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Design and Evaluation Process of A Herbal Cough Syrup

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ABSTRACT

The systematic process for the design and evaluation of a herbal cough syrup, focusing on a methodology that ensures product efficacy, safety, and quality. The process begins with the selection of active herbal ingredients based on their traditional use and scientific evidence for antitussive, expectorant, and demulcent properties. The formulation is developed to optimize the synergistic effects of these ingredients while ensuring stability and palatability. A key aspect of this process is the establishment of robust quality control measures, including the standardization of raw materials and the final product.

The evaluation phase involves a multi-pronged approach. Pre-clinical studies, such as in-vitro assays for antimicrobial and anti-inflammatory activity, are conducted to support the traditional claims. Clinical evaluation, including a randomized, double-blind, placebo-controlled trial, is proposed to assess the syrup's efficacy in reducing cough frequency and severity, as well as its safety profile. Patient-reported outcomes, such as symptom relief and quality of life, are also considered. Stability studies are performed to determine the product's shelf-life under various storage conditions. The ultimate goal is to create a well-documented and scientifically validated herbal cough syrup that provides a safe and effective alternative for cough relief.

KEYWORDS :- Herbal medicine, Cough syrup, Quality control, Efficacy, Safety, Stability, Pharmacognosy, Natural products, Expectorant, Antitussive, Demulcent, Pre-clinical studies.

INTRODUCTION

Apart from medicinal use, Ayurvedic herbs can also be used for purposes like pest control, natural dyes, and formulation of food items, teas and perfumes among others. If we look at various researches from across the world, a sudden spurt in cases of people turning to natural herbs for treatments and usage in everyday life has gone up significantly. Going back to the basics, people have realized the threat chemically treated products pose to their life and are rightly so adopting healthier ways of life by including Ayurveda and its principals as the mainstay of their life. Cough is one of the most common symptoms of respiratory tract infections and other underlying conditions affecting the lungs, throat, and airways. While often dismissed as a minor ailment, a persistent or severe cough can significantly affect quality of life and may indicate serious health issues. Medically, a cough is a reflex action to clear the airways of mucus, irritants, foreign particles, or microbes. It is a vital protective mechanism of the respiratory system but can become problematic if it is chronic, painful, or associated with other serious symptoms.

MATERIALS AND METHOD

Selection of plant materials:

Some plants viz. *Thymus vulgaris* (leaves), *Zingiber officinale* (rhizome), *Glycyrrhiza glabra* (roots), *Ocimum basilicum* (leaves) were selected for the present study, based on their utility as antifungal agent, also these form common ingredients in many polyherbal formulations available as antitussive agents. There are no scientific reports available depicting their efficacy or bioavailability, hence the present study was aimed at preparation of formulation containing these plant parts.

Table No. 5.1.: Plants parts make to use polyherbal formulations.

S. No.	Plant	Part Used
1.	<i>Thymus vulgaris</i>	Leaves
2.	<i>Zingiber officinale</i>	Rhizomes
3.	<i>Glycyrrhiza glabra</i>	Roots
4.	<i>Ocimum basilicum</i>	Leaves

Plants parts were mixed together in equal ratio at first than hot extraction process has been done. 50 gms of dried shade powder was exhaustively extracted with chloroform, ethyl acetate, ethanol and water using soxhlet extraction apparatus. The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Preliminary phytochemical screening of plants extract

The powder extracts were individually evaluated for the presence of different phytoconstituents as per the below mentioned methods:

● **Test for terpenes:**

To the 5ml of the extract, 2ml of chloroform and 3ml of conc. H₂SO₄ was added. The formation of a reddish brown ring confirmed the presence of terpenes.

Test for flavonoids:

A few drops of conc. HCl were added in the small amount of the prepared extracts. The red colour was immediately developed, which confirmed the presence of flavonoids.

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Test for saponins (Frothing test):

0.5ml of the extract was taken into a test tube and dissolved in distilled water. Frothing was persisted on warming, which preliminary shows as evidence of saponins.

Test for steroids (Liebermann–Burchard reaction):

2ml of acetic anhydride and 2 ml conc. H₂SO₄ was added into 5ml of the extract in a test tube. Change of colour from violet to blue confirms the presence of steroids.

Test for glycosides:

2ml of glacial acetic acid containing one drop of ferric chloride solution and 1 ml of conc. H₂SO₄ was added into 5ml of the extract in a test tube. The appearance of a brown ring indicates the presence of glycosides.

Test for proteins (Biuret test):

4% of NaOH and few drops of 1% CuSO₄ solution were added into 3ml of the extract in a test tube. Formation of violet or pink color indicates the presence of proteins.

Test for reducing sugars (Fehling test):

1ml of Fehling's A and Fehling's B solutions was mixed in a test tube, boiled for one minute then added an equal volume of test solution (2ml extract). The mixed solution was then heated on boiling water bath for 5–10 min. First a yellow then a red brick precipitate was observed.

