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Formulation and Evaluation of Polyherbal Antiasthmatic Cough Syrup

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Abstract: This study developed and evaluated a polyherbal anti-asthmatic cough syrup using *Glycyrrhiza glabra, Withania somnifera, Cinnamomum verum* and *Bauhinia racemosa*. These plants were chosen for their documented anti-asthmatic, anti-inflammatory, mucolytic, antioxidant, and antimicrobial effects. Extraction was carried out by Soxhlet method with ethanol for *Withania somnifera* and *Cinnamomum verum*, while *Bauhinia racemosa* and *Glycyrrhiza glabra* were extracted by cold maceration using ethanol and distilled water, respectively. The extracts were concentrated into semi-solid residues for formulation. Phytochemical analysis confirmed the presence of Quercetin in *Bauhinia racemosa*, Glycyrrhizin in *Glycyrrhiza glabra*, and Cinnamaldehyde in *Cinnamomum verum*. UV-visible spectroscopy verified extract purity, with maximum absorbance values matching reported standards and calibration curves showing strong linearity, confirming reliability of the method. FTIR spectroscopy and DSC indicated no significant interaction between extracts and excipients, establishing stability of the formulation. Three formulations were developed and tested, among which the third formulation was most compliant with Indian Pharmacopoeial standards. Each 10 milliliters contained *Bauhinia racemosa* 100 milligrams, *Cinnamomum verum* 100 milligrams, *Withania somnifera* 200 milligrams, and *Glycyrrhiza glabra* 200 milligrams in a sugar syrup base with preservatives. The formulation showed significant pharmacological activity. Anti-inflammatory testing revealed 65 to 74 percent inhibition, with *Glycyrrhiza glabra* reaching 87.4 percent, comparable to diclofenac sodium. Antioxidant activity was highest in *Withania somnifera* at 88.3 percent, while the syrup demonstrated 59 to 65 percent inhibition. Antibacterial testing showed *Cinnamomum verum* with the strongest effect at 18.7 millimeters of inhibition, followed by *Bauhinia racemosa* at 9.8 millimeters, and the formulation at 8.2 millimeters against *Klebsiella pneumoniae*. *Ex-vivo* studies on goat trachea showed that the syrup inhibited 77.3 percent of histamine-induced contraction, confirming bronchodilatory and antihistaminic potential. Overall, the formulation demonstrated strong anti-inflammatory, antioxidant, antimicrobial, and bronchodilatory effects, supporting its use as an anti-asthmatic cough syrup.

Keywords: *Polyherbal Formulation, Antiasthmatic, Bauhinia racemosa, Cinnamomum verum, Withania somnifera, Glycyrrhiza glabra.*

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I. INTRODUCTION

Asthma is a chronic inflammatory disease of the airways marked by reversible obstruction, bronchial hyperresponsiveness, and episodes of wheezing, coughing, chest tightness, and breathlessness. It occurs when the airways swell, constrict, and produce excess mucus, often triggered by allergens, infections, cold air, exertion, pollution, or stress. While asthma cannot be cured, it can be controlled with medications, lifestyle adjustments, and increasingly, herbal formulations that strengthen respiratory function and reduce inflammation. Early management is essential because poorly controlled asthma can lead to frequent hospitalizations, reduced quality of life, and progressive airway damage over time.

Cough, one of the major symptoms of asthma and other respiratory disorders, is a protective reflex that clears the airways of irritants, microbes, and mucus. It may be dry, linked to irritation or allergies, or productive, associated with infections, bronchitis, or asthma. Persistent cough usually reflects airway inflammation or hyperreactivity, making it an important therapeutic target. In herbal medicine, cough is managed with a holistic approach that combines antitussive, expectorant, mucolytic, demulcent, and anti-inflammatory herbs. *Glycyrrhiza glabra* soothes irritation and suppresses the cough reflex, *Cinnamomum verum* helps expel phlegm, mucolytic herbs break down thick secretions, and protective

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herbs coat and heal inflamed mucosa.

An asthmatic cough is a persistent cough that is usually dry and non-productive and which is related with bronchial asthma. It is caused by the inflammation and hypersensitivity of the airways that causes coughing even without the usual symptoms of asthma such as wheezing or coughing of breath. In some individuals, known as cases of cough-variant asthma, cough may even be the sole symptom, making early diagnosis and effective management vital.

Asthma is not limited to airway constriction but is also driven by oxidative stress and immune dysregulation. Excessive production of cytokines, leukotrienes, and reactive oxygen species perpetuates inflammation and damages bronchial tissues, leading to long-term complications like airway remodelling. Antioxidant-rich herbs such as *Bauhinia racemosa* and *Cinnamomum verum* counteract this damage by scavenging free radicals, while *Withania somnifera* supports immune balance and reduces stress-induced bronchial constriction. This dual action on inflammation and oxidative stress makes herbal formulations particularly valuable in long-term asthma management, as they not only provide symptomatic relief but also protect lung tissues from chronic damage.

Another advantage of polyherbal remedies lies in their patient-friendly nature. Unlike synthetic drugs, which often cause side effects like dependency, throat irritation, or immune suppression, herbal syrups are generally well tolerated, culturally familiar, and suitable for children, the elderly, and sensitive individuals. Their natural taste, non-alcoholic base, and minimal side effects enhance patient compliance, ensuring consistent use. By combining *Bauhinia racemosa*, *Withania somnifera*, *Cinnamomum verum*, and *Glycyrrhiza glabra*, a balanced polyherbal syrup can address multiple aspects of asthma and cough simultaneously relieving bronchospasm, reducing mucus, modulating immunity, and protecting airway tissues. This multi-targeted approach aligns with modern trends in holistic and preventive healthcare, making polyherbal cough syrups a promising adjunct in the comprehensive management of asthma and other chronic respiratory conditions.

II. MARTIALS AND METHODS

➢ *Preparation of the Sample*

The collected parts of *Bauhinia racemosa*, *Withania somnifera*, *Cinnamomum verum* and *Glycyrrhiza glabra* were washed thoroughly with distilled water, shade-dried, and subsequently oven-dried at 50 °C before being pulverized to obtain a coarse powder suitable for extraction.

➢ *Determination of Solubility*

The solubility of the plant extracts in various solvents (water, methanol, ethanol, and phosphate buffer) was determined using the shake-flask method. An excess quantity of each extract was added to 2 mL of the respective solvents in separate volumetric flasks, and the mixtures were vigorously agitated to ensure uniform dispersion. The flasks were placed in a shaking water bath maintained at 25 °C and were continuously agitated for 24 h to attain equilibrium solubility. Following incubation, the samples were withdrawn, filtered through a 0.22 µm membrane filter, and centrifuged to eliminate any undissolved particles. The clear filtrates were then analyzed using a UV– visible spectrophotometer at their respective wavelengths to determine the concentration of dissolved extract in each solvent.

➢ *Extraction Procedure:*

A total of 20 g of *Bauhinia racemosa* leaf powder was mixed with 500 ml of ethanol and soaked for 120 hours, after which the extract was filtered through Whatman No. 1 filter paper and air-dried in an aseptic area. Similarly, healthy plants of *Glycyrrhiza glabra* were selected, shade-dried, and powdered, from which 100 g of dried root powder was soaked in 500 ml of distilled water under controlled aseptic conditions for 120 hours. The extract was then filtered using a percolator setup to obtain a clear filtrate.

Healthy plants were selected and processed, and 30 g of dried powder was used for extraction. The powdered samples were separately subjected to Soxhlet extraction in 90% ethanol (200 ml per gram dry weight) on a heating mantle for 15–20 cycles. The extracts were then concentrated and re-concentrated in ethanol to remove fatty substances. After cooling and filtration through Whatman filter paper, the *Cinnamomum verum* bark extract was condensed to reduce solvent volume.

The Soxhlet extraction and cold maceration method was selected on the basis of literature reports demonstrating its efficiency and reliability for herbal material extraction.

➢ *Preliminary Phytochemical Screening:*

Preliminary phytochemical tests were carried out on the obtained plant extracts to detect the presence of primary and secondary metabolites. Standard qualitative methods were employed to evaluate the extracts for alkaloids, saponins, flavonoids, carbohydrates, tannins, phenols, proteins, glycosides, and steroids.

