

Evaluation of Antitussive and Mast Cell Stabilizing of Some Indigenous Plants

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Abstract

The primary objective of this study was to evaluate the phytochemical composition, antioxidant capacity, and the antitussive and mast cell stabilizing potential of three indigenous medicinal plants—*Nyctanthes arbor-tristis* (flowers), *Argemone mexicana* (flowers), and *Clitoria ternatea* (flowers). Additionally, the study aimed to formulate and characterize a polyherbal syrup using these extracts for respiratory care. The research employed an experimental design involving successive solvent extraction, comprehensive phytochemical screening, chromatographic fingerprinting (TLC and HPTLC), and *in vivo* pharmacological assessment using specialized animal models [1]. Ethyl acetate and ethanolic extracts were prepared from the flower parts. A polyherbal syrup was formulated incorporating 5% w/v of each ethyl acetate extract. The efficacy was measured via cough frequency reduction in ammonium hydroxide-induced models, percentage of mast cell granulation in sensitized rats, and radical scavenging activity (IC₅₀) in DPPH and ABTS assays. Phytochemical analysis confirmed a high concentration of flavonoids and phenolics, particularly in the ethanolic extracts. *Clitoria ternatea* and *Nyctanthes arbor-tristis* demonstrated significant antitussive effects, with percentage inhibitions of 83.28% and 82.89% respectively at 500 mg/kg [2]. The polyherbal syrup showed a dose-dependent reduction in cough bouts, achieving an 81.07% inhibition at the highest dose (3 ml). Mast cell stabilization was most pronounced in the ethyl acetate fractions, significantly protecting against antigen-induced degranulation. The findings validate the synergistic therapeutic potential of the selected herbs in managing cough and allergic inflammatory conditions, providing a scientific basis for their traditional use [3].

Keywords: *Nyctanthes arbor-tristis*, *Argemone mexicana*, *Clitoria ternatea*, Antitussive activity, Mast cell stabilizers, HPTLC, Polyherbal syrup, Phytochemicals.

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Introduction

Medicinal plants (MP) represent a legacy of human health care that transcends the boundaries of recorded history, serving as the foundational tools for ancient medical practitioners across diverse cultures. Even in the contemporary era, these plants continue to be utilized in their crude forms or as standardized extracts for the prevention and treatment of a myriad of physiological disorders. The formal definition of a medicinal plant encompasses any botanical species possessing therapeutic properties suitable for use within traditional or allopathic healthcare systems, often also finding utility in the food, cosmetic, and aromatic industries[4]. Early literature, some dating back over 6,000 years, offers a profound glimpse into the medicinal applications of plants, with the ancient Egyptians recognized as one of the earliest societies to develop systematic collections categorized by therapeutic benefits. Similarly, the use of medicinal herbs in Greece between 3,000 and 1,500 years ago culminated in the influential text *De Materia Medica* during the 1st century AD. Between 1 AD and 1000 AD, evidence suggests the sophisticated development of therapeutic methods utilizing MPs in India, China, and Tibet, which eventually paved the way for modern pharmacological science[5].

The transition from traditional botany to modern pharmacognosy was spearheaded by several key historical figures. Nicholas Culpeper, a 17th-century herbalist, is often hailed as the father of contemporary alternative medicine for his efforts to make herbal remedies both affordable and accessible. Samuel Hahnemann, in the late 18th century, introduced the concepts of Homeopathy, which frequently employed dilutions of plant extracts for treatment. Simultaneously, the English botanist William Withering discovered the clinical utility of *Digitalis purpurea* for heart conditions, which led to the isolation of the cardiac medication Digitalis [6]. The systematic categorization of these species was further refined by the Swedish naturalist Carolus Linnaeus, whose *Systema Naturae* (1735) established the biological taxonomy essential for documenting the evolution of medicinal flora. Today, the rediscovery of these plants through modern technology provides promising avenues for the development of new drug candidates.

The efficacy of herbal medicines is primarily driven by bioactive phytoconstituents—secondary metabolites synthesized by plants to resist environmental stress and facilitate growth. When introduced into other organisms, these compounds trigger pharmacological changes that can be carminative, immunomodulatory,

cardioprotective, or anti-inflammatory. Medicinal plants are particularly valuable for their antimicrobial actions, with over 1,300 species containing alkaloids, phenols, and hydrocarbons known for inhibiting pathogenic growth. Furthermore, the scientific search for affordable cancer treatments has highlighted that approximately [7] 60% of current antineoplastic agents are plant-derived, with polyphenols, flavonoids, and brassinosteroids playing crucial roles in inducing apoptosis and suppressing tumor proliferation.

