bacteria	*Escherichia coli*	ATCC 25922
	Klebsiella pneumoniae	NCIM 2719
Fungi	*Candida albicans*	-
	Aspergillus niger	ATCC 16404

Sterile discs (6 mm diameter) were impregnated with 20 µL of each plant extract (100 mg/mL) and allowed to air-dry at ambient temperature. Plant extracts were prepared using a Soxhlet apparatus with various solvents, including water, methanol, acetone, and petroleum ether. Each disc was estimated to carry around 2 mg of crude extract. Streptomycin was designated as the positive control, whereas DMSO functioned as the negative control.[9-12] The bacterial inoculum was standardized to 0.5 McFarland standard (~1×10^8 CFU/mL). Plates were incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungi.

Molecular docking: Molecular docking was performed using AutoDock Vina implemented through AutoDock Tools (ADT 1.5.6) to evaluate the interaction of gallic acid with the DNA gyrase B subunit of *Escherichia coli* (PDB ID: 1AM6). The crystal structure of the target protein was retrieved from the Protein Data Bank and prepared by: removal of co-crystallized water molecules, addition of polar hydrogens, assignment of Kollman charges, conversion to PDBQT format.

Grid Box Parameters: The docking grid was centered on the ATP-binding pocket based on the co-crystallized ligand position with the following dimensions: Center: x = −9.6661, y = −1.7246, z = 16.1232, Grid size: 47.33 × 44.13 × 54.79 Å, Exhaustiveness: 8, Number of modes: 9

Gallic acid and the control ligand ciprofloxacin were energy-minimized and converted to PDBQT format prior to docking.

Validation: Method validation was performed by re-docking the native ligand into the active site of 1AM6.The reproduced pose showed RMSD < 2.0 Å, confirming the reliability of the docking protocol for predicting biologically relevant orientations.

Results:

GC-MS ANALYSIS:

GC–MS analysis of the aerial parts of *Mukia maderaspatana* revealed chemically diverse metabolite profiles, which predominantly include fatty acids, phenolic derivatives, long-chain hydrocarbons, and bioactive amides. These groups of plant chemicals are frequently associated with medicinal plants and are recognized for their antimicrobial and antioxidant potential, which is achieved through mechanisms such as disruption of cell membranes, inhibition of enzymatic activities, and regulation of oxidative balance.[13-15]

The fatty acid derivatives identified here including 2-hydroxy-hexadecanoic acid ester (13.18%), methyl palmitate (8.44%), and n-hexadecanoic acid (0.73%) are consistent with reports in which similar compounds in plant extracts exhibit antimicrobial and antioxidant activities. Their amphipathic nature can disrupt microbial membrane integrity and enhance cell permeability, thereby contributing to the significant inhibitory zones observed in this study.[16]

Identification of 2,4-di-tert-butylphenol (4.38%), a phenolic compound known for its antioxidant and

antimicrobial properties, further confirms the bioactive potential of the extract. Additionally, phenolic acids and related compounds are known to exhibit broad-spectrum antimicrobial activity by interacting with microbial proteins and promoting oxidative damage.[2] The presence of 13-docosenamide (6.41%), a known bioactive fatty amide with anti-inflammatory and anticancer properties, may synergistically enhance antimicrobial efficacy through interference with microbial metabolic pathways. Comparable amide derivatives have been reported to exhibit a range of biological activities in extracts of various medicinal plants. Long-chain hydrocarbons such as dotriacontane and tetrapentacontane, though less directly antimicrobial, contribute to the extract's complex chemical milieu and may support extraction efficiency and compound stability. Moreover, the presence of these compounds is consistent with phytochemical patterns observed in other plant species, where fatty hydrocarbons occur alongside bioactive phenolics and esters. Notably, the GC–MS findings are corroborated by HPTLC analysis, which confirmed the presence of gallic acid and rutin well-established phenolic compounds known for their antioxidant and antimicrobial activities. The stronger antimicrobial activity of methanolic extract can be partly attributed to the higher solubility of phenolic acids in polar solvents, facilitating their extraction and bioavailability. Moreover, molecular docking of gallic acid with DNA gyrase B (PDB: 1AM6) demonstrated favorable binding interactions, providing mechanistic evidence that phenolic acids may inhibit essential microbial enzymes, thereby reinforcing the *in-vitro* findings. Thus, the metabolite composition and associated biological activities suggest that the antimicrobial efficacy of *M. maderaspatana* aerial parts is likely due to a multi-targeted action of phenolics, fatty acid esters, and amides, rather than a single active compound. This complex synergy underscores the therapeutic potential of the extract and supports its traditional use.