➢ *Thin Layer Chromatography:*

TLC was carried out for the identification of flavonoids in the extracts of *Bauhinia racemosa*, *Cinnamomum verum*, and *Glycyrrhiza glabra*. Pre-coated silica gel 60 F_{254} plates were used as the stationary phase, and the extracts were dissolved in ethanol. Standard markers such as quercetin, cinnamaldehyde and glycyrhizzin were co-spotted for comparison. The plates were developed, air-dried, and visualized under UV light after spraying with aluminium chloride, and Rf values were calculated using the standard formula.

$$Rf\ value = \frac{Distance\ travelled\ by\ compound\ (Spot)}{Distance\ travelled\ by\ solvent}$$

➢ *Determination of λmax:*

All extracts (*Bauhinia racemosa* leaves, *Cinnamomum verum* bark, *Glycyrrhiza glabra* roots and *Withania*

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somnifera roots) were prepared precisely to a weight of 10mg and put into separate 100-mL volumetric flasks. Samples were dissolved in limited amount of ethanol and then the volume was brought to 100 mL by adding more ethanol to make 100 ug mL stock solutions. Proper dilutions were then made into ethanol, and the resultant solutions were scanned between the wavelength 200 400nm wavelength ranges with the UV Visible spectrophotometer to identify the wavelength of maximum absorption (λ-max) of each extract.

➢ *Determination of Calibration Curve:*

Accurately weighed 10 mg of each extract (*Bauhinia racemosa* leaves, *Cinnamomum verum* bark, *Glycyrrhiza glabra* roots, and *Withania somnifera* roots) was transferred into separate 100 mL volumetric flasks, dissolved in ethanol, and diluted to obtain stock solutions (100 µg/mL). From these, serial dilutions (1–10 µg/mL) were prepared using ethanol as the solvent. The UV absorbance of each dilution was recorded at the respective λmax of each extract using ethanol as blank. Calibration curves were then constructed by plotting absorbance against concentration, and linearity was assessed by least-squares regression analysis (R^2).

➢ *Determination of in-Vitro Anti-Inflammatory Activity*

- *Protein Denaturation Method:*

This method is used to evaluate the anti-inflammatory property of a compound by its ability to inhibit heat-induced protein denaturation.

- Materials Required: Bovine serum albumin (BSA), Phosphate buffer (pH 6.3), Test samples (extracts or drugs), Distilled water, Spectrophotometer[62]

- *Procedure:*

✓ A 1% solution of BSA in phosphate buffer (pH 6.3) was prepared.
✓ Combine 1 mL of the test sample with BSA solution (5 mL) at various concentrations.
✓ Allow the mixture to incubate at room temperature in 30 minutes.
✓ Heat the samples in 57°C in 15 minutes.
✓ Allow the samples to cool down and record the absorbance at 560 nm using a spectrophotometer.
✓ Compare using a standard (e.g., diclofenac sodium) and control sample.

- *Calculation:*

$$\%\ Inhibition\ = \left(\frac{A_{\ control}\ -\ A_{sample}}{A_{control}}\right) \times 100$$

Where, $A_{control}$ = Absorption of control (standard)

A_{sample} =Absorption of sample (herbal extract)

- *Human Red Blood Cell (HRBC) Membrane Stabilization Method:*

This is a method of determining the anti-inflammatory activity of test compounds in terms of their ability to stabilize human red blood cell membrane. Materials Needed Fresh human blood (collected in EDTA tubes), Isotonic phosphate buffer (pH 7.4), Hypotonic solution (distilled water), Centrifuge and test tubes and Spectrophotometer.

- *Procedure:*

✓ Take human blood and wash using isotonic phosphate buffer by centrifuging at 3000 rpm.
✓ Re-suspend the packed cells in the isotonic buffer to get a 10 percent v/v suspension of the cells.
✓ Combine 1 mL test sample, 1 mL HRBC suspension and 8 mL Hypotonic solution.
✓ Incubate at 37°C for 30 minutes.
✓ The mixture is centrifuged and the absorbance of the supernatant is measured at 560 nm.
✓ Compare findings with standard drug and control.

- *Calculation:*

$$\text{Percentage inhibition of hemolysis} = 100 - \left(\frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\right)$$

Where, OD_{sample} = Absorption of control (standard)

$OD_{control}$ = Absorption of sample (herbal extract)

➢ *Determination of Antioxidant Activity by in-Vitro Method:*

- *DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay:*

The DPPH assay is a widely used method for evaluating the free radical scavenging ability of antioxidants present in the test samples.

- *Materials Required:*

✓ DPPH (2,2-diphenyl-1-picrylhydrazyl)
✓ Methanol
✓ Test samples (Ascorbic acid)
✓ Spectrophotometer

- *Procedure:*

✓ Make 0.1 mM solution of DPPH in methanol.
✓ To 1 mL of test sample/standard, add 1 mL of DPPH solution at various concentrations.
✓ Allow the reaction mixture to incubate in darkness at room temperature (30min).
✓ Record the absorbance at 517 nm, with a spectrophotometer.
✓ Take the controls with methanol and DPPH solution in the absence of the test sample.

- *Calculation:*

$$\%\ Inhibition\ = \left(\frac{A_{\ control}\ -\ A_{sample}}{A_{control}}\right) \times 100$$

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Where, $A_{control}$ = Absorption of control (standard)

A_{sample} =Absorption of sample (herbal extract)

➢ *In-Vitro Antibacterial Activity – Cup Plate Method:*

The cup plate is a simple and dependable procedure that is applied to determine the antibacterial activity of plant extracts or pharmaceutical formulations. The principle of operation is to quantify as a zone of inhibition of a well (cup) containing the test compound on a bacterial inoculated agar plate. Materials needed- Nutrient agar medium, Sterile Petri dishes, Test microorganisms (e.g *Klebsiella pneumoniae*), Plant extracts and standard antibiotics (Streptomycin IP), Sterile cork borer or metal borer, Micropipette, sterile dropper, sterile swabs, Incubator.

- *Procedure-*

Preferably prepare nutrient agar media and autoclave it. Put the sterilized medium into sterile Petri plates and solidify. Transfer the test bacterial culture to the agar surface that has been solidified with a sterile swab in an even manner. Punch holes (cups) into the agar using a sterile cork borer which have the same diameter. Inoculate each of the wells with a set volume of test sample (usually 100 uL), control (solvent), and standard antibiotic. Allow the plates to cool to room temperature during a period of approximately 1 hour to enable diffusion of the sample into the agar. Incubate the plates at 37°C 18 24 hrs. Measure the diameter of the zone of inhibition (clear area) of each well in milli-meters after incubation.

- *Observation-*

Optical evidence of an antibacterial activity is a clear area around the well. The area of the zone is proportional to the effectiveness of the test substance.

➢ *Ex-Vivo Anti-Asthmatic Activity:*

- *Materials Required:*

Fresh goat trachea (collected from a slaughterhouse), Krebs's solution, Standard bronchodilator drugs: Bilastine, Polyherbal syrup/formulation, Histamine (to induce contraction), Kymograph or student organ bath with lever, Aerator, Thread, hooks, surgical tools, Stopwatch and scale.

- *Procedure:*

✓ *Tissue Preparation:*

Take goat trachea immediately after slaughter and put in cold Tyrode solution. Take good care not to cut the trachea, cut it into transverse rings (~2-3 mm wide). Connect 4-5 rings with a thread of cotton to make a chain of trachea.

✓ *Mounting the Tissue:*

Install the tracheal chain on a student organ bath with Tyrode solution at 37degC. The tissue is bound to one end of a fixed hook and to the other to a lever or isotonic transducer with a resting tension of 1 g. Continuously stir the bath with carbogen.

✓ *Equilibration:*

Equilibrate the tissue in the 45 minutes with a 10-15 minutes wash.

✓ *Dose-Response Study:*

- Get contraction with a spasmogenic agent such as histamine.
- Record the contraction.

When the optimum contraction has been attained, inject regular drug (e.g. Bilastine) and note the effect of relaxation. Repeat using the second standard drug and then using the polyherbal formulation at varying concentrations (e.g., 0.1 mL, 0.2 mL, 0.4 mL). Each dose is to be followed by washing of the tissue using the Tyrode solution, followed by recovery.

✓ *Observation and Recording:*

Determine the percentage of relaxation of the tissue following treatment with each agent in comparison to contraction brought about by only histamine. As hundred percent maximum contraction has been obtained. determine percent contraction obtained at each dose.

➢ *Formulation of Polyherbal Cough Syrup:*

The dried and powdered plant materials were extracted separately by maceration or Soxhlet extraction using suitable solvents. The extracts were then filtered, concentrated by distillation, and further dried to obtain semi-solid masses. For the syrup base, 66.7 g of sucrose was dissolved in an appropriate quantity of purified water with gentle heating using the double boiler method to prepare a sugar syrup of approximately 66.66% w/v, which was allowed to cool to room temperature. Preservatives were prepared by dissolving 0.1 g each of methyl paraben and propyl paraben in a small amount of warm water, and this solution was added to the cooled syrup with continuous stirring. The accurately weighed quantities of each herbal extract were then incorporated into the syrup base under slow stirring to ensure uniform distribution. To adjust the pH to an optimal range of 5–6, 0.3 g of citric acid was added, which also contributed to taste enhancement. The final volume of the formulation was adjusted with purified water, followed by filtration through clean muslin cloth or filter paper to remove any undissolved particles. The prepared syrup was then transferred into amber-colored bottles, properly labeled, and stored in a cool, dry place to protect it from light and maintain stability.