Respiratory conditions, particularly those involving cough and allergic reactions, are major areas of interest for phytotherapy. Cough is a protective reflex essential for airway health, yet it can become debilitating when persistent. It is evoked by the activation of vagal afferent nerves (A-delta and C-fibers) in the respiratory tract. While synthetic antitussives like codeine and dextromethorphan are used, they often carry risks of sedation and dependence[8]. Mast cells (MCs), hematopoietic cells derived from bone marrow, are pivotal in these processes, participating in allergic responses, inflammation, and asthma through the release of preformed mediators such as histamine and tryptase. The activation of MCs occurs via surface receptors like Fc ϵ RI and MRGPRX2, leading to degranulation and the release of inflammatory cytokines. Traditional systems like Ayurveda, Siddha, and Traditional Chinese Medicine (TCM) have long utilized plants like *Nyctanthes arbor-tristis*, *Argemone mexicana*, and *Clitoria ternatea* to balance these physiological responses and treat bronchial diseases[9].

Materials and Methods

Selection, Collection, and Authentication

The plants NATF (*Nyctanthes arbor-tristis* flowers), AMF (*Argemone mexicana* flowers), and CTF (*Clitoria ternatea* flowers) were selected based on their traditional repute in treating cough and bronchial disorders. The flowers were collected during their peak blooming season (July–September 2021) from the Malwa region of Madhya Pradesh, India. Authentication was conducted by the Department of Botany at Janata PG College, Rewa, and voucher specimens were deposited (J/Bot./NAT-F/111, J/Bot./AM-F/112, and J/Bot./CT-F/113) [10].

Physico-chemical Characterization

Dried flower parts were subjected to standard procedures for the determination of various physicochemical parameters to ensure the quality and purity of the crude drugs. Foreign organic matter (FOM) was detected by thin-layer inspection and lens use, while moisture content (LOD) was calculated by drying 10 g of the sample at 150°C for 5 hours[11]. Ash values, including total ash, acid-insoluble ash, and water-soluble ash, were determined to detect low-

grade products or earthy matter. The swelling index was measured to evaluate the presence of mucilage in the plant tissues.

Extraction Protocol

The powdered plant materials (shattered and screened with 40 mesh) were extracted using a Soxhlet apparatus with ethyl acetate and ethanol. For each extraction, 250 g of powdered drug was used until the extraction was complete [12]. Solvents were removed via distillation and extracts were further dried using a rotary evaporator. The residue was stored in a desiccator, and the percentage yield was determined for each solvent.

Preliminary Phytochemical Screening

A series of qualitative tests were performed on the ethyl acetate and ethanolic extracts to identify the chemical classes present. Carbohydrates were detected via Molisch's and Fehling's tests, while glycosides were identified through Legal's and Borntrager's tests [13]. Alkaloids were screened using Dragendorff's, Wagner's, Mayer's, and Hager's reagents. Proteins were identified via Million's, Ninhydrin, and Biuret's tests, and phenolic compounds were tested with ferric chloride and lead acetate. Additional tests for flavonoids (Shinoda's), fixed oils (spot test), and steroids (Liebermann-Burchard) were also conducted [14].

Quantitative Analysis and Chromatography

Total flavonoid content (TFC) was quantified using the aluminum chloride method with quercetin as the standard, and results were expressed as quercetin equivalents (QE) per gram. Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay with gallic acid as the standard, expressed as gallic acid equivalents (GAE). Fingerprint profiles were established using thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) [15]. HPTLC analysis utilized silica gel 60°C sheets and a CAMAG Linomat 5 applicator, with biomarkers such as ferulic acid, quercetin, and stearic acid used for densitometric estimation.

In Vitro Antioxidant Assays

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was employed to track radical scavenging interactions, where a change from deep violet to yellow indicates reduction by an antioxidant. Total antioxidant activity was also measured using the ABTS cation radical scavenging assay, with TROLOX as the standard. The TROLOX equivalent antioxidant concentration (TEAC) was calculated based on the inhibition percentage.

In Vivo Pharmacological Evaluation

Acute oral toxicity was assessed following OECD Guideline 423, using female Swiss albino mice to determine the LD₅₀. For the antitussive study, mice were placed in a glass chamber and exposed to 25%

NH₄OH to induce coughing. Frequency and latent periods were recorded over 2 minutes of exposure and 5 minutes of observation, with Dextromethorphan as the reference. Mast cell stabilizing activity was evaluated in male Wistar rats sensitized with horse serum and triple antigen. After 14 days of treatment, mesenteries were challenged with horse serum and examined microscopically for degranulation [16].