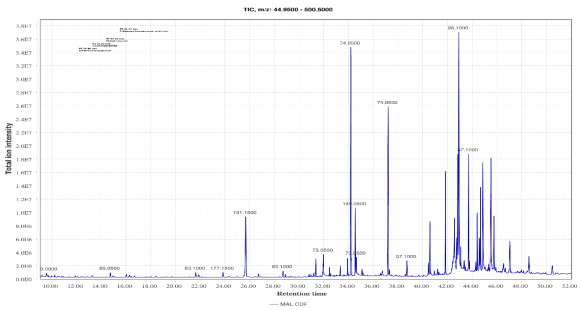
Table 2: GC–MS analysis of major phytochemical constituents identified in *Mukia maderaspatana*

Peak No	Retention Time (min)	Identified Compound	Molecular Formula	Peak Area (%)	Chemical Class	Reported Biological Activity
63	42.92	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ester	$C_{17}H_{34}O_4$	13.18	Fatty acid ester	Antimicrobial, antioxidant
40	37.20	Methyl stearate	$C_{19}H_{38}O_2$	8.84	Fatty acid ester	Anti-inflammatory
32	34.18	Hexadecanoic acid, methyl ester (Methyl palmitate)	$C_{17}H_{34}O_2$	8.44	Fatty acid ester	Antioxidant, antibacterial
75	44.85	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	6.58	Fatty acid ester	Antimicrobial
79	45.52	13-Docosenamide (Z)	$C_{22}H_{43}NO$	6.41	Fatty acid amide	Anti-inflammatory, anticancer
15	25.68	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	4.38	Phenolic compound	Antioxidant, antimicrobial
68	43.71	Tetrapentacontane	$C_{54}H_{110}$	3.81	Long-chain hydrocarbon	Structural lipid, antibacterial
56	41.84	Dotriacontane	$C_{32}H_{66}$	3.30	Long-chain hydrocarbon	Antimicrobial
60	42.55	1,3,5-Trisilacyclohexane	$C_6H_{18}Si_3$	3.52	Siloxane derivative	
73	44.57	Stearic acid, TMS derivative	$C_{18}H_{36}O_2Si$	1.63	Fatty acid derivative	Antimicrobial
61	42.74	Lauryl di-lactate (TMS derivative)	$C_{18}H_{34}O_5Si$	1.24	Ester	Antimicrobial
23	31.97	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.34	Fatty acid	Antibacterial
35	34.62	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	0.73	Fatty acid	Anti-inflammatory

***** Reported biological activities are based on previously published literature.

Note: Siloxane derivatives detected in GC–MS analysis may originate from column bleed, septa, or derivatization artifacts and are therefore not considered primary bioactive constituents.

Figure1: GC–MS total ion chromatogram (TIC) of methanolic extract of *Mukia maderaspatana* aerial parts showing major identified phytoconstituents with their respective retention times.



HPTLC fingerprinting:

Figure 2: HPTLC Quantification of Phenolic Markers in *Mukia maderaspatana* Aerial Part

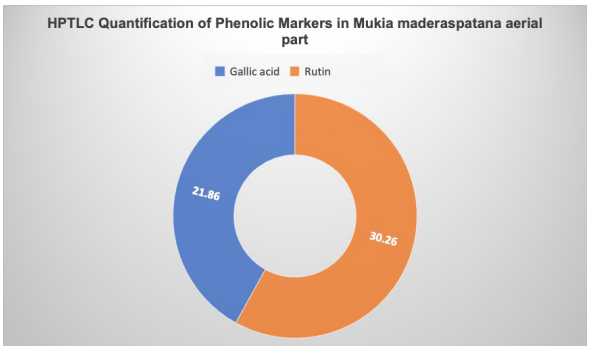


Table 3: HPTLC quantification and interpretation of phenolic markers in sample MML