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Table 1 Formulation Table

Herb	F_1 (Qty in milligram per 10 ml)	F_2 (Qty in milligram per 10 ml)	F_3 (Qty in milligram per 10 ml)
Bauhinia racemosa	50	75	100
Cinnamomum verum	50	75	100
Withania somnifera	100	150	200
Glycyrrhiza glabra	100	150	200
Methyl paraben	0.01	0.01	0.01
Propyl paraben	0.01	0.01	0.01
Citric acid	0.03	0.03	0.03

➢ *Evaluation of Polyherbal Cough Syrup:*

- *Organoleptic Test:*

✓ Colour, odour, and taste of the syrup were evaluated.

- *pH Test:*

✓ The pH of formulations was measured using a digital pH meter.

- *Viscosity Test:*

✓ Viscosity was determined using a Brookfield viscometer with spindle type 3.

- *Specific Gravity:*

✓ Specific gravity was calculated using a specific gravity bottle weight measurement method.

- *Density:*

✓ Density was determined in g/cm³ by standard calculation methods.

- *Water Content Determination / Loss on Drying:*

✓ 10 mL of syrup was placed in a petri dish and kept for 6 hours at 100°C in a hot air oven, then reweighed to determine water content.

- *Determination of Ash Values:*

✓ Total ash, acid-insoluble ash, and sulphated ash were measured to assess inorganic content.

III. RESULTS AND DISCUSSION

➢ *Authentication of Plant Material*

Bauhinia racemosa leaves, *Cinnamomum verum* bark, *Withania somnifera* roots, and *Glycyrrhiza glabra* roots were collected from Ratnagiri district, Maharashtra, and authenticated by the Head, Department of Botany, Sharadchandraji Pawar Krushi Mahavidyalaya, Kharawate. Voucher specimens were deposited for future reference.

➢ *Extraction of Herbs*

A total of 20 g of *Bauhinia racemosa* leaf powder was extracted with 500 ml ethanol by maceration for 120 hours, filtered through Whatman No. 1, and air-dried under aseptic conditions. For *Glycyrrhiza glabra*, 100 g of shade-dried root powder was soaked in 500 ml distilled water for 120 hours and filtered using a percolator. Meanwhile, 30 g of *Cinnamomum verum* bark powder was Soxhlet-extracted in 90% ethanol (200 ml/g) for 15–20 cycles, concentrated to remove fatty substances, filtered, and condensed to reduce solvent volume. Extraction yields are summarized in Table No:

Table 2 Description of Herbal Extracts

Sample	% Yield	Description
Bauhinia racemosa ethanolic extract	11.7	Deep green colored extract was obtained, with bitter smell.
Glycyrrhiza glabra aqueous extract	12.4	Brownish colored extract was obtained, with sweet smell.
Cinnamomum verum ethanolic extract	10.9	Deep red colored extract was obtained, with spicy aromatic smell.
Withania somnifera ethanolic extract	9.6	Light Brown colored extract was obtained, with bitter and earthy smell.

➢ *Solubility of Herbal Extracts Various Solvents*

Table 3 Solubility of Herbal Extracts

Solvent	Percent (%) solubility of *Bauhnia racemosa*	Percent (%) solubility of *Cinnamomum verum*	Percent (%) solubility of *Withania somnifera*	Percent (%) solubility of *Glycyrrhiza glabra*
Ethanol	22.92	29.69	17.74	24.69
Methanol	21.53	25.69	13.97	21.84
Distilled Water	14.38	18.23	13.54	19.62
DMSO	15.46	14.92	12.25	10.92

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➢ *Phytochemical Screening of Herbal Extracts*

Table 4 Phytochemical Screening of Herbal Extracts

Phytoconstituents	***Bauhinia racemosa***	***Cinnamomum verum***	***Withania somnifera***	***Glycyrrhiza glabra***
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Glycosides	+	+	+	-
Carbohydrates	+	+	+	+
Steroids	–	–	+	+

➢ *Thin Layer Chromatography of Herbal Extracts*

The Thin Layer Chromatography (TLC) was performed to confirm the presence of key phytoconstituents in the herbal extracts. *Bauhinia racemosa* was analyzed using a mobile phase of ethyl acetate: formic acid: glacial acetic acid: water (10:1:1:2.5), *Glycyrrhiza glabra* using chloroform: methanol: water (6.5:3.5:1), and *Cinnamomum verum* using toluene: ethyl acetate: formic acid (5:4:1). Each extract was compared with its respective standard reference compound and visualized under UV light, confirming the presence of bioactive phytochemical constituents in the selected herbal extracts.

Table 5 RF Value of Herbal Extracts

Sr. No	**Extract**	RF Value	**Standard**	RF Value
1	*Bauhinia racemosa*	0.40	Quercetin	0.42
2	*Cinnamomum verum*	0.43	Ferulic acid	0.45
3	*Glycyrrhiza glabra*	0.26	*Trigonella foenum-graecum*	0.31

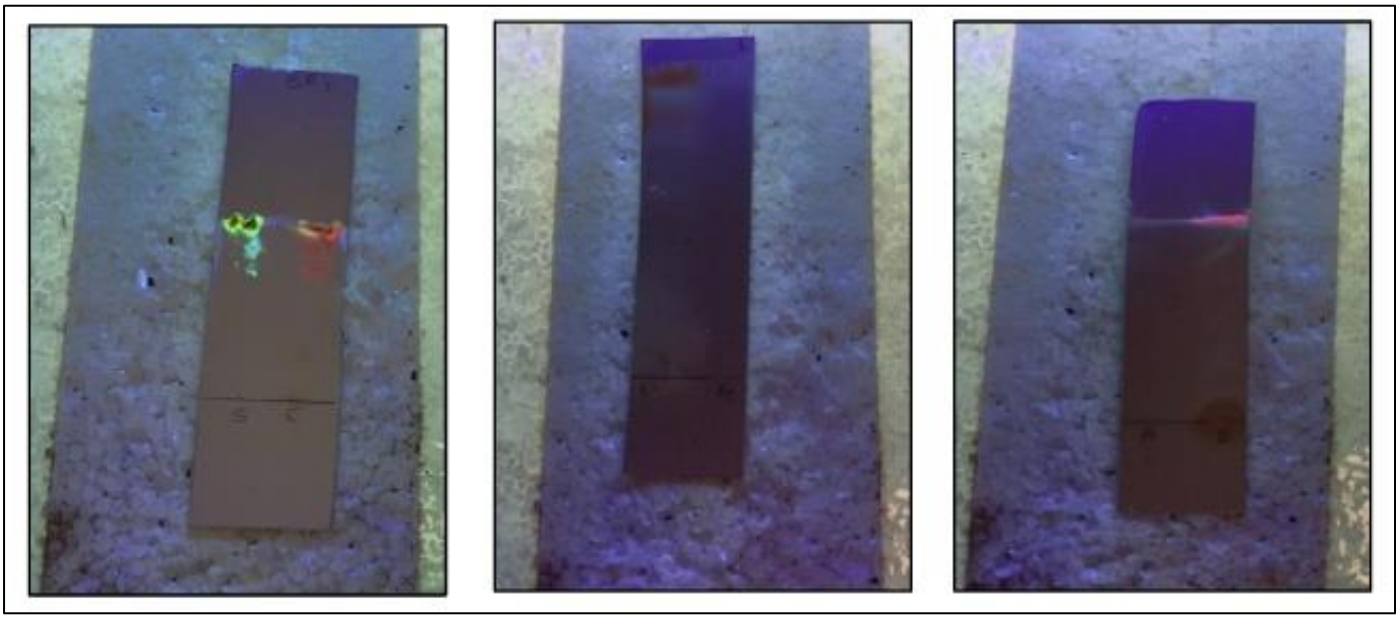

Fig 1 Thin Layer Chromatogram of Herbs 1) *Bauhinia racemosa* 2) *Glycyrrhiza glabra* 3) *Cinnamomum verum*

➢ *Determination of λmax and Construction of Calibration Curve*

The UV spectrophotometric analysis of the plant extracts revealed characteristic λmax values corresponding to their major phytoconstituents. The ethanolic extract of *Bauhinia racemosa* leaves showed absorption maxima at 235 nm, indicating the presence of quercetin-like flavonoids, regression coefficient R^2 was found to be 0.8813, and showed linearity, indicating compliance with Beer's Law.

