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DEVELOPMENT AND EVALUATION OF HERBAL HARD CANDY CONTAINING MUKIA MADERASPATANA L. WITH PHYTOCHEMICAL AND IN SILICO IMMUNOMODULATORY STUDIES

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ABSTRACT

The present study aimed to evaluate the pharmacognostical, physicochemical, phytochemical, and immunomodulatory potential of Mukia maderaspatana L. leaves, along with the formulation and evaluation of an herbal hard candy. The plant material was collected, authenticated, and subjected to macroscopic and microscopic analysis for proper identification. Physicochemical parameters such as ash values, extractive values, moisture content, and foaming index were determined to assess purity and quality. The powdered leaves were extracted using successive solvent extraction, and the aqueous extract was further analyzed for preliminary phytochemical screening, which revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic compounds. GC–MS analysis identified several bioactive compounds including γ-bisabolol and ergost derivatives, indicating potential pharmacological activity. In silico molecular docking studies were carried out to evaluate the immunomodulatory potential of selected phytoconstituents, which showed favorable binding interactions with target proteins. An herbal hard candy formulation containing Mukia maderaspatana extract was successfully prepared and evaluated for organoleptic, physical, and physicochemical parameters. The formulation exhibited acceptable hardness, uniform weight, low moisture content, and good stability. Dissolution studies indicated rapid drug release (>90% within 20 minutes), and drug content was within acceptable limits (91–99%). The formulation also demonstrated moderate antimicrobial activity against Escherichia coli. Overall, the study confirms that Mukia maderaspatana possesses significant phytochemical and potential immunomodulatory properties, and the developed herbal candy may serve as a promising and patient-friendly dosage form for therapeutic applications.

Key words: Mukia maderaspatana, Herbal candy, Phytochemical screening, GC–MS analysis, Immunomodulatory activity, in silico docking, Pharmacognostical studies, Antimicrobial activity.

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INTRODUCTION

Medicinal plants have played a pivotal role in human healthcare since ancient times and continue to serve as a primary source of therapeutic agents worldwide. According to the World Health Organization (WHO), nearly 80% of the global population relies on traditional plant-based medicines for primary healthcare, particularly in developing regions where access to modern medicine is limited *(WHO, 2013)*. Herbal

medicines are rich in bioactive phytochemicals such as flavonoids, alkaloids, tannins, saponins, and terpenoids, which exhibit diverse pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects *(Sharma et al., 2024; Hooda et al., 2024)*. In recent years, there has been growing scientific interest in immunomodulation, particularly through plant-derived compounds. Immunostimulants are substances that enhance the body's immune response by activating both innate and adaptive immune mechanisms, including macrophage activation, cytokine production, and lymphocyte proliferation *(Parbat et al., 2021)*. The increasing prevalence of antibiotic resistance, adverse effects of synthetic drugs, and emerging infectious diseases have accelerated the search for natural immunomodulators derived from medicinal plants *(Maheshwari et al., 2022)*. Phytochemicals have been shown to regulate immune pathways and improve host defense, making them promising candidates for preventive and therapeutic applications *(Sharma et al., 2024)*. Among medicinal plants, *Mukia maderaspatana* (L.) Roem**.** (family: Cucurbitaceae) has gained attention due to its extensive use in traditional medicine systems across Asia and Africa. It is commonly known as *Madras pea pumpkin* and is traditionally used for treating respiratory disorders, fever, inflammation, gastrointestinal disturbances, and metabolic disorders *(Kumar et al., 2022)*. Phytochemical investigations of this plant have revealed the presence of flavonoids, phenolics, steroids, saponins, and triterpenes, along with bioactive compounds such as phytol, eugenol, and β-sitosterol, which contribute to its pharmacological properties *(Kumar et al., 2022)*. Preliminary studies suggest that extracts of *Mukia maderaspatana* exhibit antioxidant, anti-inflammatory, and potential immunomodulatory activities, supporting its ethnomedicinal use *(Jayasakthi & Murugaian, 2011)*. Parallel to the growing interest in herbal therapeutics, there is an increasing demand for functional food formulations that combine nutritional and medicinal benefits. Herbal confectionery products, such as herbal hard candies**,** represent an innovative delivery system for phytochemicals. These formulations not only improve patient compliance, especially among pediatric and geriatric populations, but also provide localized soothing effects for respiratory ailments such as cough and sore throat *(Maheshwari et al., 2022)*. Hard candies, characterized by low moisture content and high stability, serve as an effective medium for incorporating plant extracts while maintaining product shelf life and palatability. Despite the recognized therapeutic potential of *Mukia maderaspatana*, there is limited scientific literature integrating its phytochemical evaluation, immunomodulatory assessment (particularly through in silico approaches), and formulation into functional dosage forms such as herbal candies**.** Therefore, the present study aims to investigate the phytochemical profile of *Mukia maderaspatana* leaves, evaluate their immunomodulatory potential using computational methods, and develop a stable and acceptable herbal hard candy formulation. This integrative approach bridges traditional knowledge with modern scientific validation and novel drug delivery systems.

