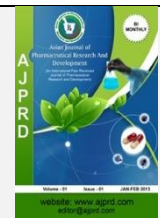


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Research Article

Antispasmodic Efficacy of Ethanolic Extract of Chamomile Flowers in Isolated Chicken Ileum Using *Ex-Vivo* Experiments

Vaibhav Dwivedi, Nisha Gautam, Priya, Avinash C. Tripathi*

R. G. S. College of Pharmacy, Lucknow, Uttar Pradesh, India – 226203

ABSTRACT

Matricaria chamomilla L. (Chamomile), a member of the Asteraceae family, has long been valued in traditional medicine for its broad therapeutic applications, including the relief of smooth muscle spasms. Its pharmacological properties are attributed to a diverse range of bioactive constituents present in its essential oils and plant extracts. Ethanolic extracts were prepared from disc florets, ray florets, and their combination. Phytochemical screening was conducted to identify major classes of constituents. The effect of the extracts on acetylcholine-induced contractions was assessed using the interpolation method, and responses were recorded with a kymograph. Dose response relationships for acetylcholine were determined in the absence as well as presence of the extracts. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, and triterpenoids, with varying intensity across flower parts. Pharmacological assays demonstrated that ethanolic extracts significantly reduced acetylcholine-induced ileum contractions in at higher dose, indicating antagonistic activity. The combined extract of ray and disc florets showed the greatest inhibitory effect. The findings support the traditional use of *M. chamomilla* L. as an antispasmodic agent. The inhibitory effect on acetylcholine-induced smooth muscle contraction suggests possible interference with parasympathetic neurotransmission. Further studies may need to identify the specific active compounds and elucidate their exact mechanisms of action.

Keywords: *Matricaria chamomilla* L., chamomile, antispasmodic activity, phytochemical screening, acetylcholine antagonism

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*Address for Correspondence:

Avinash C. Tripathi, R. G. S. College of Pharmacy, Lucknow, Uttar Pradesh, India – 226203

INTRODUCTION

The term “chamomile” is derived from the Greek words *chemos* and *melos*, meaning “ground apple,” a reference to its characteristic aroma (1). Belonging to the Asteraceae family, *M. chamomilla* is native to southern and eastern Europe but is now cultivated in diverse climates, including Germany, Hungary, France, Russia, Brazil, and the Xinjiang region of China (2). The annual plant possesses slender spindle-shaped roots and highly branched erect stems reaching 10–80 cm in height. Leaves are bi-to-tri innate, and the solitary flower heads, 10–30 mm in diameter, are pediculate and Heterogamous (3). Distinctive features include a hollow receptacle and yellow disc florets surrounded by white ray florets. The fruit is a yellowish-brown achene (4). The plant contain diverse bioactive compounds, including; Organic acids- palmitic, linoleic, oleic, stearic, and isobutyric acids; Coumarins-

umbelliferone, scopoletin, esculetin, and daphnetin; Volatile oils- 3-methyl-2-butanol, isoamyl alcohol, benzaldehyde; Terpenes- camphene, α -pinene, γ -terpinene, carvone; Sterols and guaianolides: β -sitosterol, stigmasterol, and related derivatives which were found effective against numerous health Issues such as gastrointestinal disorders, insomnia, anxiety, arthritis, eczema, wounds, burns, menstrual cramps etc (5,6). Gastrointestinal (GI) disorders constitute a significant proportion of health complaints in paediatric populations worldwide. Common conditions include functional abdominal pain, ulcerative colitis, irritable bowel syndrome (IBS), infantile colic, constipation, gastroenteritis, and acute gastrointestinal disturbances (7). These disorders can substantially impair quality of life and are frequently associated with heightened risks of anxiety and depressive symptoms (8). The pathophysiology of GI spasms involves dysregulated neurotransmission, notably via the cholinergic system, aberrant calcium influx through L-type channels, and

altered membrane excitability mediated by potassium channels (9). Conventional antispasmodic agents, including anticholinergics and calcium channel blockers, are effective but often limited by adverse effects such as dry mouth, blurred vision, and cardiovascular risks (10,11). Given the limitations of current pharmacotherapies and the increasing demand for natural remedies, systematic studies comparing extraction methodologies and their pharmacological outcomes are essential. This investigation aims to delineate the comparative antispasmodic efficacy using standardized acetylcholine-induced contraction assays in isolated chicken ileum preparations. The findings will present the basis to develop optimized, standardized herbal formulations for clinical management of GI spasms (12, 13).