Cinnamomum verum bark extract exhibited a λmax at 283 nm, suggesting the presence of cinnamaldehyde-related phenolic compounds, regression coefficient R^2 was found to be 0.9687, and showed linearity, indicating compliance with Beer's Law.. The aqueous extract of *Glycyrrhiza glabra* roots showed a λmax at 262.5 nm, supporting the presence of glycyrrhizin-like saponins, regression coefficient R^2 was found to be 0.9507, and showed linearity, indicating compliance with Beer's Law., while the ethanolic extract of *Withania somnifera* roots displayed a λmax at 273.5 nm, indicating the presence of withanolide-type steroidal lactones, regression coefficient R^2 was found to be 0.9425, and showed linearity, indicating compliance with Beer's Law.. These results demonstrate the reliability of UV spectrophotometric analysis for the standardization of herbal extracts.

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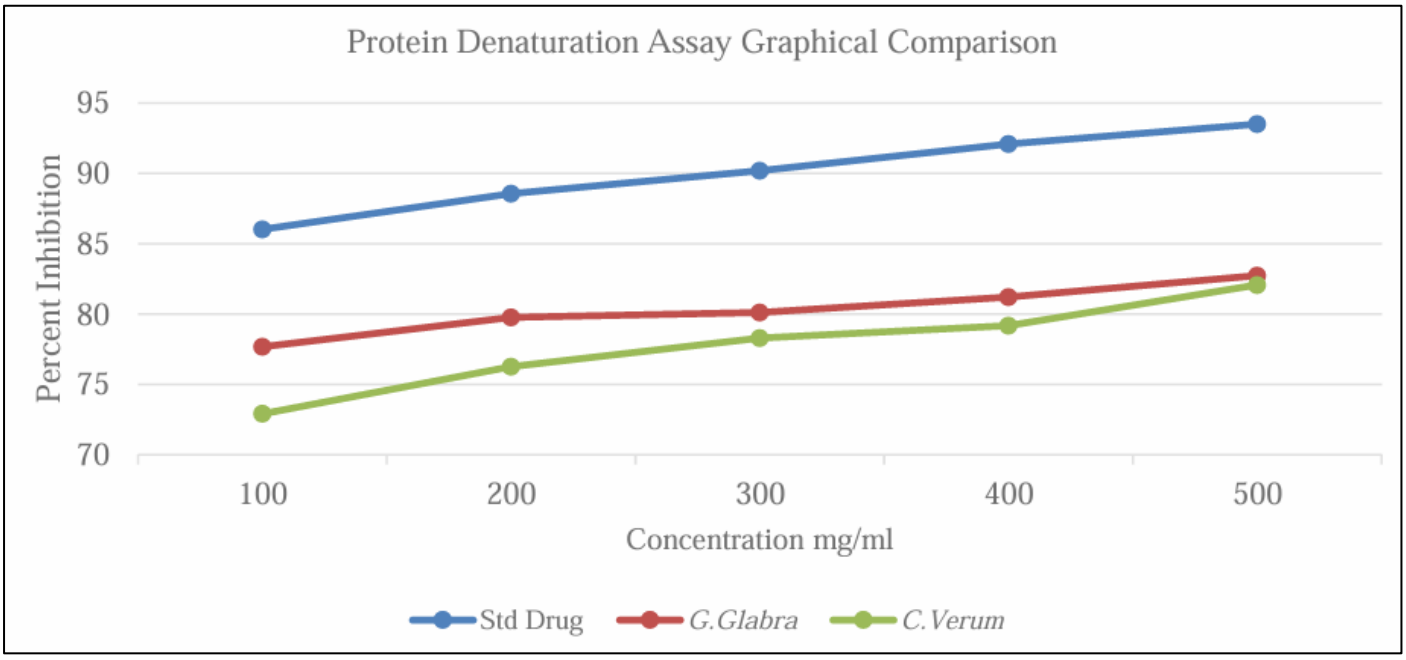
➢ *Determination in-Vitro Anti-Inflammatory Activity:*

- *Protein Denaturation Method:*

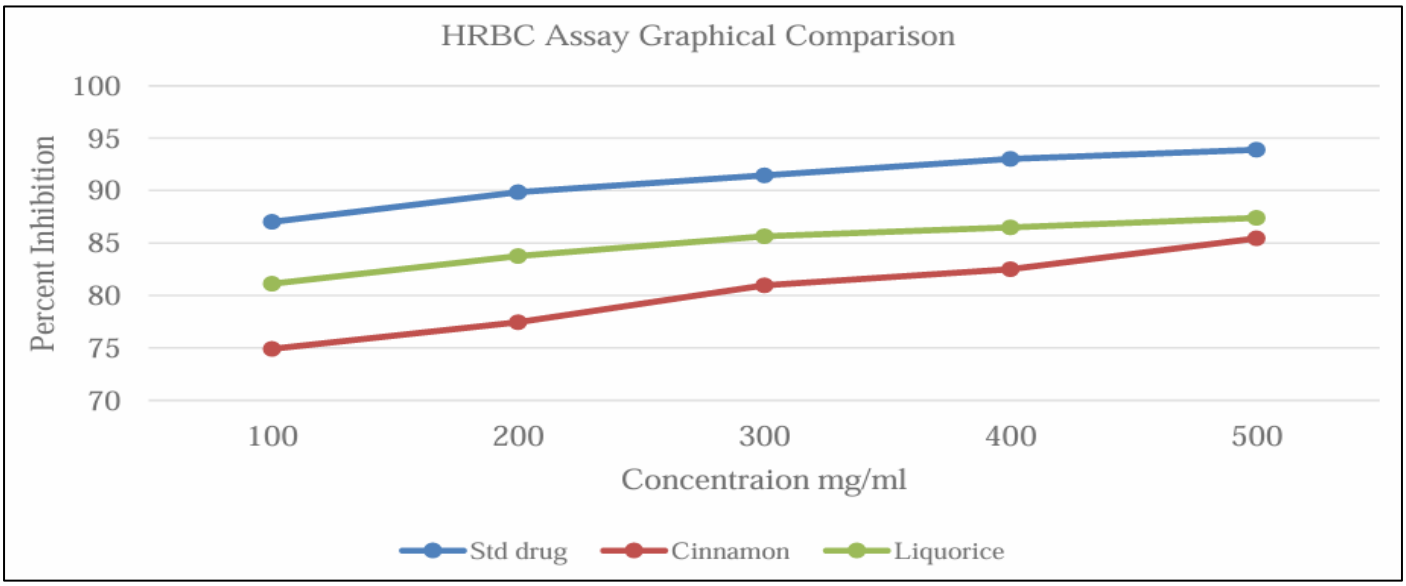
The protein denaturation test showed that all the test samples inhibited heat induced denaturation of bovine serum albumin dose dependently. The assay was validated by the highest inhibition, which was produced by diclofenac sodium (85.05%to 92.33% at 0.1-0.5g/mL). In the two botanical extracts, *Glycyrrhiza glabra* performed better, showing better the anti-inflammatory activity (76.55% to 81.42%) in all the concentrations used than *Cinnamomum verum* (73.01% to 81.23%). *Glycyrrhiza glabra* showed a tendency to inhibit the same (81.42%) at the highest concentration (0.5g /ml) compared to the standard, which demonstrated that it has the potential to be used as a natural anti-inflammatory agent.



Graph 1 Protein Denaturation Assay Graphical Comparison

- *Human Red Blood Cell (HRBC) Membrane Stabilization Method:*

The HRBC membrane stabilization assay showed that Cinnamomum verum and Glycyrrhiza glabra had dose dependent anti-inflammatory effects since both the plants were able to stabilize red blood cell membrane against hypotonic-induced lysis. The reliability of the method was proved after the standard drug, Diclofenac sodium, showed the highest inhibition (between (88.32% and 92.46%) was used. *Glycyrrhiza glabra* had better membrane stabilization activity (81.26% to 86.57 %) than *Cinnamomum verum* (75.67 % to 84.72%) at all concentrations of the two extracts. The *G. glabra* activity at 0.5g/mL was similar to the standard drug which shows that the plant has a strong potential of anti-inflammatory action by stabilizing the membranes.



