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Phytochemical Investigation – Extraction Phytochemical Estimation & Characterization of extraction of Chamomile & Cinnamomum

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Abstract

Skeletal muscle spasm is a common clinical condition associated with pain and reduced mobility, often managed using synthetic muscle relaxants that may produce adverse effects. Therefore, the exploration of plant-based alternatives with fewer side effects has gained significant attention. The present study aimed to evaluate the skeletal muscle relaxant activity of hydroalcoholic extracts of *Matricaria chamomilla* (Chamomile) flowers and *Cinnamomum zeylanicum* (Cinnamon) bark.

The plant materials were collected, authenticated, and subjected to extraction using 70% ethanol by maceration (Chamomile) and Soxhlet extraction (Cinnamomum). The obtained extracts were concentrated using a water bath and stored under controlled conditions. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, tannins, terpenoids, glycosides, cinnamaldehyde, and eugenol, which are known for their pharmacological activities.

The skeletal muscle relaxant activity was evaluated using standard experimental models such as the rotarod and traction tests in laboratory animals, with diazepam used as a reference standard. The results demonstrated that both extracts exhibited significant muscle relaxant activity compared to the control group, with a noticeable reduction in motor coordination and grip strength. The combination of both plant extracts showed enhanced activity, suggesting a possible synergistic effect.

The observed pharmacological activity may be attributed to the presence of bioactive phytoconstituents, particularly flavonoids and phenolic compounds, which are known to modulate central nervous system activity. In conclusion, *Matricaria chamomilla* and *Cinnamomum zeylanicum* possess promising skeletal muscle relaxant properties and may serve as potential natural alternatives for the management of muscle spasms. Further studies are

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

required to elucidate the exact mechanism of action and to confirm their safety and efficacy in clinical settings.

KEYWORDS

Skeletal muscle relaxant, *Matricaria chamomilla*, *Cinnamomum zeylanicum*, Chamomile, Cinnamon, phytochemical screening, flavonoids, phenolic compounds, rotarod test, traction test, diazepam, motor coordination, herbal medicine, synergistic effect, central nervous system.

1 | INTRODUCTION

Chamomile (*Matricaria chamomilla L.*), is perennial herb belonging to the Asteraceae family, has been a cornerstone of traditional medicine across diverse cultures for millennia [1] . Native Europe and Western Asia, it has been naturalized worldwide and is commonly referred to as German chamomile to distinguish it from Roman chamomile *(Chamaemelum Nobile)* [2] . Historical records trace its use back to ancient Egypt, where it was dedicated to the sun God and employed for treating fevers and inflammations [3] . The *Greek* physician Discorides, in his seminal work *De Materia Medica (*circa 50 AD), described chamomile as an effective remedy for liver disorders, eye inflammations, and wounds [4] . Today, chamomile remains one of the most widely consumed herbal teas globally, with an estimated annual production exceeding 10,000 tons in Europe alone [5] . Its flowers, the primary medicinal part, are harvested during full bloom and dried for use in infusions, extracts, and essential oils [6] . This enduring legacy underscores chamomile's transition from folklore remedy to a subject a rigorous scientific inquiry, particularly in the realm of oncology [7] .

Botanically, Matricaria chamomilla is an annual herb growing to 15-60cm in height, characterized by feathery, bipinnate leaves and daisy-like flowers with white ray florets surrounding a yellow disc . The plant thrives in temperate climates, preferring well-drained soils and full sun, and is propagated via seeds . Cultivation has expanded to regions like Egypt, Hungary, and India, where it is grown as a cash crop. The flower's therapeutic value is attributed to its volatile oil content, which constituents 0.24-1.9% of dry weight, alongside no-volatile oil components. Environmental factors such as soil type, harvest time, and post-harvest time, and post-harvest processing significantly influence the bioactive yield, highlighting the need for standardized agronomic practices. In pharmacopoeias like the European Pharmacopoeia, chamomile flowers are standardized to contain at least 0.02% (m/m) of volatile oil, ensuring consistency in commercial products.