MATERIALS AND METHODS

Plant material selection and collection

Fresh flower heads of *Matricaria chamomilla* L. were harvested from the RGS Herbal Garden, Itaunja, Lucknow,

Uttar Pradesh, India. Selection of fully matured flowers ensured optimal phytoconstituent content (Figure 1a). The selection of this plant is scientifically justified on phytochemical, pharmacological, and research grounds. Ray florets are particularly enriched with flavonoids and phenolic compounds, which are well documented to produce smooth muscle relaxation through phosphodiesterase inhibition (elevating cAMP), calcium influx blockade, and antioxidant activity that stabilizes gastrointestinal tissue responses. On the other hand, disc florets contain alkaloids, triterpenoids, and tannins, which modulate muscarinic receptors and ion channels, providing additional protective and astringent effects on the gastrointestinal mucosa. Separating and testing disc and ray florets enables differential profiling of bioactive constituents, aiding in the standardization of herbal medicines. Such findings provide a strong basis for targeted extract formulation, mechanistic studies on phytochemical interactions, and quality-controlled development of plant-based antispasmodic agents.



Figure 1: a. Chamomile Flower b. Disc Florets c. Ray Florets

Drying

Post-harvest, the flowers were divided into two parts i.e., Disc Florets (Yellow Parts) as shown in Figure 1 b and Ray Florets (White Parts) as shown in Figure 1 c. Both parts were shade dried in a well-ventilated environment to minimize the degradation of volatile constituents and photosensitive compounds. Direct sunlight and elevated temperatures were avoided to prevent the loss of essential oils and bioactive metabolites (4, 14).

Size Reduction

The both dried plant material were ground separately using a mechanical grinder to achieve a coarse powder. The powdered material was sieved through No. 40 mesh (0.420 mm) to ensure uniformity and stored in airtight containers under controlled conditions for extraction. Particle size reduction will increase the surface area available for solvent contact, thereby enhancing extraction efficiency in Soxhlet apparatus (15).

Preparation of Ethanolic Extract

The coarse powdered plant material were packed in a thimble with the help of filter paper and extracted separately with ethanol using a Soxhlet extractor (16). The solvent in the round-bottom flask was heated to reflux, condensed, and percolated through the plant matrix, facilitating extraction of soluble constituents. The process was continued until the solvent in the siphon tube appeared clear. The extract was

concentrated under reduced pressure and stored at 4 °C for further use (17, 18).

Phytochemical Screening

Preliminary phytochemical screening was conducted to identify major secondary metabolites using standard qualitative tests such as; Dragendorff's, Mayer's, and Wagner's tests for alkaloids; Molisch's and Barfoed's tests for carbohydrates; ferric chloride and ammonia tests for flavonoids; gelatin and Braymer's tests for tannins; and Salkowski's test for triterpenoids (18, 19).

Procurement of Biological Tissue

Chicken ileum segment (2-3 cm length) was obtained from healthy broiler, breed male chicken (2.5 kg-3 kg) from Karaundi village, Itaunja, Lucknow immediately after sacrifice and transported to the laboratory in Tyrode's solution with continuous aeration. The terminal 10-20 mm of ileum nearest to the ileocecal junction was discarded to ensure tissue uniformity (20).

Antispasmodic Assay (Ex-vivo)

Chicken ileum was selected as the experimental model based on several established advantages (21, 22): ethical considerations (use of food industry animals), pharmacological equivalence (similar EC₅₀ values to guinea pig ileum), and validated reliability (multiple studies confirm suitability for antispasmodic testing). The choice of acetylcholine (0.4 mL) as the standard spasmogenic agent is

based on its role as the primary neurotransmitter in gastrointestinal motility and its ability to produce reproducible, submaximal contractions suitable for evaluating relaxant effects (23).