Formulation and Characterization of Polyherbal Syrup

A polyherbal syrup was formulated using ethyl acetate extracts of NATF, AMF, and CTF (5% w/v each). The syrup base consisted of 60% sucrose, with glycerin as a co-solvent, sodium benzoate as a preservative, and citric acid as a pH adjuster. Evaluation parameters included organoleptic properties [17], pH, density, specific gravity, viscosity, and microbial content (total viable count, *E. coli*, and *Salmonella*). The syrup was subsequently tested for antitussive activity in the ammonium hydroxide-induced model at doses of 1 ml, 2 ml, and 3 ml [18].

Results and Discussion

Pharmacognostical Evaluation

Morphological analysis revealed that NATF and AMF are tubular and cup-shaped yellow flowers, respectively, while CTF is an oval deep-blue flower. All surfaces were smooth with absent fractures. The physicochemical data indicated that AMF had the highest total ash (8.52%) and swelling index (6.91), while CTF showed the lowest swelling index (4.12). Foreign organic matter was minimal across all samples, staying below 2.22%.

Table 1: Physicochemical Evaluation of Selected Plant Material (Mean SEM, n=3)

Parameters	NATF	AMF	CTF
Foreign Organic Matter (FOM)	1.15±0.22	1.01±0.32	2.22±0.21
Loss on Drying (LOD)	1.02±0.02	4.16±0.29	3.11±0.08
Total Ash (TA)	4.17±0.11	8.52±0.21	6.01±0.19
Acid Insoluble Ash (AIS)	1.23±0.32	1.08±0.10	1.22±0.32
Water Soluble Ash (WSA)	1.49±0.20	2.11±0.11	2.54±0.17
Swelling Index (SI)	5.21±0.07	6.91±0.14	4.12±0.11

Extraction Yield and Phytochemical Profile

Ethanol proved to be a more efficient solvent for all three plants, providing significantly higher yields than ethyl acetate. The highest yield was obtained from the

ethanolic extract of *Clitoria ternatea* (EECTF) at 14.82%, while the ethyl acetate extract of *Nyctanthes arbor-tristis* (EAENATF) yielded only 4.32%. Preliminary screening indicated that tannins, phenolic compounds, and flavonoids were present in all extracts. Glycosides and alkaloids were predominantly found in the ethanolic extracts (EENATF, EEAMF, EECTF), while fixed oils were detected only in AMF and CTF extracts.

Table 2: Estimation of Percentage Yield of Various Extracts

Nature of Extract	Color	pH	% Yield
EAENATF (Semi-solid)	Cream	6.88	4.32
EENATF (Solid)	Off-white	7.10	8.19
EAEAMF (Semi-solid)	Dark orange	6.90	5.13
EEAMF (Solid powder)	Light yellow	7.00	10.29
EAECTF (Semi-solid)	Bluish black	7.10	5.11
EECTF (Sticky solid)	Light blue	7.02	14.82

Quantitative Phytoconstituents and HPTLC

Ethanolic extracts demonstrated a higher concentration of bioactive compounds compared to ethyl acetate fractions. EEAMF recorded the highest total flavonoid content (4.28 mg QE/g) and total phenolic content (3.23 mg GAE/g). HPTLC fingerprinting at 254 nm identified several peaks corresponding to ferulic acid (R_f 0.28–0.77) and quercetin (R_f 0.77–0.80) in the extracts.

Table 3: Total Flavonoid and Phenolic Content of Extracts (Mean SD, n=3)

Extract	TFC (mg QE/g)	TPC (mg GAE/g)
EAENATF	0.29 ±0.12	0.29 ±0.27
EENATF	2.42 ±0.12	1.36 ±0.11
EAEAMF	1.19 ±0.02	0.31 ±0.28
EEAMF	4.28 ±0.08	3.23 ±0.11
EAECTF	1.28 ±0.11	0.48 ±0.17
EECTF	3.29 ±0.21	2.14 ±0.19

Antioxidant Activity

In the DPPH assay, ethanolic extracts showed superior radical scavenging activity. EAEAMF had an IC₅₀ of 67.17 µg/mL, which was the most potent among the test extracts, although less potent than ascorbic acid (33.92 µg/mL). Total antioxidant activity measured via ABTS also favored the ethanolic fractions, with EECTF showing an IC₅₀ of 101.74 µg/mL compared to standard TROLOX (37.76 µg/mL).

Antitussive Potential

All tested extracts at 500 mg/kg dose demonstrated significant ($P<0.01$) antitussive activity when

compared to the control group. EAECTF and EAENATF showed the highest percentage inhibition of the cough reflex at 120 minutes (83.28% and 82.89% respectively), which is highly comparable to the standard dextromethorphan (85.46%).