Graph 2 HRBC Assay Graphical Comparison

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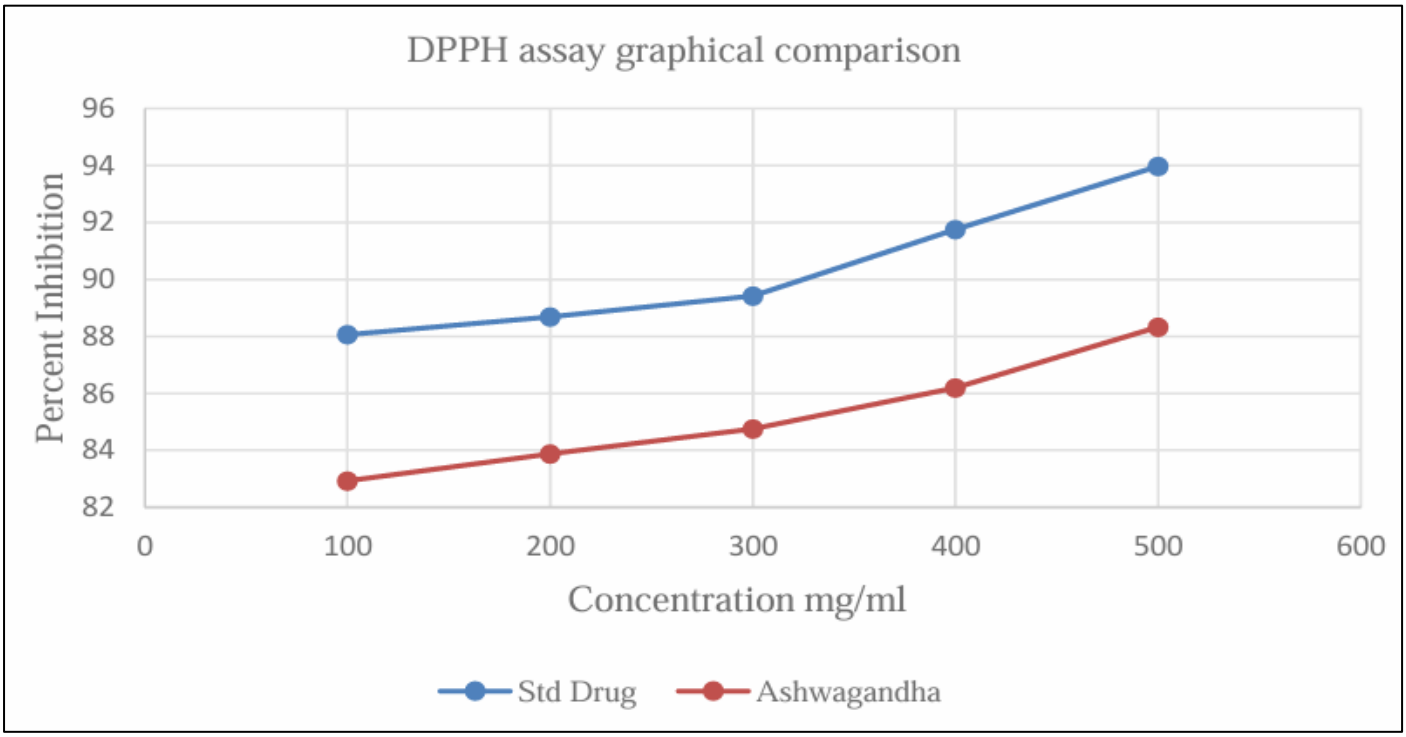
➢ *Determination of Antioxidant Activity by in-Vitro Method:*

- *DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay:*

The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging test proved that the concentration-dependent in-vitro antioxidant activity was significant in *Withania somnifera.* Ascorbic acid, the standard antioxidant, had good inhibition of 87.93 the standard 88.13% to 94.09%, thus proving the assay. The free radical scavenging ability *Withania somnifera* of extract showed a gradual rise in the inhibition values of 83.01% to 87.93% respectively at 0.1 and 0.5g/mL. Although the activity was a bit low compared to that of the standard at all the concentrations, findings suggest that ashwagandha is important in antioxidant activity probably because of its bioactive phytoconstituents like withanolides.



Graph 3 DPPH Assay Graphical Comparison

➢ *Determination in-Vitro Antibacterial Activity – Cup Plate Method:*

The anti-microbial test *in vitro*, performed with the cup plate technique against *Klebsiella pneumoniae* showed that the specimens *Bauhinia racemosa* and *Cinnamomum verum* have quantifiable anti-microbial effect as shown by the zone of inhibition. Owing to strong antibacterial effect, Streptomycin was the standard drug and showed a prominent zone of inhibition of 31.3 mm resulting in positive control. Comparatively, *Cinnamomum verum* exhibited a moderate range of inhibition of 19.1 mm which means that the antimicrobial activity is extensive. *Bauhinia racemosa* had a smaller zone of 10.8 mm indicating quite weak antibacterial activity. These findings indicate that *Cinnamomum verum* possesses better prospects of anti-microbial action than *Bauhinia racemosa* against *Klebsiella pneumoniae*. These results justify the further research of bioactive compounds that have this activity and how they may be synergetic in polyherbal preparations.

➢ *Evaluation of Polyherbal Cough Syrup:*

- *Organoleptic Test:*

All three batches showed same results which are as follows

✓ A) Colour Test - Reddish Brown B) Oduor Test - Spicy Aromatic C) Taste Test – Sweet

- *pH Test:*

Table 6 pH Test

F_1	F_2	F_3
6.10	6.05	5.98

- *Viscosity Test:*

Table 7 Viscosity Test

F_1	F_2	F_3
729mPa.s.	742mPa.s.	758mPa.s.

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- *Specific Gravity:*

Table 8 Specific Gravity

F_1	F_2	F_3
1.240	1.258	1.270

- *Density:*

Table 9 Density

F_1	F_2	F_3
1.240g/cm³	1.258g/cm³	1.270g/cm³

- *Water Content Determination/Loss on Drying:*

Table 10 Water Content Determination/Loss on Drying

F_1	F_2	F_3
61.92%	63.23%	64.96%

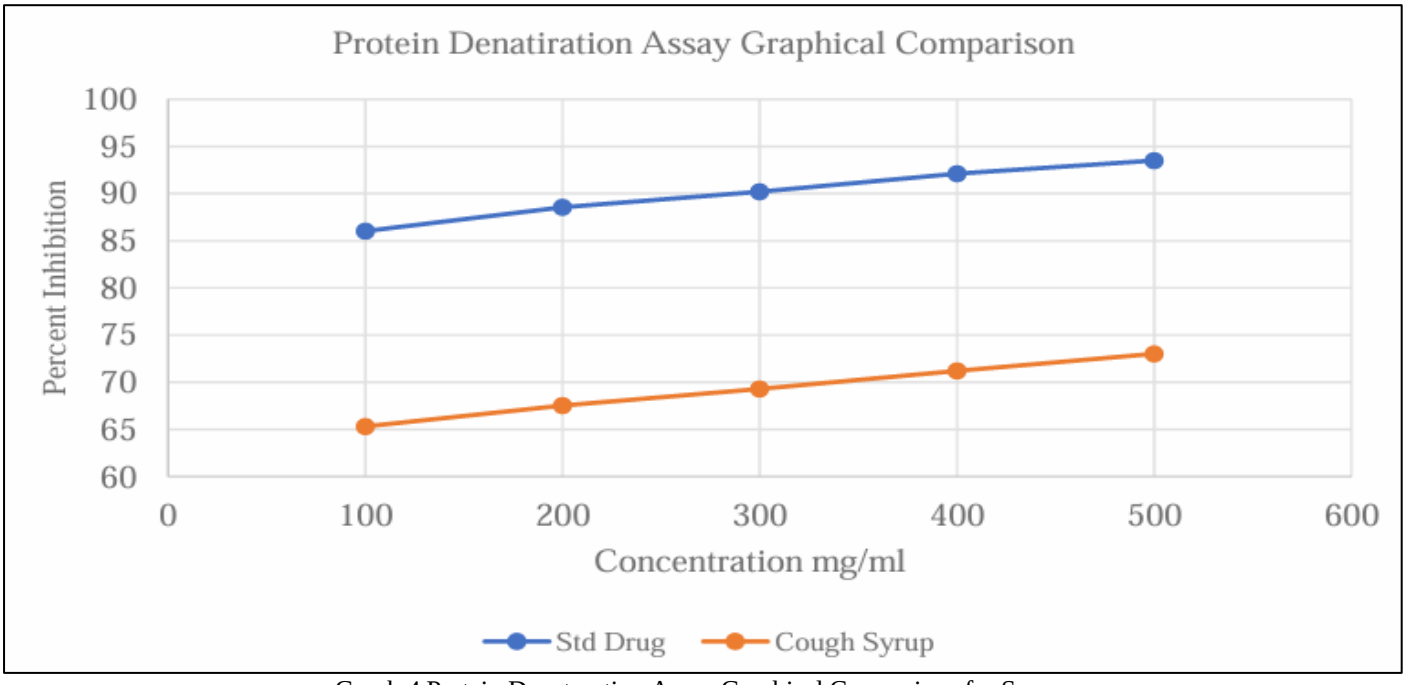
- *Determination of Ash Values:*

Table 11 Determination of Ash Values

Types of Ash Values	F_1	F_2	F_3
Total Ash	1.66% w/w	1.89% w/w	2.04% w/w
Acid Insoluble Ash	0.46% w/w	0.71% w/w	0.97% w/w
Sulphated Ash	2.29% w/w	2.59% w/w	2.85% w/w

➢ *Determination of in-Vitro Anti-Inflammatory Activity of Polyherbal Cough Syrup:*

- *Protein Denaturation Method:*



Graph 4 Protein Denaturation Assay Graphical Comparison for Syrup

- *Human Red Blood Cell Assay Membrane Stabilization Method*

Polyherbal cough syrup had a significant membrane stabilization activity, and the percentage of inhibition was (66.93%-73.89%) at a concentration of 0.1 to 0.5g/ml. Even though it was slightly weaker than Diclofenac Sodium (85.05%to 92.33), the formulation exhibited an apparent dose-dependent reaction. This implies the existence of the stabilizing phytoconstituents of the membrane, which confirms the prospective anti-inflammatory effects of the syrup in terms of the protection of lysosomal membranes, which are applicable in such conditions as asthma.