The terminal 2 cm segment of chicken ileum was mounted in a 20 mL organ bath containing Tyrode solution maintained at 37 °C, aerated with a gas mixture of 95% O₂ and 5% CO₂. After a 30-minute equilibration period with bath solution changes every 10 minutes, dose response curves for acetylcholine (0.2 and 0.4 mL) were recorded using a kymograph. Subsequently, cumulative concentration–response curves were obtained for acetylcholine in the absence and presence of chamomile ethanolic extract (1 mg/mL) prepared from disc florets, ray florets, or their combination. Contact time was fixed at 60 s with a baseline of 30 s and a 5-minute cycle, using a drum speed of 0.25 rpm. Percentage inhibition of contraction was calculated relative to control.

RESULTS

Phytochemical Analysis

The ethanolic extracts of chamomile disc and ray florets tested positive for several secondary metabolites (Table 1). Alkaloids, flavonoids, phenolic compounds, triterpenoids, and carbohydrates were prominently detected, with higher intensity (+++) for flavonoids and phenolics in ray florets compared to disc florets.

Alkaloids

Distinct responses were observed in tests for alkaloids. In disc florets, the Dragendorff's, Hager's, and Mayer's tests indicated the presence of alkaloids, as evidenced by the positive reactions (+). Ray florets yielded a moderately strong positive (++). These results suggest that alkaloids are distributed in both parts, possibly contributing to physiological activity.

Carbohydrates

Disc florets showed weak or negative results for carbohydrates, with Molisch's and Selivanoff's tests displaying faint or negative responses. In contrast, ray florets

presented a strong positive (+++) in the resorcinol test, indicating higher carbohydrate content compared to disc florets. This contrast highlights potential differences in primary metabolism between these floret types.

Flavonoids

Flavonoid presence was clearly established using both ferric chloride and ammonia tests which demonstrated strong reactions (++) and (+++), particularly prominent in ray florets, suggesting flavonoids are more abundant or easily extractable from this part of the plant. Flavonoids are well-known for their antioxidant properties, lending further biological significance.

Phenolic Compounds

Positive results for phenolic compounds were noted with both iodine and ferric chloride tests, though with varying intensities. The disc florets registered a single positive reaction for iodine, while ray florets showed a strong (+++) response to the ferric chloride test. This indicates a richer phenolic content in the ray florets.

Tannins

Both types of florets yielded positive reactions for tannin tests, especially Braymer's and sodium hydroxide tests. These findings pointed towards the presence of hydrolysable or condensed tannins, likely contributing to astringent properties and defence mechanisms of the plant.

Triterpenoids

Salkowski's test showed clear, strong positives in both disc and ray florets, suggesting the presence of triterpenoids across floral tissues. Triterpenoids are associated with various pharmacological effects, supporting further interest in these plant parts.

The screening underscores broad phytochemical diversity of tested extract from the isolated tissues. Alkaloids, flavonoids, phenols, tannins, and triterpenoids were found to be present, though their abundance appears to differ in disc and ray florets. This differential distribution is pivotal for understanding the plant's medicinal properties guiding the need of further phytochemical or pharmacological studies.

Table 1: Phytochemical profile of *Matricaria chamomilla* extracts

Category	Test Name	Ethanolic Extract	
		Disc Florets	Ray Florets
Alkaloids	Dragendorff's Test	++	++
	Hager's Test	-	-
	Mayer's Test	++	++
	Picric Acid Test	++	+
	Iodine Test	-	-
Carbohydrates	Barfoed's Test	+	+
	Molish's Test	+	+
	Seliwanoff's Test	-	-
	Resorcinol Test	++	+++
Flavonoids	Ferric Chloride Test	++	+++
	Ammonia Test	++	++
	Conc. H ₂ SO ₄ Test	++	+++
Phenolic Compounds	Iodine Test	+	++
	Ferric Chloride Test	++	+++
Tannins	Braymer's Test	+	-
	10% NaOH Test	-	-
Triterpenoids	Salkowski's Test	+++	++

Effect of Acetylcholine-Induced Contraction on Isolated Tissue Preparations

Acetylcholine elicited a dose-dependent increase in ileal smooth muscle contraction (1.3 cm and 1.8 cm for 0.2 ml and 0.4 ml doses, respectively as shown in Figure 2, 3). In the presence of chamomile extracts, contraction amplitude was significantly reduced across all extract types (Table 2,3).