Marker	Standard Rf Range	MML Rf	Peak Area (AU)	Peak Height (AU)	Concentration / Quantity	Interpretation
Gallic acid	0.946–1.050	1.026	0.01229	1.047	21.86 µg/mL; 87.42 ng	Detected; strong match with standard Rf, moderate content
Rutin	0.184–0.322	0.184	0.00178	0.0434	30.23 µg/mL	Detected; strong match with standard Rf, moderate content

HPTLC analysis was performed to establish a reproducible chemical fingerprint of the methanolic extract of *Mukia maderaspatana* aerial parts and to quantify selected phenolic markers. Chromatographic development using ethyl acetate:formic acid:acetic acid:water (100:11:11:26, v/v/v/v) resulted in well-resolved bands under UV detection at 254 and 366 nm. The chromatographic system produced consistent Rf values and peak symmetry across applied volumes, confirming method robustness.

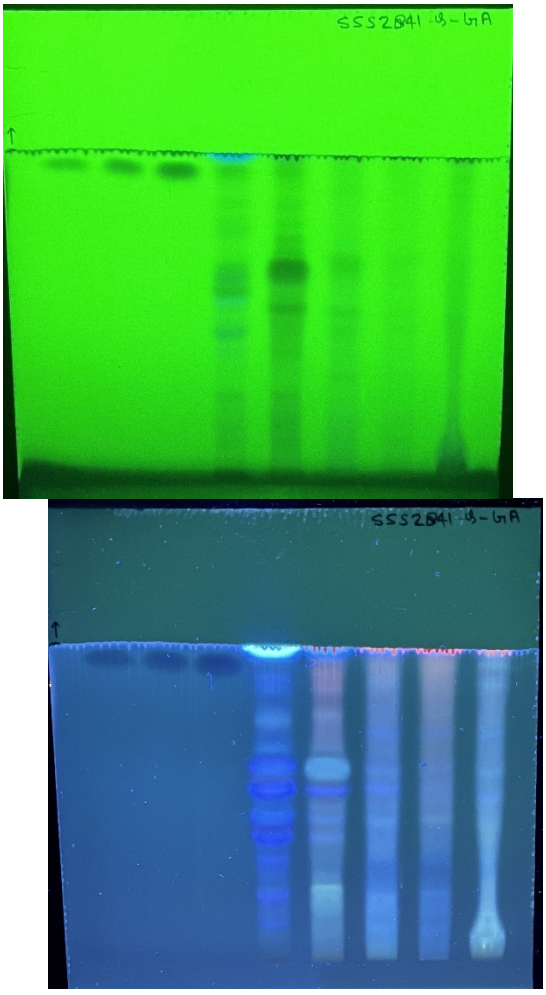
Densitometric evaluation identified gallic acid and rutin in the extract, with Rf values matching those of authenticated standards. Quantification revealed gallic acid at 21.86 µg/mL (87.42 ng/band) and rutin at 30.23 µg/mL. Calibration curves exhibited strong linearity ($R^2 > 0.99$), supporting analytical reliability and suitability for quality control applications.

The presence of these phenolics is mechanistically relevant to the observed antimicrobial activity. Gallic acid, owing to its trihydroxybenzene scaffold, can form multiple hydrogen bonds within enzyme active sites and participate in redox cycling, leading to oxidative stress in microbial cells.[17] Its documented interaction with the ATP-binding domain of DNA gyrase B supported here by docking analysis suggests interference with ATP-dependent DNA supercoiling, a critical step in bacterial replication. Rutin, a flavonol glycoside, contributes through membrane destabilization, metal chelation, and modulation of quorum-sensing pathways, collectively impairing microbial survival and virulence.

The enrichment of these polar phenolics in the methanolic extract correlates with its superior antimicrobial performance, indicating that phenolic abundance may serve as a predictive marker of bioactivity. The reproducible HPTLC fingerprint therefore provides not only a tool for authentication but also a chemically validated framework for standardization of *M. maderaspatana* aerial parts.