METHODOLOGY

Collection and Authentication of Plant Material

Fresh leaves of *Mukia maderaspatana* (L.) were collected from Masakalipatty, Rasipuram, Namakkal (Tamil Nadu, India) in July 2025. The plant was taxonomically identified and authenticated by a qualified taxonomist (Dr. P. Radha, Ministry of AYUSH, Government of India). Only healthy, disease-free specimens were selected to ensure accuracy in pharmacognostical and phytochemical investigations *(Kumar et al., 2022)*.

Pharmacognostical Evaluation

Macroscopic analysis:

Organoleptic characteristics such as color, odor, taste, size, and shape were evaluated using standard procedures for crude drug identification *(WHO, 2011)*.

Microscopic analysis:

Transverse sections of fresh leaves were prepared, stained with phloroglucinol–HCl, and examined under a compound microscope to study anatomical features such as epidermis, trichomes, vascular bundles, and stomata. Powder microscopy of shade-dried leaves (60–80 mesh) was performed to identify diagnostic characters including lignified fibers, calcium oxalate crystals, starch grains, and trichomes *(Evans, 2009)*.

Physicochemical Analysis

Standard physicochemical parameters including total ash, acid-insoluble ash, water-soluble ash, sulphated ash, loss on drying, foaming index, and extractive values (alcohol- and water-soluble) were determined as per pharmacopeial guidelines *(Indian Pharmacopoeia, 2018; WHO, 2011)*. These parameters ensure quality, purity, and standardization of the plant material.

Extraction of Plant Material

Shade-dried powdered leaves (100 g) were subjected to successive Soxhlet extraction using petroleum ether followed by solvents of increasing polarity. The aqueous extract was prepared by decoction. Extracts were concentrated, and percentage yield and physical characteristics were recorded *(Harborne, 1998)*.

Preliminary Phytochemical Screening

Qualitative phytochemical tests were carried out on extracts to detect major secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, saponins, carbohydrates, proteins, steroids, and triterpenoids using standard chemical tests (e.g., Dragendorff's, Shinoda, Molisch's, and Salkowski tests) *(Trease & Evans, 2009)*. GC–MS analysis was performed to identify bioactive compounds present in the extract, confirming the presence of key phytoconstituents reported in *Mukia maderaspatana (Kumar et al., 2022)*.

In Silico Pharmacological Screening

Drug-likeness and ADME properties of selected phytoconstituents were evaluated using the SWISS ADME tool based on Lipinski's rule of five *(Lipinski et al., 2001)*. Molecular docking studies were conducted using SWISS DOCK to evaluate binding affinity of bioactive compounds with selected protein targets (PDB IDs: 5F19, 4G8A, 2Z7X, 1NFK). Docking analysis employed the Lamarckian Genetic Algorithm to determine ligand–protein interactions *(Morris et al., 2009)*.

Formulation of Herbal Hard Candy

Herbal hard candy was prepared using sucrose and jaggery as base materials. The mixture was heated to the hard crack stage (135–145°C), followed by addition of plant extract, citric acid, and flavoring agents. The mass was poured into molds, cooled, and packed in aluminum foil *(Desai & Park, 2005)*.

Evaluation of Herbal Hard Candy

Physical evaluation:

Color, texture, odor, and shape were assessed for organoleptic acceptability.

Table 1: Macroscopic Evaluation

Parameter	Observation
Color	Greenish
Odor	Odorless
Taste	Slightly bitter
Texture	Dried leaf/powder

These organoleptic features are consistent with earlier reports and serve as preliminary quality control parameters (Kumar et al., 2022).