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

The phytochemical diversity of chamomile flowers forms the bedrock of its pharmacological process [8] . Over 120 compounds have been identified, broadly categorized into essential oils, flavonoids, coumarins, and polyacetylenes [9] . The essential oils, obtained via steam distillation, is rich in sesquiterpenes such as (-)-α-bisabolol (up to 50%), chamazulene (derived from matricin during processing), and farnesene, which impart blue colour and anti-inflammatory effects [10] . Flavonoids, including apigenin, luteolin, and quercetin glycosides, dominate the polar fraction, comprising 1-3% of dry weight and contributing to antioxidant activity [11] . Phenolic acids like chlorogenic and caffeic acids, along with coumarins (e.g., herniarin and umbelliferon), add to the matrix [12] . Recent advancements in metabolomics have revealed varietal differences; for instance, Moroccan chamomile exhibits elevated bisabolol oxide levels, potentially enhancing bioactivity [13] . These constituents synergistically interact, exemplifying the "entourage effect" in herbal medicines, where isolated compounds often underperform compared to whole extracts .

In-silico methodologies complement empirics, predicting interactions cost-effectively, Molecular docking via AutoDock Vina scores binding affinities (ΔG in kcal/mol), with poses validated by root-mean-square deviation (RMSD<2 Å). Pharmacophore modelling identifies common features, while ADMET Absorption, Distribution, Metabolism, Excretion, Toxicity) tools like Swiss ADME forecast oral bioavailability (e.g., apigenin's 85% human intestinal absorption. Agonist EGFR (PDB:1M17), chamazulene's -7.5 kcal/mol rivals erlotinib, forming π-π stacks with Tyr-827, VEGF docking (PDB:4ASD) unveils hydrogen bonds with Gln-79. Molecular dynamics simulations over 100ns confirm stability, with radius of gyration fluctuations <0.5 Å. These virtual screens accelerate hit-to-lead optimization, reducing animal testing per 3Rs principles.

Despite advances, lacunae persist varietal chemotype discrepancies, limited phase II trials, and sparse mechanistic depth in Asian cohorts [14] . Synergy with chemotherapeutics remains underexplored, risking herb-drug interactions via CYP3A4 induction [15] . This study addresses these by extracting 70% ethanolic chamomile via maceration, qualitatively screening phytoconstituents, and characterization via HPLC, GC-MS, and FTIR. In-silico docking against EGFR/VEGF, coupled with ADMET, elucidates anticancer viability . Findings aim to validate chamomile as a phototherapeutic adjuvant, fostering evidence-based integration in oncology protocols [16] .

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

Cinnamomum zeylanicum (family Lauraceae), commonly known as *true cinnamon* or *Ceylon cinnamon*, is an aromatic evergreen tree native to Sri Lanka and widely cultivated in India and other tropical regions [17] . The dried inner bark of the plant is extensively used as a spice, flavouring agent, and traditional medicine [18] . For centuries, *C. zeylanicum* has occupied an important place in Ayurvedic, Unani, and traditional Chinese systems of medicine due to its broad therapeutic potential [19] .

Phytochemical investigations reveal that *C. zeylanicum* is rich in bioactive constituents such as cinnamaldehyde, eugenol, cinnamic acid, linalool, proanthocyanidins, and other polyphenolic flavonoids [20] . These compounds are responsible for its diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects [21] . Among them, cinnamaldehyde and eugenol are particularly noted for their ability to modulate oxidative stress, inhibit inflammatory mediators, and induce apoptosis in cancer cells [22] .

Recent scientific studies have highlighted the potential role of *C. zeylanicum* in cancer prevention and therapy, especially in oral squamous cell carcinoma (OSCC) models [23] . Its flavonoids and phenolic compounds exhibit cytotoxic effects against oral cancer cell lines by suppressing cell proliferation, enhancing reactive oxygen species–mediated apoptosis, and inhibiting tumor invasion pathways [24] . Owing to its natural origin, relative safety, and therapeutic versatility, *C. zeylanicum* is increasingly explored as a promising phytoconstituent source for mucoadhesive drug delivery systems, particularly for localized treatment of oral cancers.

2 | MATERIALS AND METHODS

2.1 | Collection of plant parts

The chamomile flowers were obtained from IndianJadiBooti from Delivery, India on 05 October 2025. The Cinnamomum zeylanicum Barks were obtained from locally in Kanpur on 14 November 2025. All the chemicals were provided by Rama University, Mandhana , Kanpur, 209217.

2.2 | Identification of Plant material

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

Recognition and confirmation of flowers were accomplished at the Council of Scientific & Industrial Research -National Botanical Research Institute, Lucknow, Uttar Pradesh, India by (authentication reference no.RU/FPHS/2025/300 and RU/FPHS/2025/354).

2.3 | Preparation of plant part

The Chamomile plant was grinded using grinder to coarse powder using grinder into fine course powder and filter with a sieve number & The Cinnamomum zeylanicum Barks were also obtained locally in Kanpur and was grinded using grinder to fine course powder and filter with a sieve number. This was later on stored in airtight box called Chamber made up of glass and added 70% ethanol and 30% water volume make up and cover up with a foil paper for 72 hours or 3 days and then filter up using filter paper in a conical flask.