Phenolic compounds such as gallic acid and rutin are widely known for their antioxidant and antimicrobial properties, and their quantification has been used as a marker for biological activity in medicinal plants. For example, gallic acid and rutin quantified through HPTLC have been associated with antioxidant activity in extracts of *Psidium guajava* and *Aegle marmelos*, indicating that phenolic markers can serve as reliable indicators of biological efficacy in complex plant matrices.[18]

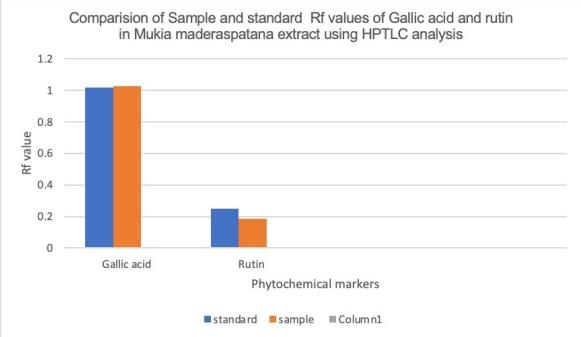
Figure 3. HPTLC fingerprint profile of methanolic extract of *Mukia maderaspatana* aerial parts developed in ethyl acetate:formic acid:acetic acid:water (100:11:11:26, v/v/v/v) and visualized at (A) 254 nm and (B) 366 nm.



(A)

(B)

Figure 4. HPTLC densitometric overlay chromatograms of standard phenolic markers (gallic acid and rutin) and methanolic extract of *Mukia maderaspatana* aerial parts scanned at 254 nm.



Antimicrobial assay:

Antibacterial acivity:

Values represent mean ± SD (n = 3). Streptomycin (10 µg/mL) and fluconazole (10 µg/mL) were used as antibacterial and antifungal positive controls, respectively. DMSO served as negative control.

Antimicrobial activity of *Mukia maderaspatana* extracts by disc diffusion method

Table 4: Zone of inhibition (mm) of aerial part extracts of *M. maderaspatana* (Values are mean ± SD, n = 3)

Microorganism	**Acetone extract (100 mg/mL)**	**Methanol extract (100 mg/mL)**	**Standard drug (10 µg)**	**Negative control (DMSO)**
Bacillus subtilis NCIM 2250	ND	ND	Streptomycin: 35 ± SD	ND
Escherichia coli ATCC 25922	25 ± 1	23 ± 1	Streptomycin: 26 ± SD	ND
Staphylococcus aureus NCIM 2079	20 ± 1	22 ± 1	Streptomycin: 27 ± SD	ND
Klebsiella pneumoniae NCIM 2719	20 ± 1	22 ± 1	Streptomycin: 22 ± SD	ND
Candida albicans	22 ± 1	26 ± 1	Fluconazole: 29 ± SD	ND
Aspergillus niger	ND	ND	Fluconazole: 24 ± SD	ND
ATCC 16404				

ND = Not Detected

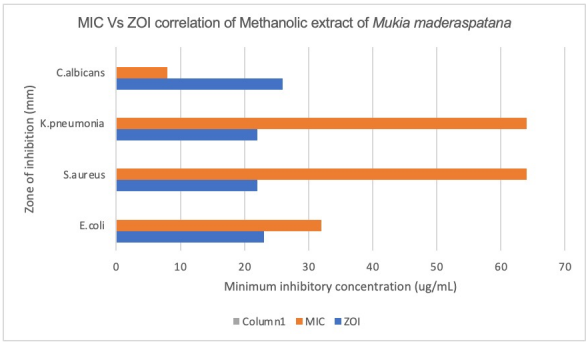
Disc diffusion performed according to **CLSI M02-A12 (2023)**

Table 5: Minimum inhibitory concentration (MIC) of *Mukia maderaspatana* extracts determined by broth microdilution