Table 2: Powder Microscopy

Diagnostic Feature	Observation
Lignified fibers	Present
Trichomes	Uniseriate multicellular
Calcium oxalate crystals	Present
Starch grains	Present
Pollen grains	Present

Table 3: Physicochemical Parameters of Leaf Powder

S.No	Parameter	Observation (% w/w)
1	Total ash	9.66
2	Acid insoluble ash	5.6
3	Water soluble ash	6.8
4	Sulphated ash	7.33
5	Water soluble extractive	6.8
6	Alcohol soluble extractive	7.2
7	Swelling index	7.2 ml

Table 4: Additional Parameters

Parameter	Observation
Moisture content	8.8%
Foaming index	125

Table 5: Extractive Yield

Extract	Appearance	Yield (% w/w)
Aqueous	Brown, powder	6.8

The aqueous extract showed appreciable yield, indicating the presence of water-soluble bioactive compounds such as flavonoids and glycosides (Harborne, 1998).

Table 6: Phytochemical Constituents

S.No	Constituent	Observation	Result
1	Alkaloids	Precipitate	+
2	Flavonoids	Pink/red color	+
3	Glycosides	Orange color	+
4	Saponins	Foam	+
5	Tannins/Phenols	Blue-green	+
6	Proteins	Violet color	+
7	Steroids	No reaction	–
8	Terpenoids	Positive	+

Table 7: Identified Compounds.

Peak	Retention Time	Area %	Compound
1	26.90	0.53	γ-Bisabolol
2	28.665	2.88	Ergost-25-ene-5,5,6,12-tetrol
3	31.842	0.40	13-Methyl-z-14-nonacosene
4	31.987	0.28	3-Hydroxy-7,8-dihydro-β-ionol
5	31.6	0.11	Fumaric cyclonox-3-methyl

Table 8: Organoleptic Evaluation.

Candy	Color	Odor	Taste	Appearance
F1	Light brown	Strong herbal	Sweet-herbal	Smooth
F2	Light brown	Mild	Sweet-herbal	Slightly smooth
F3	Light brown	Faint	Slightly bitter	Slightly rough

Table 9: Physical Evaluation

Candy	Shape	Texture	Surface	Hardness
F1	Square	Smooth	Uniform	Good
F2	Square	Slightly smooth	Uniform	Good
F3	Square	Grainy	Uniform	Hard

Table 10: Mechanical Properties

Candy	Weight (g)	Hardness	Thickness (mm)	Moisture (%)	Friability
F1	3.01	9.65	14.25	0.125	0.2
F2	3.02	10.5	14.58	0.054	0.1
F3	3.02	9.7	14.14	0.108	0.1
F4	3.03	10.15	14.81	0.05	0.3
F5	3.05	9.85	14.45	0.2	0.2

Table 11: Dissolution Study

Time	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
5 min	38.4	42.6	46.8	49.1	51.3
10 min	62.1	65.8	71.2	74.6	78.1
15 min	84.7	88.3	93.0	95.8	98.5
20 min	96.5	99.1	93.2	99.5	98.7

All formulations showed >90% drug release within 20 minutes, complying with pharmacopeial standards *(USP, 2020)*.

Table 12: Drug Content and Antimicrobial Activity

Candy	Drug Content (%)	pH	Zone of Inhibition	Disintegration
F1	91.2	4.5	9 mm	4 min 10 sec
F2	93.5	4.8	10 mm	4 min 25 sec

F3	96.8	5.5	9 mm	4 min 5 sec
F4	98.4	4.7	10 mm	4 min 5 sec
F5	99.1	5.3	11 mm	4 min 10 sec

The formulation exhibited moderate antimicrobial activity (9–11 mm) against *E. coli*, consistent with phytochemical composition. Drug content (91–99%) indicates uniform distribution and stability of active constituents *(Bauer et al., 1966)*.