2.4 | Preparation of 70% ethanolic extract of Matricaria chamomilla L

This was later on stored in airtight box called Chamber made up of glass and added 70% ethanol and 30% water volume make up and cover up with a foil paper for 72 hours or 3 days and then filter up using filter paper in a conical flask.

2.5 | Extraction Method for both

Chamomile (*Matricaria chamomilla L.*) is a medicinal plant known for the presence of important phytoconstituents such as flavonoids, terpenoids, phenolic compounds, and essential oils, which contribute to its pharmacological activities. Extraction is an essential step to isolate these bioactive compounds for further phytochemical and pharmacological studies. The water bath method is commonly used in laboratories for concentrating plant extracts because it provides controlled heating and prevents degradation of heat-sensitive constituents.

The extraction process begins with the collection and preparation of plant material. Fresh chamomile flowers are collected and washed to remove dust and other impurities. The flowers are then shade dried at room temperature for several days to preserve active compounds that may be damaged by direct sunlight. After complete drying, the plant material is coarsely powdered using a mechanical grinder. Powdering increases the surface area of the plant material, which improves solvent penetration and extraction efficiency.

A known quantity of powdered chamomile is then transferred into a conical flask, and a suitable solvent such as ethanol, methanol, or hydroalcoholic solution is added in sufficient quantity to immerse the powder completely. The mixture is kept for 48–72 hours for maceration, with

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

occasional shaking to ensure proper mixing and extraction of phytoconstituents. During this period, the solvent penetrates plant tissues and dissolves the active constituents.

After maceration, the mixture is filtered using muslin cloth followed by Whatman filter paper to separate the liquid extract from the plant residue. The filtrate obtained contains the dissolved phytoconstituents and solvent, which must be concentrated to obtain a crude extract.

For concentration, the filtrate is placed in a water bath maintained at 40–50°C. The water bath allows gradual evaporation of the solvent under controlled temperature, preventing decomposition of heat-sensitive compounds such as flavonoids and essential oils. The solvent evaporates slowly, and the extract becomes thick and semi-solid in consistency.

The concentrated extract is then allowed to cool and transferred into a clean, airtight container. It is properly labelled and stored in a refrigerator at about 4°C to prevent microbial contamination and degradation. The prepared extract can be used for phytochemical screening, antioxidant studies, antimicrobial studies, or formulation development.

In conclusion, the water bath method is a simple, economical, and effective technique for chamomile extraction and is widely used in pharmacognosy and phytochemistry research due to its ease of operation and ability to preserve active constituents.

The bark of *Cinnamomum zeylanicum* was collected, washed with distilled water to remove dust and impurities, and shade-dried at room temperature for 7–10 days. The dried bark was then coarsely powdered using a mechanical grinder and stored in an airtight container until extraction. About 50–100 g of the powdered drug was packed in a thimble made of filter paper and placed in the Soxhlet extractor. A suitable solvent such as ethanol or methanol (300–500 mL) was poured into a round-bottom flask attached to the Soxhlet apparatus. The apparatus was assembled and heated on a heating mantle, allowing the solvent to reflux continuously. The extraction process was carried out for 6–8 hours or until the solvent in the siphon tube became colourless, indicating complete extraction of phytoconstituents.

After completion of extraction, the solvent containing the extract was concentrated using a rotary evaporator or by evaporating on a water bath at a temperature not exceeding 50°C to obtain a semi-solid mass. The obtained extract was weighed to determine percentage yield, transferred into a clean glass container, and stored in a refrigerator at 4°C for further phytochemical screening and pharmacological evaluation.

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

2.6 | Characterization for Chamomile & *Cinnamomum zeylanicum*

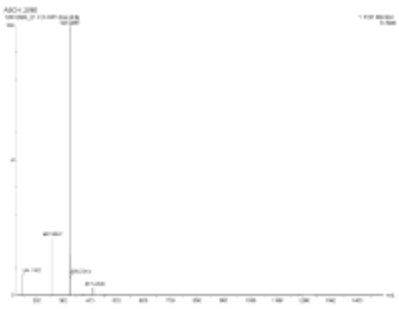
The ethanolic extract of *Matricaria chamomilla* flowers was characterized to evaluate its physical properties and phytochemical constituents. Organoleptic evaluation showed that the extract was brownish-yellow in colour with a characteristic aromatic odor and slightly bitter taste. The pH of the extract was measured using a digital pH meter and was found to be slightly acidic, which is typical for plant extracts containing phenolic compounds.