Microorganism	**Methanol extract MIC (µg/mL)**	**Acetone extract MIC (µg/mL)**	**Standard drug (µg/mL)**
Escherichia coli ATCC 25922	32	16	Streptomycin
Staphylococcus aureus NCIM 2079	64	64	Streptomycin
Klebsiella pneumoniae NCIM 2719	64	64	Streptomycin
Bacillus subtilis NCIM 2250	>128	>128	Streptomycin
Candida albicans	8	8	Fluconazole

MIC by broth microdilution **CLSI M07-A1**

Figure 5: Correlation between minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) of Mukia maderaspatana extracts



The methanolic extract demonstrated pronounced antifungal efficacy against *Candida albicans*, while no detectable inhibition was observed in the case of *Aspergillus niger*. In addition, the extract displayed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*,

indicating a broad spectrum of action against both Gram-negative and Gram-positive pathogens.

Molecular docking: Molecular docking analysis demonstrated that gallic acid exhibited a strong binding affinity of −6.2 kcal/mol toward the ATP-binding domain of DNA gyrase B from *Escherichia coli* (PDB ID: 1AM6). The best docked pose showed an RMSD value of 0.0 Å, indicating a stable and reliable ligand–protein conformation.

The 2D interaction analysis revealed that gallic acid formed multiple hydrogen bonds with key active-site residues ASP73, ARG76, and GLY77**,** which are critical for gyrase catalytic activity. The presence of these polar interactions suggests effective occupancy of the nucleotide-binding pocket and potential inhibition of enzyme function.

The three-dimensional binding orientation confirmed deep embedding of gallic acid within the active cavity, stabilized predominantly by hydrogen bonding and electrostatic interactions. These findings support the potential of gallic acid as a gyrase B inhibitor and correlate with the observed *in-vitro* antibacterial activity against *E. coli*.

To validate the docking results, the binding affinity of gallic acid was compared with the standard antibiotic ciprofloxacin. Ciprofloxacin exhibited a binding energy of −7.8 kcal/mol, which was higher than that of gallic acid (−6.2 kcal/mol), indicating stronger binding. However, gallic acid demonstrated stable hydrogen bonding interactions with key active-site residues (ASP73, ARG76, GLY77), suggesting its potential as a natural inhibitor of DNA gyrase B.

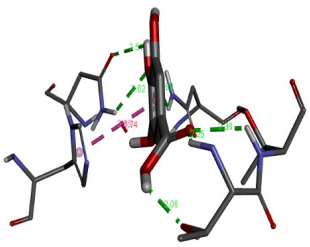


*Figure 6: Three-dimensional binding orientation of gallic acid within the active site of [*DNA Gyrase B Subunit from *Escherichia coli,* 1AM6*]. Hydrogen bonds are shown as green dashed lines with interacting residues labeled.*

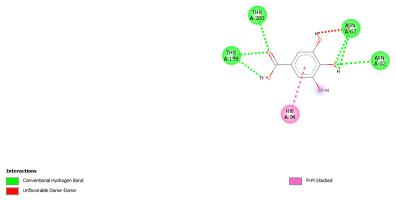


Figure 7: 2D interaction map showing H-bonds and distances

Table 6: Docking interaction profile of gallic acid with DNA gyrase B (1AM6)

Ligand	**Binding energy (kcal/mol)**	**RMSD (Å)**	**Interacting residues**	**Interaction type**
Gallic acid	**−6.2**	**0.0**	**ASP73, ARG76, GLY77**	Hydrogen bond, polar

Discussion: According to this research study, aerial parts of *Mukia maderaspatana* display significant activity, with an increased amount of activity seen with methanol extracts. Additionally, the MIC values reported for leaves alone are now shown to be lower than previously reported when stem and petiole were included in the study because both of those plant parts were also extracted in methanol where additional bioactive compounds would have been included resulting in a higher level of activity for the whole plant material. Past research examined only basic antimicrobial activity without any further study; however, this research utilizes an approach that has depth/complexity due to the addition and subsequent use of GC–MS analysis, quantification via the HPTLC method, the use of MIC and ZOI relationships, and the utilization of docking analyses to tie together all of these results.