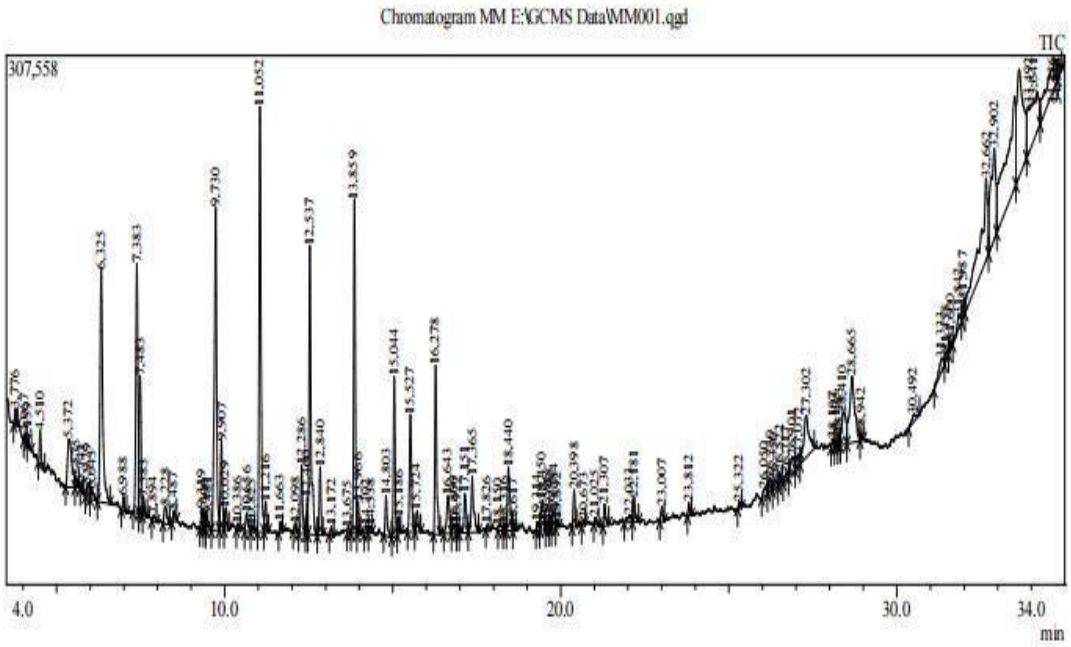
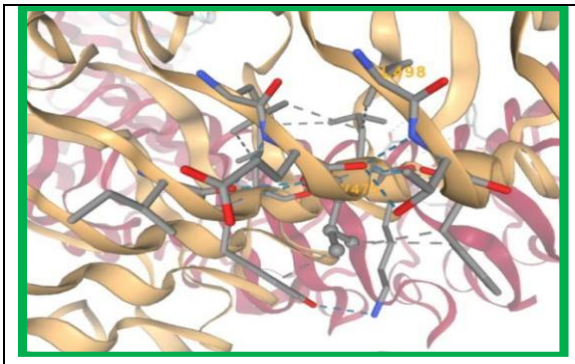
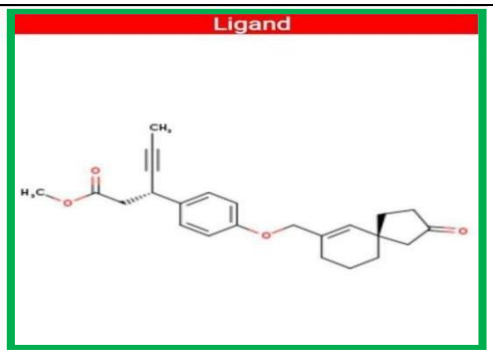
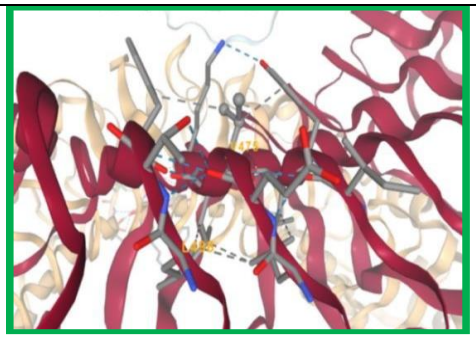
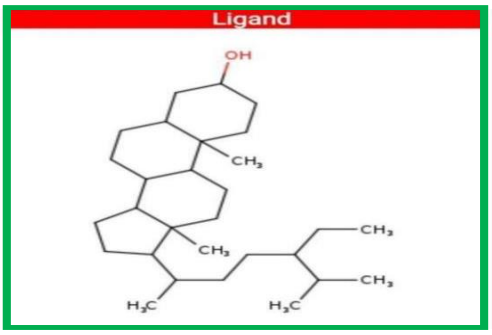