Table 4: Effect of Extracts on Ammonium Hydroxide-Induced Cough Frequency

Treatment (500 mg/kg)	Cough Frequency (30 Mts)	Cough Frequency (120 Mts)	% Inhibition (120 Mts)
Control	78.11	76.21	-
Standard	31.23	11.23	85.46
EAENATF	61.13	13.22	82.89
EENATF	66.33	14.26	81.54
EAECTF	60.43	12.92	83.28
EECTF	63.19	14.71	80.96

Mast Cell Stabilizing Activity

The extracts demonstrated a notable protective effect on sensitized mast cells. While the control group had a high granulated mast cell count (84.29 ± 1.18), treatment with EAENATF, EAEAMF, and EAECTF significantly reduced granulation and increased the count of non-granulated (intact) cells to approximately 47. This stabilizing effect is indicative of anti-allergic potential, likely through the inhibition of histamine and cytokine release.

Polyherbal Syrup Characterization and Efficacy

The formulated syrup exhibited clear organoleptic properties with a neutral pH (6.7) and appropriate viscosity (885 cp). Microbial analysis confirmed the absence of *E. coli* and *Salmonella*, with a total aerobic viable count of 220 CFU/gm, safely below pharmacopoeial limits. When tested for antitussive activity, the syrup showed a dose-dependent effect. The 3 ml dose inhibited 81.07% of cough bouts, while the 1 ml dose inhibited 74.78%. This high level of efficacy at the 3 ml dose suggests a synergistic interaction between the three active herbal extracts in the formulation.

Table 5: Evaluation Parameters of Formulated Polyherbal Syrup

Parameters	Inference
Appearance	Clear, Brown
pH	6.7
Density	1.42 g/ml
Viscosity	885 cp
Total Aerobic Count	220 CFU/gm
Pathogens (<i>E. coli</i> , <i>Salmonella</i>)	Absent

Discussion of Phytochemical and Pharmacological Synergisms

The results of this study underscore the intricate relationship between plant secondary metabolites and their therapeutic manifestations in respiratory health.

Phytochemicals, primarily flavonoids and phenolics identified through quantitative estimation and TLC/HPTLC profiling, appear to be the primary drivers of the observed antioxidant and anti-inflammatory actions. The high phenolic content in *Argemone mexicana* and *Clitoria ternatea* correlates with their significant IC₅₀ values in DPPH and ABTS assays, suggesting that these extracts can effectively neutralize reactive oxygen species (ROS) that contribute to airway inflammation.

The physiological mechanism of the cough reflex involves a complex signaling path through vagal afferent nerves. The extracts, particularly the ethyl acetate fractions of *Nyctanthes arbor-tristis* and *Clitoria ternatea* [19], likely exert their antitussive effects by either desensitizing these peripheral receptors in the bronchial mucosa or by interacting with the cough center in the nucleus tractus solitarius (nTS). The comparable efficacy of these extracts to Dextromethorphan—a centrally acting non-opioid antitussive—suggests a potent inhibitory influence on the cough signaling cascade.

Furthermore, the mast cell stabilizing results provide a mechanistic explanation for the anti-asthmatic and anti-allergic properties traditionally attributed to these herbs. Mast cells are pivotal in early-phase allergic responses, releasing preformed mediators like histamine that trigger immediate bronchoconstriction. By increasing the percentage of intact mast cells, the extracts effectively block the degranulation machinery, which may involve the modulation of G-protein coupled receptors like MRGPRX2 or the stabilization of KIT receptors. This dual action—suppressing the cough reflex and stabilizing the cellular drivers of inflammation—positions the polyherbal syrup as a comprehensive therapeutic intervention for respiratory disorders. [22]

The successful formulation of the polyherbal syrup and its proven dose-dependent efficacy further advocate for the use of multi-botanical preparations [20]. Synergistic interactions often allow for lower doses of individual components while achieving a greater overall effect, potentially reducing the risk of side effects associated with any single constituent. The physicochemical stability and microbial safety of the syrup ensure its suitability as a clinical-grade alternative to traditional crude drug preparations. [23]

Conclusion

This research confirms that *Nyctanthes arbor-tristis*, *Argemone mexicana*, and *Clitoria ternatea* flowers are valuable sources of bioactive phytochemicals with significant antioxidant, antitussive, and mast cell stabilizing properties. The individual extracts demonstrate potency comparable to standard pharmaceuticals, while the polyherbal syrup formulation effectively harnesses their synergistic

potential to provide substantial cough suppression and anti-allergic protection [21]. The safety profile established through acute toxicity studies further supports the clinical viability of these indigenous remedies. [24] These findings provide a robust scientific validation for the traditional use of these plants and offer a promising foundation for the development of novel, safe, and effective herbal treatments for chronic and allergic respiratory conditions.

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