The methanolic extracts were definitely responsible for some of the observed activity against both *Candida albicans* (26 mm zone of inhibition at 8 µg/mL) and *Escherichia coli* (23 mm zone of inhibition at 32 µg/mL). The pattern of activity observed was, in fact, quite consistent with what has been seen regarding similar polarity of phenolic compounds extracted from other Cucurbitaceae family members, with methanol being known to provide a higher concentration of both flavonoids and phenolic acids that may disrupt the membrane of many microorganisms (including *Candida albicans* as well as *Escherichia coli*) or inhibit several enzymes necessary for the cellular function of various microorganisms. Furthermore, it is interesting (but perhaps not surprising) that there was no observed activity against *Bacillus subtilis*. The thick peptidoglycan layer may merely keep things out. It

appears to provide built-in resistance. a type of built-in resistance found in many plants. Given that microbial resistance has also increased due to the increased concentration of crude extracts compared to standard antibiotics, the level of inhibition suggests that the crude extracts had something that could cause microbial inhibition. From the chemist's perspective, examining the GC–MS data reveals the presence of fatty acid esters, phenolic derivatives, and amide compounds. While metadata may show some of these compounds are known for their antimicrobial properties due to destabilizing cell membranes or affecting the metabolism of microorganisms (and the studies supporting these correlations), the use of crude extracts as a source for these compounds leads to the isolation of either of these compilation points. The HPTLC findings indicate additional consistency. The presence of gallic acid and rutin was shown to be measurable levels of 21.86 µg/mL for gallic acid, and 30.23 µg/mL for rutin, and they correlate reasonably well with previously characterized biological activity. While it may be argued that these correlations are not as straightforward, there appear to be findings elsewhere that support growing interest in developing gallic acid as a useful marker for standardizing and evaluating both activity and activity levels.

While the uptick in stability at docking ultimately leads to just one conclusion, the results from this study do indicate that gallic acid binds to the active site of DNA gyrase B via multiple stable hydrogen bonds that are likely to inhibit supercoiling through ATP-dependent mechanisms. This does lend some credibility to the previously reported mode of action for phenolic acids, but the findings are at least plausible at this stage. The SwissADME data also support the possibility of having acceptable drug-like characteristics, but the confidence provided by SwissADME is relatively low.

When combined, the chemical profiling of potential use of the *M. maderaspatana* components and the in vitro antimicrobial testing of the extracts as well as the molecular docking studies lend themselves to a fairly coherent storyline. However, there are some major limitations that are hard to overlook. There was only one protein target that was investigated and the research was done on crude extracts rather than isolated compounds. Future contributions to this body of evidence would aid in strengthening the findings and would likely provide a more complete picture of the biological activities of these different parts of the *M. maderaspatana* plant. Future studies such as time kill studies or biofilm models would be beneficial and will add more detail to the data currently available. For the most part, these findings yield scientific evidence to support the traditional uses of the *M. maderaspatana* components. More specifically, gallic acid and rutin appear to be more than merely incidental compounds. These two compounds may serve as both markers for standardization and have potential for future development of antimicrobial agents. [19,20]

Conclusion

The use of gas chromatography-mass spectrometry (GC-MS), high-performance thin-layer chromatography (HPTLC), antimicrobial testing and molecular docking together creates a cohesive result. From GC-MS, it can be seen that the extract from methanolic extraction contains some fatty acid ester, phenolic compounds and amides; however, through HPTLC analysis, it was found that gallic acid and rutin are the compounds responsible for these effects. In particular, the methanolic extract exhibited superior antimicrobial activity against both *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*), with the low minimum inhibitory concentration (MIC) values indicating that these compounds would not need to act cumulatively in order to display sufficient levels of antimicrobial activity. Molecular docking analysis supports a possible mechanism of action wherein gallic acid binds to the ATP-binding site of DNA gyrase B, but this should be considered an indication but not definitive proof of the mechanism. There were a number of little siloxane peaks that were ignored, as these are more likely to be a function of the system than of the plants. Combined with traditional usage data, the results suggest that gallic acid and rutin can be employed as good markers. However, isolating compounds and then conducting in vivo tests would add considerable credibility to the understanding of these compounds**.**

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