Figure: 1 GCMS-Chromatogram









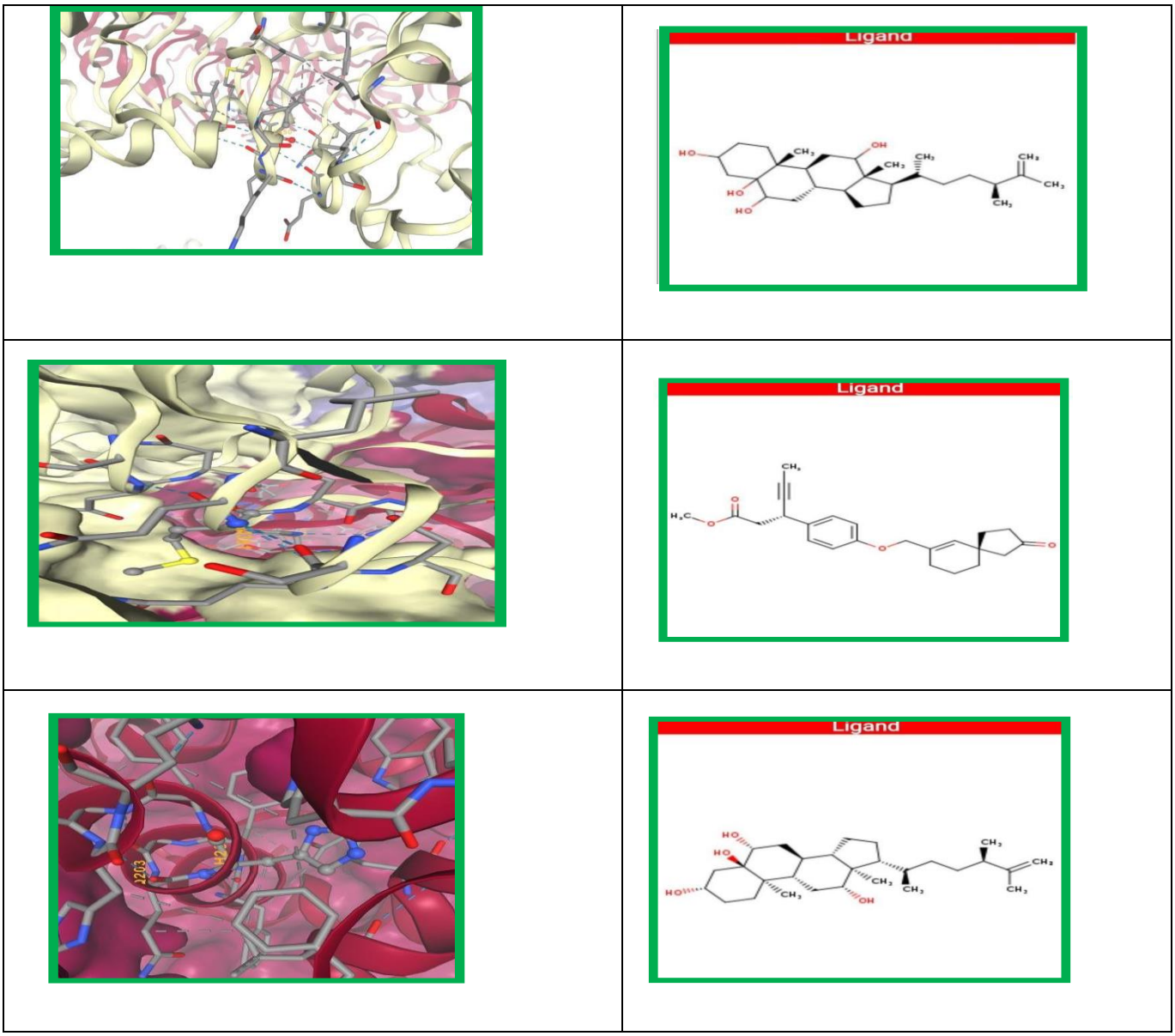


Figure 2: Insilico molecular analysis of Ligands and targets

Physicochemical evaluation:

Parameters including weight variation, hardness (Monsanto tester), and thickness, moisture content (LOD), pH, and drug content (UV–Vis spectrophotometry at 294.3 nm) were determined *(USP, 2020)*.

In vitro dissolution study:

Conducted using USP Type II apparatus in simulated salivary fluid (pH 6.8) at 37 ± 0.5°C and 50 rpm to determine drug release profile *(USP, 2020)*.

Disintegration and friability tests:

Performed to evaluate mechanical strength and release characteristics of the formulation.

Antimicrobial Activity (Cup Plate Method)

Antimicrobial activity was assessed using the agar well diffusion method. Nutrient agar plates inoculated with test microorganisms were prepared, and wells were filled with test samples. After incubation at 37°C for 18–24 h, zones of inhibition were measured to evaluate antimicrobial efficacy *(Bauer et al., 1966)*.