The contractile response of isolated tissue to acetylcholine and its modulation by ethanolic flower extracts was evaluated (Table 1,2).

Acetylcholine Response (Control)

Administration of acetylcholine at 0.2 ml produced contraction amplitude of 1.3 cm, which increased to 1.8 cm when the dose was doubled to 0.4 ml. This dose-dependent rise in contractile force confirmed the sensitivity of the preparation to cholinergic stimulation.

Effect of Ethanol Extract of Disc Florets

When acetylcholine (0.2 ml) was co-administered with 0.2 ml of disc floretsethanolic extract, the contractile response decreased to 0.7 cm, indicating a marked attenuation of cholinergic activity. Similarly, the combination of acetylcholine (0.4 ml) with extract (0.4 ml) resulted in a response of 0.9 cm, further supporting an inhibitory influence.

Effect of Ethanol Extract of Ray Florets

Co-administration of acetylcholine (0.2 ml) with ray florets ethanolic extract (0.2 ml) yielded a response of 0.6 cm, while the higher combination dose (0.4 ml + 0.4 ml) produced 0.8 cm. These results suggest that the ray florets extract exerted a slightly stronger inhibitory effect than the disc florets extract at comparable doses.

Effect of Combined Ray and Disc Florets Extracts

The simultaneous administration of both ray and disc Florets ethanolic extracts with acetylcholine (0.2 ml each) resulted in a response of 1.1 cm, while higher doses (0.4 ml each) elicited 1.5 cm as shown in Figure 4. This partial recovery in contractile amplitude compared with individual extracts suggests a possible interaction or modulation between constituents present in the two floral parts.

The overall outcome of the study revealed that acetylcholine caused spasm by contracting the smooth muscle of chicken ileum but when given in presence of extract, there was a marked decrease in the muscle contraction. This revealed that ethanolic extract of *Matricaria chamomilla* flower possess a high degree of spasmolytic (antispasmodic) activity by blocking cholinergic muscarinic receptors.

Table 2: Antispasmodic activity (at 0.2 ml dose) on the isolated chicken ileum

S. N	Drug	Dose (ml)	Response (cm)	Percentage Inhibition (%)	Antispasmodic Activity
1	Acetylcholine	0.2	1.3	-	Control
2	Acetylcholine + Ethanol Extract (Disc Florets)	0.2+0.2	0.7	46.15%	Moderate
3	Acetylcholine + Ethanol Extract (Ray Florets)	0.2+0.2	0.6	53.84%	High
4	Acetylcholine + Ethanol Extract (Ray Florets + Disc Florets)	0.2+0.2	1.1	15.38%	Low

Table 3: Antispasmodic activity (at 0.4 ml dose) on the isolated chicken Ileum

S. N	Drug	Dose (ml)	Response (cm)	Percentage Inhibition (%)	Antispasmodic Activity
1	Acetylcholine	0.4	1.8	-	Control
2	Acetylcholine + Ethanol Extract (Disc Florets)	0.4+0.4	0.9	50%	Moderate
3	Acetylcholine + Ethanol Extract (Ray Florets)	0.4+0.4	0.8	55.55%	High
4	Acetylcholine + Ethanol Extract (Ray Florets + Disc Florets)	0.4+0.4	1.5	16.66%	Low

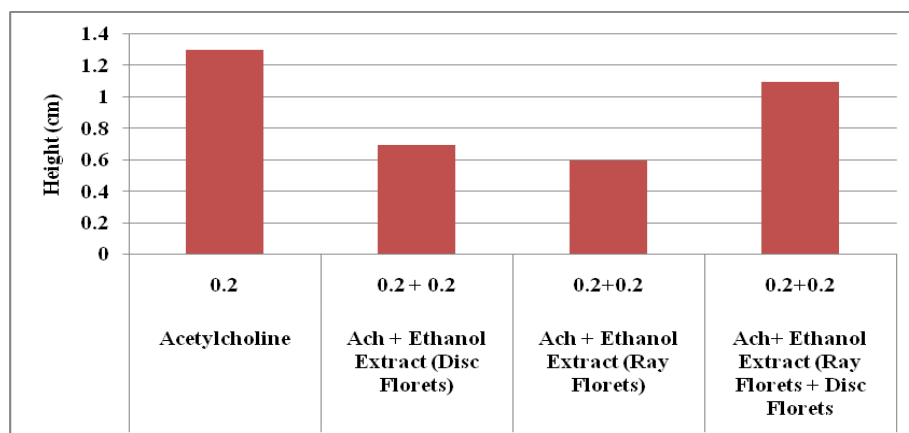


Figure 2: Response to Acetylcholine and Extract at 0.2 ml dose on the isolated chicken ileum