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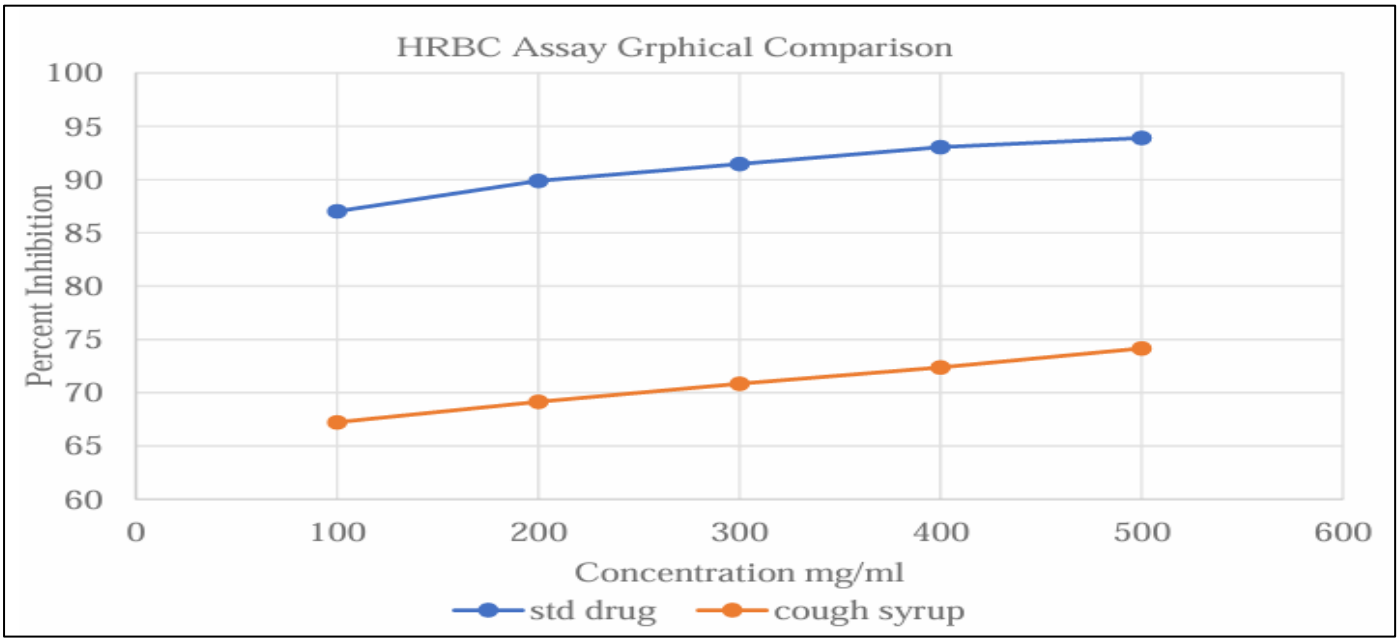
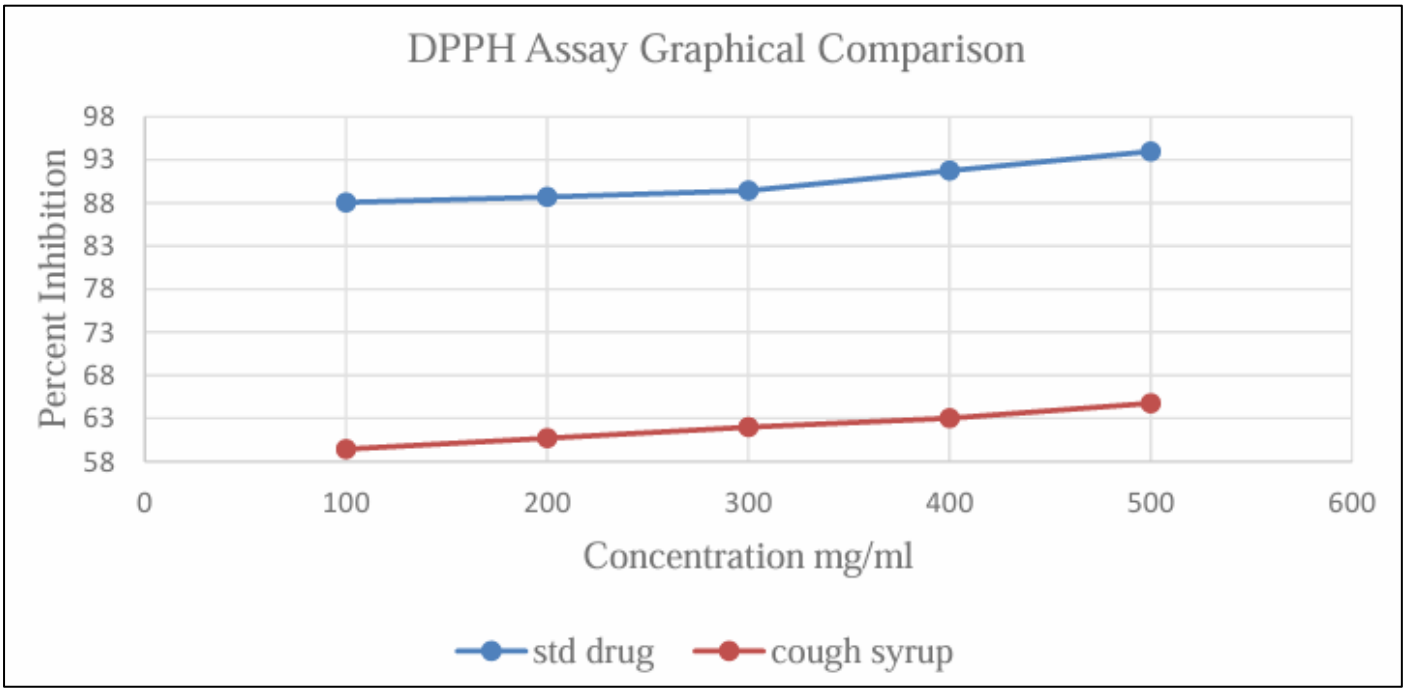


Graph 5 HRBC Assay Graphical Comparison for Syrup

- *In-Vitro Anti-Oxidant Activity of Polyherbal Cough Syrup by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay:*

The polyherbal cough syrup exhibited the concentration dependent antioxidant effect with an increasing percentage of inhibition (60.26% to 65.12%) at the concentration of 0.1 to 0.5g/mL. In spite of the fact that the activity was low in relation to the conventional antioxidant Ascorbic Acid (88.13% to 94.09%), the formulation was uniform in the free radical scavenging activity. This shows that there are phytochemicals that have antioxidant properties and this could be one of the contributing factors to the therapeutic effects of the syrup in oxidative stress related respiratory diseases such as asthma.



Graph 6 DPPH Assay Graphical Comparison for Syrup

- *In-Vitro Anti-Microbial Activity of Polyherbal Cough Syrup by Cup Plate Method:*

The polyherbal cough syrup exhibited significant in-vitro antimicrobial effects in *Klebsiella pneumoniae*, and the zone of inhibition was 8.7 mm. Although Streptomycin presented a bigger zone 31.3 mm, the presence of herbal constituents of antibacterial potential can be emphasized by the activity presented in the syrup. The outcomes indicate that the formulation has potential supportive antimicrobial properties, which have a general add-on effect in the overall treatment value of respiratory tract infections and other symptoms of asthma.