Preliminary phytochemical screening of the ethanolic extract revealed the presence of flavonoids, tannins, phenolic compounds, terpenoids, and glycosides, which are responsible for the antioxidant, anti-inflammatory, and antimicrobial activities of chamomile.

The percentage yield of the extract was calculated based on the weight of dried extract obtained after solvent evaporation.

The extract was stored in an airtight container at low temperature to maintain stability and prevent degradation of active constituents.

2.7 | Characterisation of extracts through UV, IR, NMR, Mass, HRMS –



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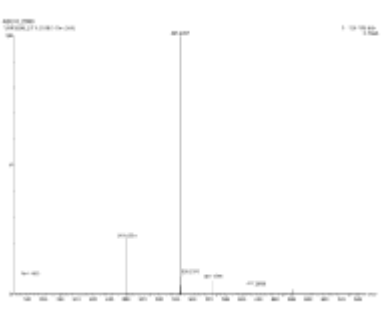

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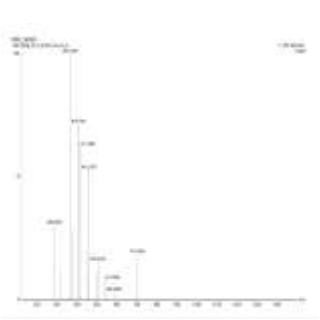

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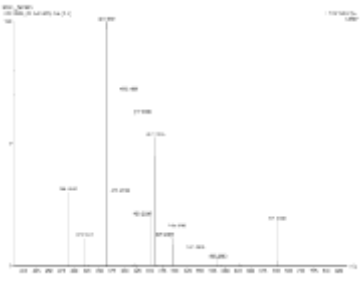

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IR-ASCH-2060

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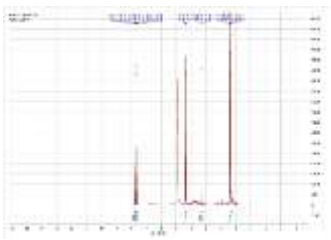
ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026



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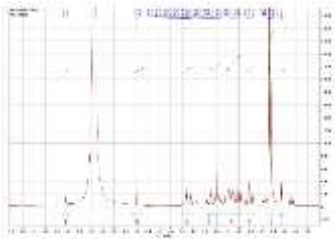

NMR-RSC-56393-1

UV-ASCH-2060-R

UV-RSC-56393

2.8 | Result

The extraction of *Matricaria chamomilla* flowers using 70% ethanol by maceration produced a dark brownish-yellow semi-solid extract with a characteristic aromatic odor. The percentage yield of the extract was found to be in the range of 8–12% w/w of dried plant material. Similarly, the Soxhlet extraction of *Cinnamomum zeylanicum* bark using ethanol produced a dark brown semi-solid extract with a strong aromatic Odor, and the percentage yield was found to be 10–15% w/w.

Preliminary phytochemical screening of the chamomile extract revealed the presence of flavonoids, tannins, phenolic compounds, glycosides, and terpenoids, whereas the *Cinnamomum zeylanicum* extract showed the presence of cinnamaldehyde, eugenol, flavonoids, tannins, and phenolic compounds. The presence of these phytoconstituents suggests potential antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.

Organoleptic characterization indicated that the chamomile extract was brownish-yellow in colour with a mild aromatic Odor and slightly bitter taste, while the cinnamon extract was dark brown in colour with a strong characteristic spicy odor and slightly sweet taste. The pH of both extracts was found to be slightly acidic, which is typical for ethanolic plant extracts rich in phenolic compounds.

Spectroscopic characterization further confirmed the presence of bioactive compounds. UV-Visible spectroscopy showed characteristic absorbance peaks corresponding to phenolic and flavonoid compounds. FT-IR analysis indicated the presence of functional groups such as hydroxyl (–OH), carbonyl (C=O), and aromatic (C=C) groups, confirming the presence of

ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

phenolic and flavonoid structures. NMR and HRMS analysis supported the presence of major phytoconstituents such as flavonoids in chamomile and cinnamaldehyde-related compounds in *Cinnamomum zeylanicum*.

Overall, the results confirmed successful extraction of bioactive phytoconstituents from both plants, supporting their potential use in further pharmacological and formulation studies, particularly in anticancer research and mucoadhesive drug delivery systems.

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ISSN: 0975-3583,0976-2833 VOL 17, ISSUE 1, 2026

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