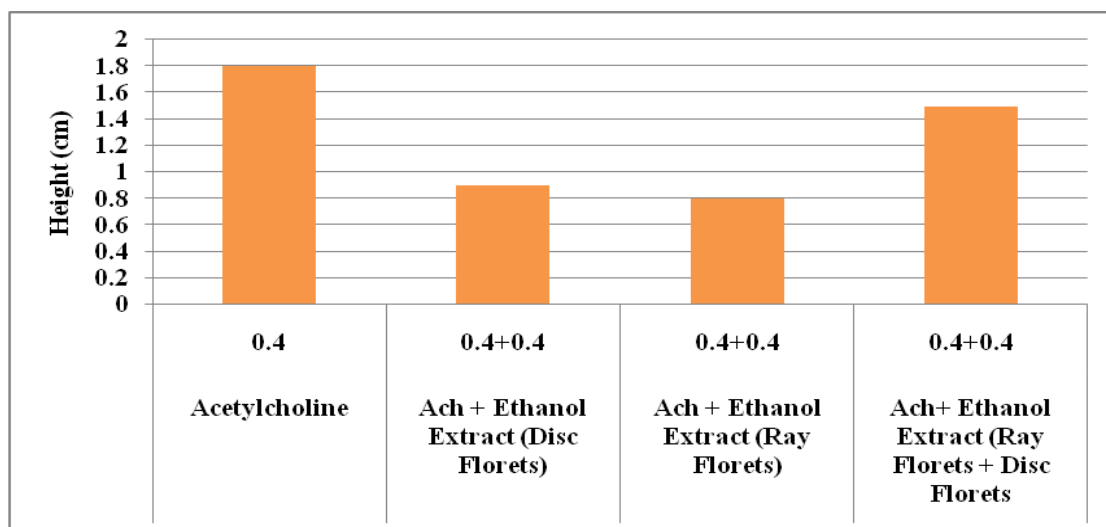


Figure 3: Response to Acetylcholine and Extract at 0.4 ml dose on the isolated chicken ileum

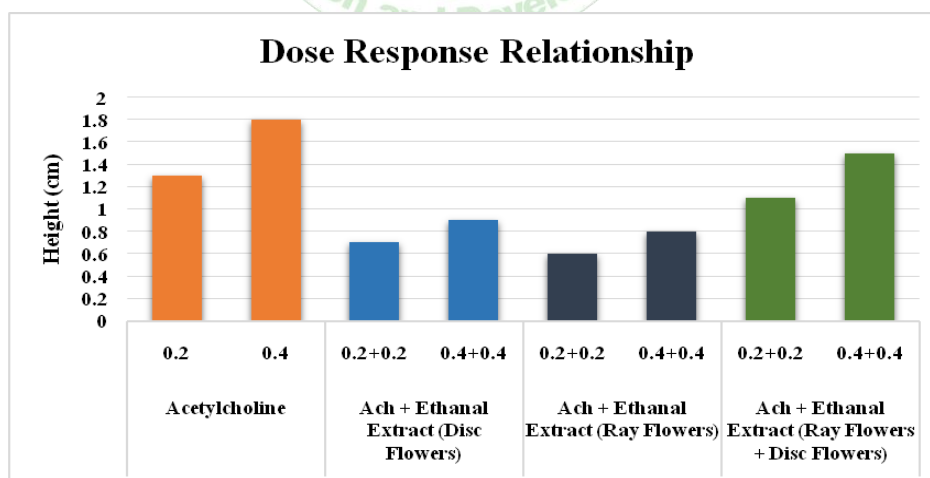


Figure 4: Combined Dose Response Relationship of Acetylcholine

DISCUSSION

The present study demonstrated significant attenuation of acetylcholine-induced contractions in isolated chicken ileum by ethanolic extracts of *Matricaria chamomilla* L. which aligns well with earlier findings and validates the research proposal using isolated tissue bioassay methodology. Competitive antagonism at muscarinic receptors has been