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➢ *Ex-Vivo Anti-Asthmatic Activity (Goat Trachea Isolation) Method:*

- *Percent Response of Histamine:*

Table 12 Ex-Vivo Anti-Asthmatic Activity (Goat Trachea Isolation) Method - Percent Response of Histamine Data

Sr. No.	Dose qty (in ml)	Dose(µg/ml)	Peak Height (in mm)	% Contraction response
1	0.1	10	7	31.82
2	0.1	10	7	31.82
3	0.2	20	10	45.45
4	0.4	40	16	72.73
5	0.8	80	21	95.45
6	1.6	160	22	100
7	3.2	320	19	86.36
8	6.4	640	17	87.27

- *Percent Response of Histamine and Standard Drug (Bilastine) (1:1):*

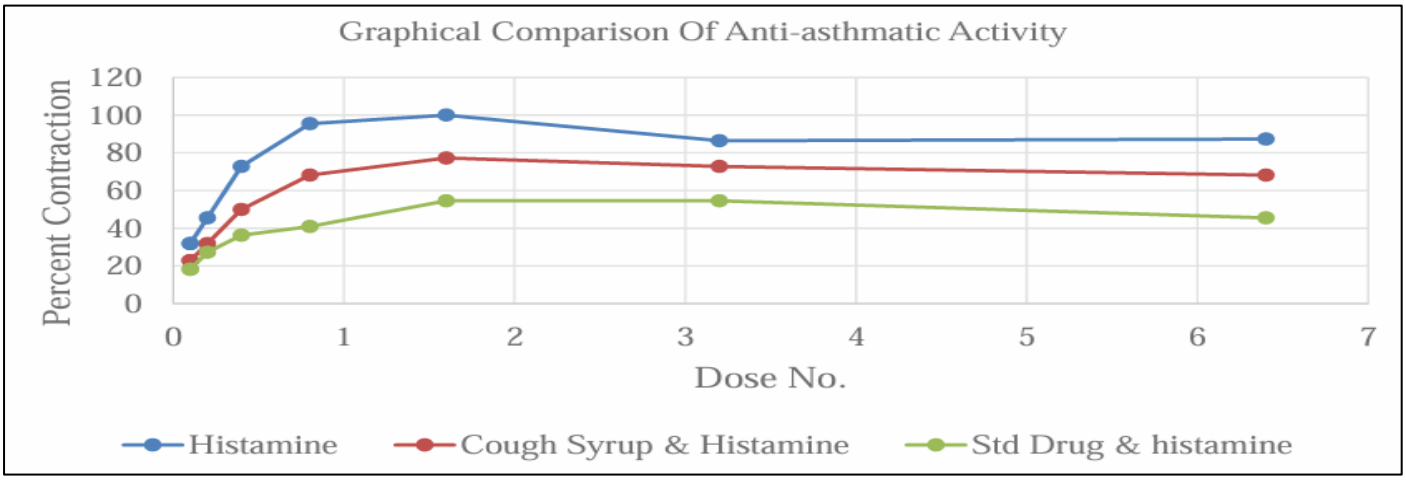
Table 13 Ex-Vivo Anti-Asthmatic Activity (Goat Trachea Isolation) Method - Percent Response of Histamine and Standard Drug Data (Bilastine) (1:1)

Sr. No.	Dose qty (in ml)	Dose(µg/ml)	Peak Height (in mm)	% Contraction response
1	0.1	10	4	18.18
2	0.1	10	4	18.18
3	0.2	20	6	27.27
4	0.4	40	8	36.36
5	0.8	80	9	40.91
6	1.6	160	12	54.55
7	3.2	320	12	54.55
8	6.4	640	10	45.45

➢ *Percent Response of Histamine and Polyherbal Cough Syrup (1:1):*

Table 14 Ex-Vivo Anti-Asthmatic Activity (Goat Trachea Isolation) Method - Percent Response of Histamine and Polyherbal Cough Syrup Data (1:1)

Sr. No.	Dose qty (in ml)	Dose (µg/ml)	Peak Height (in mm)	% Contraction response
1	0.1	10	5	22.73
2	0.1	10	5	22.73
3	0.2	20	7	31.82
4	0.4	40	11	50
5	0.8	80	15	68.18
6	1.6	160	17	77.27
7	3.2	320	16	72.73
8	6.4	640	15	68.18



Graph 7 Graphical Comparison of Anti-Asthmatic Activity

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The *ex-vivo* goat trachea model effectively demonstrated the contractile response of airway smooth muscle to histamine, with a peak response of 100% contraction at 160 µg/mL, confirming the model's suitability for evaluating bronchodilatory agents.

When co-treated with the standard drug bilastine (1:1), the contraction response was significantly reduced, peaking at only 54.55%, validating its known antihistaminic and bronchodilatory effects.

Similarly, co-administration of the polyherbal cough syrup (F_3) (1:1) with histamine resulted in a notable reduction in contraction, with a peak response of 77.27%, compared to 100% with histamine alone.

IV. CONCLUSION

The present study focuses on the formulation and evaluation of a polyherbal anti-asthmatic cough syrup. Roots of *Withania somnifera* and bark of *Cinnamomum verum* were extracted using Soxhlet with ethanol, while leaves of *Bauhinia racemosa* and roots of *Glycyrrhiza glabra* were extracted by cold maceration using ethanol and distilled water, respectively. The concentrated extracts were dried to obtain semi-solid residues for formulation.

Phytochemical confirmation was carried out by TLC, which indicated the presence of quercetin in *Bauhinia racemosa* (analgesic, antimicrobial), glycyrrhizin in *Glycyrrhiza glabra* (mucolytic, anti-inflammatory), and cinnamaldehyde in *Cinnamomum verum* (antimicrobial, anti-inflammatory). UV–Visible spectroscopy revealed λmax values consistent with reported literature, and calibration curves showed strong linearity ($R^2 > 0.99$), validating accuracy and purity of the extracts.

Anti-inflammatory activity was demonstrated through protein denaturation and HRBC membrane stabilization assays, with *Glycyrrhiza glabra* showing the highest inhibition (up to 82.73% and 87.40%), comparable to diclofenac sodium. Antioxidant evaluation revealed *Withania somnifera* had strong free radical scavenging activity (88.33% in the DPPH assay), attributed to Withaferin-A. Antimicrobial studies by the cup plate method showed *Cinnamomum verum* exhibited the strongest inhibition against *Klebsiella pneumoniae* (18.7 mm), while *Bauhinia racemosa* also showed measurable activity (9.8 mm).

The polyherbal cough syrup was formulated in three batches (F1, F2, F3) with varying extract concentrations. All formulations showed acceptable organoleptic and physicochemical properties, with moisture content ranging 61.92–64.96% and ash values within pharmacopeial limits. F3 was found most compliant with IP standards and selected for further studies. The final formulation contained per 10 mL: *Bauhinia racemosa* (100 mg), *Cinnamomum verum* (100 mg), *Withania somnifera* (200 mg), and *Glycyrrhiza glabra* (200 mg), blended in 66.7% sugar syrup with preservatives.

The formulation exhibited dose-dependent in-vitro anti-inflammatory activity (65.31–73.01% in protein denaturation and 67.23–74.16% in HRBC stabilization), significant antioxidant potential (59.44–64.76% scavenging in DPPH assay), and antimicrobial activity against *Klebsiella pneumoniae* (8.2 mm inhibition zone). In the ex-vivo goat trachea model, the syrup reduced histamine-induced contraction by 77.27%, indicating bronchodilatory and antihistaminic properties.

These findings collectively support the polyherbal cough syrup's multi-targeted therapeutic role in managing asthma and related respiratory conditions. Future work will focus on in-vivo pharmacological screening and advanced preclinical and clinical studies to further validate efficacy, safety, and stability.

REFERENCES

[1]. Boers, E.; Barrett, M.; Su, J. G.; Benjafield, A. V.; Sinha, S.; Kaye, L.; Zar, H. J.; Vuong, V.; Tellez, D.; Gondalia, R.; Rice, M. B.; Nunez, C. M.; Wedzicha, J. A.; Malhotra, A. global burden of chronic obstructive pulmonary disease through 2050. jama netw open 2023, 6 (12), e2346598. https://doi.org/10.1001/jamanetworkopen.2023.46598.

[2]. Umamaheswari, D.; Arun, V.; Jeeva, K.; Venkateswarlu, B. S.; Kumar, M. Comparative evaluation of herbal cough syrup available in market. International journal of research and analytical reviews 2020.

[3]. Prabhakar Bangar, A.; Jatale, A.; Deshmukh, S. Poly herbal formulation and evaluation of herbal cough syrup. www.ijirct.org.

[4]. Sharma, V.; Singh, S.; Dixit, A.; Saxena, A. formulation and evaluation of herbal cough syrup from seeds extract of hedge mustard. International journal of research in pharmacy and chemistry 2020, 10 (1). https://doi.org/10.33289/ijrpc.10.1.2020.10(3).

[5]. Sumedh Sanjay, K. Formulation and evaluation of herbal cough syrup abstract; 2023; vol. 11. www.ijcrt.org.

[6]. Panda, P.; Sahu, A. formulation and evaluation of herbal cough syrup. asian journal of pharmaceutical research and development 2023, 11 (2), 28–33. https://doi.org/10.22270/ajprd.v11i2.1234.

[7]. Pranjal Nimba, P.; Tejashree Sudam, Z.; Kanchan Bhausaheb, T.; Rakesh Manik, W.; Pramod Anna, D. Formulation and evaluation of herbal cough syrup; 2023. www.ijnrd.org.