RESULTS

The present study integrates pharmacognostical standardization, phytochemical profiling, in silico immunomodulatory screening, and formulation of a novel herbal hard candy containing *Mukia maderaspatana* L. extract. The findings collectively confirm the authenticity of the plant material, presence of

biologically active phytoconstituents, and the feasibility of developing a stable and effective herbal dosage form. Similar studies have highlighted the importance of pharmacognostical and physicochemical standardization in ensuring quality and reproducibility of herbal drugs *(WHO, 2011; Evans, 2009)*, while phytochemicals such as flavonoids and saponins are known contributors to immunomodulatory activity *(Hooda et al., 2024; Sharma et al., 2024.*

Microscopic Evaluation

Microscopic analysis revealed:

- Dorsiventral leaf structure
- Single-layered epidermis with cuticle
- Presence of uniseriate multicellular trichomes
- Collateral vascular bundles
- Abundant stomata on abaxial surface

These anatomical features confirm diagnostic characteristics of the species and are in agreement with standard pharmacognostic descriptions *(Evans, 2009)*.

The ash values indicate the presence of inorganic constituents and minimal contamination. Extractive values suggest the presence of moderately polar phytoconstituents. Moisture content below 10% indicates good stability and reduced microbial growth risk *(Indian Pharmacopoeia, 2018)*.

Preliminary Phytochemical Screening

The presence of flavonoids, saponins, and phenolic compounds suggests antioxidant and immunomodulatory potential, as supported by previous studies *(Hooda et al., 2024; Sharma et al., 2024)*.

GC–MS Analysis

These compounds are reported to possess anti-inflammatory, antioxidant, and immunomodulatory activities, supporting the therapeutic potential of the plant *(Maheshwari et al., 2022)*.

In Silico Pharmacological Study

Docking studies demonstrated favorable binding interactions of selected phytoconstituents with target proteins (PDB IDs: 5F19, 4G8A, 2Z7X, 1NFK), suggesting potential immunomodulatory activity. Compounds like γ-bisabolol exhibited significant binding affinity, indicating possible biological relevance *(Morris et al., 2009)*.

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DISCUSSION

The present study was carried out to evaluate the pharmacognostical, phytochemical, and immunomodulatory potential of *Mukia maderaspatana* L., along with the development of an herbal hard candy formulation. The findings were compared with previously reported studies to validate the results. The pharmacognostical studies confirmed the identity of the plant through its characteristic macroscopic and microscopic features such as dorsiventral leaf structure, presence of trichomes, and calcium oxalate crystals. These observations are similar to earlier reports on *Mukia maderaspatana* and other medicinal plants, where such anatomical features are used for authentication *(Kumar et al., 2022; WHO, 2011)*. The physicochemical parameters such as ash values, extractive values, and moisture content were within acceptable limits. These results are in agreement with standard pharmacopoeial guidelines and previous studies, indicating good quality and purity of the crude drug *(Indian Pharmacopoeia, 2018)*. Preliminary phytochemical screening revealed the presence of important constituents like alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic compounds. Similar phytoconstituents have been reported in earlier studies on *Mukia maderaspatana* and are known for their medicinal properties, especially immunomodulatory and antioxidant activities *(Sharma et al., 2024; Hooda et al., 2024)*. GC–MS analysis identified bioactive compounds such as γ-bisabolol and ergost derivatives, which are known for anti-inflammatory and antimicrobial activities. These findings are comparable with previous phytochemical studies on medicinal plants, supporting the therapeutic potential of the plant *(Maheshwari et al., 2022)*. The in silico docking study showed good binding interactions of selected compounds with target proteins, indicating possible immunomodulatory activity. Similar computational studies have reported that plant-derived compounds can effectively interact with immune-related targets *(Morris et al., 2009)*.

The formulated herbal hard candy showed good organoleptic properties, uniform weight, adequate hardness, and acceptable stability. The drug release was rapid (>90% within 20 minutes), which is comparable to standard formulations *(USP, 2020)*. The drug content was within the acceptable range (91–99%), indicating uniform distribution of the extract. The antimicrobial study showed moderate activity against *E. coli* (zone of inhibition 9–11 mm). Similar moderate antimicrobial effects have been reported in other herbal formulations due to the presence of phenolics and flavonoids *(Bauer et al., 1966)*.

CONCLUSION

The study confirms that *Mukia maderaspatana* L. leaves possess significant phytochemical constituents with potential immunomodulatory activity. Pharmacognostical and physicochemical evaluations established the quality and authenticity of the plant material. The formulated herbal hard candy showed acceptable stability, uniformity, and rapid drug release, along with moderate antimicrobial activity. Overall, the findings suggest that the developed herbal candy is a promising and convenient dosage form, though further in

vivo studies are required to confirm its therapeutic efficacy.

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