widely reported as a principal mechanism underlying chamomile's antispasmodic effect (24, 25). The ethanolic extracts of *Matricaria chamomilla* L. flowers exhibited clear, dose-dependent antispasmodic activity in isolated chicken ileum preparations. In control tissues, acetylcholine induced smooth muscle contractions measuring 1.3 cm at 0.2 mL and 1.8 cm at 0.4 mL, confirming robust cholinergic

responsiveness consistent with prior models (21). Co-administration of disc florets extract reduced the 0.2 mL acetylcholine response to 0.7 cm (46.15% inhibition) and the 0.4 mL response to 0.9 cm (50% inhibition), indicating moderate antagonism. Ray florets extract was more potent, decreasing responses to 0.6 cm (53.84%) and 0.8 cm (55.55%), respectively, reflecting high antispasmodic efficacy (Table 2). Surprisingly, the combined disc-plus-ray extract yielded only 15.38% and 16.66% inhibition at the two doses, suggesting intraplant constituent interactions may attenuate individual extract potency.

Phytochemical profiling revealed that rayflorets extract possessed the highest levels of flavonoids and phenolic compounds (+++), compounds known to inhibit phosphodiesterase, elevate intracellular cyclic AMP, and impede calcium influx in smooth muscle cells (2, 26). The stronger inhibition by ray- florets extract correlates with its richer flavonoid content, particularly apigenin and luteolin, which relax smooth muscle by reducing intracellular Ca^{2+} availability and modulating receptor-mediated pathways (17). Disc florets extract, though containing similar phytoconstituents at lower contents, still produced significant inhibition, confirming that both flower parts contribute to spasmolysis. The unexpectedly low efficacy of the combined extract may arise from competitive binding at muscarinic receptors or physical-chemical interactions between phytochemicals that alter solubility or receptor affinity. Similar findings in other botanicals indicate that mixing plant fractions does not always yield additive effects and can sometimes diminish bioactivity route (27).

Comparison with other antispasmodic botanicals, such as *Thymus vulgaris* and *Atropa belladonna*, which rely predominantly on tropane alkaloids for receptor blockade (28), highlights chamomile's multifaceted mechanism. Rather than a single class of compounds, a synergistic interplay between flavonoids, phenolics, and terpenoids appears to underpin its efficacy (1). This synergy likely explains why the combined disc and ray florets extract exhibited a modest inhibitory effect compared to individual extracts; constituent interactions may alter receptor binding kinetics or influence membrane permeability.

The strong inhibitory effects of chamomile ethanolic extracts on intestinal smooth muscle corroborate traditional uses in gastrointestinal spasmodic disorders and extend the pharmacological understanding of *M. chamomilla*. The prominent role of flavonoids and phenolic, combined with alkaloid and terpenoid contributions, underscores a multi-targeted mechanism involving receptor antagonism and calcium modulation. Future research should isolate specific active principles, explore potential pharmacokinetic interactions, and validate this *ex-vivo* findings *in-vivo* to facilitate clinical translation.

CONCLUSION

The present investigation confirms that *Matricaria chamomilla* possesses significant antispasmodic potential, as evidenced by its ability to attenuate acetylcholine-induced contractions in isolated chicken ileum. The activity appears to be mediated through competitive antagonism at muscarinic receptors, likely supported by the presence of flavonoids, phenolic compounds, alkaloids, and terpenoids.

Among the tested extracts, ray florets demonstrated greater inhibitory effects compared to disc florets, correlating with their higher concentrations of phenolic and flavonoid constituents. These findings support the traditional use of chamomile in gastrointestinal disorders characterized by smooth muscle spasms and provide a pharmacological basis for its therapeutic application. Future studies should focus on isolating specific active principles, elucidating precise molecular mechanisms, and conducting *in-vivo* validation to establish clinical relevance.

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