[8]. Anitha, P.; Ravichandhiran, E.; Krishnan, R. HPTLC fingerprinting analysis of the ethanolic extract of *bauhinia racemosa* leaves; 2023; vol. 10. www.ejpmr.com.

[9]. Prabhu, S.; Vijayakumar, S.; Ramasubbu, R.; Praseetha, P. K.; Karthikeyan, K.; Thiyagarajan, G.; Sureshkumar, J.; Prakash, N. Traditional Uses, Phytochemistry and Pharmacology of *Bauhinia Racemosa* Lam.: A Comprehensive Review. *Futur J Pharm Sci* 2021, *7* (1).

https://doi.org/10.1186/s43094-021-00251-1.
[10]. Prabhakar Bangar, A.; Jatale, A.; Deshmukh, S. Polyherbal formulation and evaluation of herbal cough syrup. www.ijirct.org.
[11]. Nasulhah Kasim, N.; Nursyimi Azlina Syed Ismail, S.; Masdar, N.; Ab Hamid, F.; Nawawi, W. Extraction and potential of cinnamon essential oil towards repellency and insecticidal activity; 2014; vol. 4. www.ijsrp.org.
[12]. Shinde, S.; Balasubramaniam, A. K.; Mulay, V.; Saste, G.; Girme, A.; Hingorani, L. Recent advancements in extraction techniques of ashwagandha (*withania somnifera*) with insights on phytochemicals, structural significance, pharmacology, and current trends in food applications. acs omega. american chemical society november 7, 2023, pp 40982–41003. https://doi.org/10.1021/acsomega.3c03491.
[13]. Bashir, A.; Nabi, M.; Tabassum, N.; Afzal, S.; Ayoub, M. An updated review on phytochemistry and molecular targets of *withania somnifera* (l.) dunal (ashwagandha). frontiers in pharmacology. frontiers media s.a. 2023. https://doi.org/10.3389/fphar.2023.1049334.
[14]. Pastorino, G.; Cornara, L.; Soares, S.; Rodrigues, F.; Oliveira, M. B. P. P. Liquorice (Glycyrrhiza Glabra): A phytochemical and pharmacological review. phytotherapy research. john wiley and sons ltd december 1, 2018, pp 2323–2339. https://doi.org/10.1002/ptr.6178.
[15]. Wahab, S.; Annadurai, S.; Abullais, S. S.; Das, G.; Ahmad, W.; Ahmad, M. F.; Kandasamy, G.; Vasudevan, R.; Ali, M. S.; Amir, M. *Glycyrrhiza Glabra* (Licorice): A comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology. plants. mdpi december 1, 2021. https://doi.org/10.3390/plants10122751.
[16]. Balekundri, A.; Shahapuri, A.; Patil, M. Poly-herbal tablet formulation by design expert tool and in vitro anti-lipase activity. futur j pharm sci 2020, 6 (1). https://doi.org/10.1186/s43094-020-00131-0.
[17]. Prabhakar Bangar, A.; Jatale, A.; Deshmukh, S. Poly herbal formulation and evaluation of herbal cough syrup. www.ijirct.org.
[18]. Prabhakar Bangar, A.; Jatale, A.; Deshmukh, S. Poly herbal formulation and evaluation of herbal cough syrup. www.ijirct.org.
[19]. Parvin, M. S.; Das, N.; Jahan, N.; Akhter, M. A.; Nahar, L.; Islam, M. E. Evaluation of *in vitro* anti-inflammatory and antibacterial potential of crescentia cujete leaves and stem bark pharmacology and toxicology. bmc res notes 2015, 8 (1). https://doi.org/10.1186/s13104-015-1384-5.
[20]. Chauhan, A.; Semwal, D.; Mishra, S.; Semwal, R. Ayurvedic research and methodology: present status and future strategies. ayu (An international quarterly journal of research in ayurveda) 2015, 36 (4), 364. https://doi.org/10.4103/0974-8520.190699.
[21]. Nama, N.; Arora, P.; Arora, N.; Jain, H. Formulation and characterization of *bauhinia racemosa* extract loaded nanoemulsion by box behnken design. journal of pharmaceutical negative results ¦ 13, 2022. https://doi.org/10.47750/pnr.2022.13.s08.648.
[22]. Bioactivity of active extracts of leaf of *Bauhinia racemosa*. International journal of clinical chemistry and laboratory medicine 2019, 5 (2). https://doi.org/10.20431/2455-7153.0502004.
[23]. Nataraj, R.; Chettiyar, B. Phytochemical screening and antimicrobial activity of "*cinnamon zeylanicum.*" International journal of pharmaceutical research and innovation 2020, 13, 22–33.
[24]. Noor, S. U.; Faridah, M. Formulation of liquorice root extract (*glycyrrhiza glabra l.)* as skin whitening cream.
[25]. Noor, S. U.; Faridah, M. Formulation of liquorice root extract (*glycyrrhiza glabra l.*) as lozenges.
[26]. Mhaske, M.; Diliprao, S.; Jagdish, P.; Vilas, S.; Holkar, M.; Uddhav, P.; Gulve, M.; Subhash, A.; Tarade, M.; Santosh, A.; Scholar, U. G.; Professor, A.; Miratai, M. jetir2206735 Journal of emerging technologies and innovative research (jetir) www.jetir.org h297 Formulation and evaluation of oral antiulcer gel from liquorice extract; 2022. www.jetir.org.
[27]. Keramatian, B.; Ara, L.; Hajimehdipoor, H. Licorice compound traditional syrup: formulation and development of analysis method. Research journal of pharmacognosy 2022, 9 (1), 111–117. https://doi.org/10.22127/rjp.2021.294636.1739.
[28]. Omkar, M.; Daware, S.; Tijare, R. D.; Ayush, M.; Chavhan, B. Preparation and evaluation of polyherbal cough syrup: a novel approach. www.ijfmr.com.
[29]. Kapgate, P. R.; Mandade, R. J.; Dhole, Y. R.; Urkuda Gondhale, M.; Ravindra Lanjewar, S.; Purushottam Bawankar, P. Review on polyherbal syrup for various pharmacological activities; 2023; vol. 26. www.ijppr.humanjournals.comwww.ijppr.humanjournals.com.
[30]. Sakshi Anant Kadam; Seema Dharmaraj Gadade; Anjali Laxman Kalunke; Dr. L. D. Hingane. Formulation and evalution of polyherbal cough syrup. international journal of advanced research in science, communication and technology 2024, 499–509. https://doi.org/10.48175/IJARSCT-22267.
[31]. Patel, K.; Patel, D. K. Medicinal importance, pharmacological activities, and analytical aspects of hispidulin: a concise report. journal of traditional and complementary medicine. National taiwan university july 1, 2017, pp 360–366. https://doi.org/10.1016/j.jtcme.2016.11.003.
[32]. Sutherland, E. R. Nocturnal Asthma. Journal of allergy and clinical immunology. mosby inc. 2005, pp 1179–1186. https://doi.org/10.1016/j.jaci.2005.09.028.
[33]. Lee, K. K.; Davenport, P. W.; Smith, J. A.; Irwin, R. S.; McGarvey, L.; Mazzone, S. B.; Birring, S. S.; Abu Dabrh, A. M.; Altman, K. W.; Barker, A. F.; Blackhall, F.; Bolser, D. C.; Brightling, C.; Chang, A. B.; Davenport, P.; El Solh, A. A.; Escalante, P.; Field, S. K.; Fisher, D.; French, C. T.; Grant, C.; Harding, S. M.; Harnden, A.; Hill, A. T.; Iyer, V.; Kahrilas, P. J.; Kavanagh, J.; Keogh, K. A.; Lai, K.; Lane, A. P.; Lim,

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K.; Madison, J. M.; Malesker, M. A.; Murad, M. H.; Narasimhan, M.; Newcombe, P.; Oppenheimer, J.; Rubin, B.; Russell, R. J.; Ryu, J. H.; Singh, S.; Smith, M. P.; Tarlo, S. M.; Vertigan, A. E. Global physiology and pathophysiology of cough: part 1: cough phenomenology – chest guideline and expert panel report. chest. elsevier inc. january 1, 2021, pp 282–293. https://doi.org/10.1016/j.chest.2020.08.2086.

[34]. Solanki, P.; Mohabe, S. issue 6 www.jetir.org (issn-2349-5162); 2024; vol. 11. www.jetir.org.

[35]. Ruiz-Alcaraz, A. J.; Baquero, L.; Pérez-Munar, P. M.; Oliva-Bolarín, A.; Sánchez-Martínez, M. A.; Ramos-Molina, B.; Núñez-Sánchez, M. A.; Moreno, D. A. *In Vitro* study of the differential anti-inflammatory activity of dietary phytochemicals upon human macrophage-like cells as a previous step for dietary intervention. int j mol sci 2024, 25 (19). https://doi.org/10.3390/ijms251910728.