0.5ml of the extract was taken into a test tube and dissolved in distilled water. Frothing was persisted on warming, which preliminary shows as evidence of saponins.

Test for steroids (Liebermann–Burchard reaction):

2ml of acetic anhydride and 2 ml conc. H₂SO₄ was added into 5ml of the extract in a test tube. Change of colour from violet to blue confirms the presence of steroids.

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Test for carbohydrates (Molisch test):

2–3ml of the aqueous extract, 2 drops of Molisch's reagent (10% alcoholic solution of α -naphthol) was added in a test tube. After mixing, a small amount of conc. H₂SO₄ is slowly added down the sides of the sloping test-tube, without mixing, to form a layer. Violet ring is formed at the interface between the acid and test layers.

Test for tannin and phenol (Ferric Chloride Test):

3ml of extract, 3ml of 5% w/w of the FeCl₃ solution was added in a test tube. The blue-black colour indicates the presence of tannins and phenols.

Test for alkaloids:

In 10g of dried extracts 20ml of dilute HCl solution was added with vigorous shaking and then filter. In the filtrate, the following tests were performed.

**Mayer's Test :**

3ml of the filtrates, 1ml of Mayer's reagent (potassium mercuric iodide) was added in a test tube. The appearance of white precipitate confirmed the presence of alkaloids.

**Wagner's Test :**

3ml of the filtrate, 1ml of Wagner's reagent (iodine in potassium iodide) was added in a test tube. The emergence of reddish-brown precipitate at the surface indicates the presence of alkaloids.

**Dragendroff's Test:**

3ml of the filtrate, 1ml of Dragendroff's reagent (potassium bismuth iodide) was added in a test tube. The appearance of red brick precipitate indicates the presence of alkaloids.

Pharmacological evaluation***In-vitro* antimicrobial activity of plant extract**

The pharmacological activity of dried powder extracts were tested against human pathogenic fungi. The chloroform, methanol, petroleum ether and aqueous extracts at different concentrations such as 25 mg/ml, 50 mg/ml and 100 mg/ml were taken for studying the efficacy. The minimum inhibitory concentration was determined by agar well diffusion method. Pure cultures of fungi were obtained from Indore (M.P.). The following fungi were selected for studies:

- Streptococcus pneumonia
- Mycoplasma pneumonia

The minimum inhibitory concentration was determined by agar well diffusion method. In the freshly prepared and sterilized potato dextrose agar medium, 1 mg streptomycin was added for preventing bacterial growth. Then 20 ml of Potato dextrose agar medium was poured into each petriplate and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a well 6 mm was made in the medium by using sterile cork borer, 0.1, 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25 mg/ml of each concentration of chloroform, methanol, petroleum ether, aqueous and Hydroalcoholic extracts were transferred into separate wells. Then these plates were incubated at 27 °C for 48-72 hours. After incubation period the results were observed and measured the diameter of inhibition zone around the each well.

Formulation of syrup

Table 5.2 Ingredients Used in Various Batches of Herbal Cough Syrup

S. No	Ingredients	F1	F2	F3	F4
1	Polyherbal extract	5ml	10ml	15ml	20ml
2	Honey	30ml	30ml	30ml	30ml
3	Sodium Benzoate	0.1	0.1	0.1	0.1
4	Purified water	Qs 100ml	Qs 100ml	Qs 100ml	Qs 100ml



Figure 5.1 Various Batches of Herbal Cough Syrup

RESULTS AND DISCUSSION

Selection of plant materials:

On the ground of literature review and deep discussion with medical practitioners of the Indore (M.P.) *Thymus vulgaris* (leaves), *Zingiber officinale* (rhizome), *Glycyrrhiza glabra* (roots), *Ocimum basilicum* (leaves) were selected for evaluation of the antimicrobial activity and formulation of Polyherbal syrup.

Preliminary phytochemical screening of plants extract

The powder extracts were individually evaluated for the presence of different phytoconstituents as per the below mentioned methods:

- Test for terpenes:
- Test for flavonoids:
- Test for saponins (Frothing test):
- Test for steroids (Liebermann–Burchard reaction):
- Test for glycosides:
- Test for proteins (Biuret test):

Table no. 6.1 Phytochemical evaluation of *plant extract*

S. No.	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
1	Carbohydrate	<i>Molisch's test</i>	+	+	+	+	++
		<i>Fehling's test</i>	-	++	+	++	+
2	Glycosides	<i>Legal's test</i>	+	+	+	+	+
		<i>Borntrager's test</i>	++	+	-	+	++
		<i>Baljet test</i>	+	+	+	+	+
3	Fixed oil and Fats	<i>Spot test</i>	+	+	+	++	++
		<i>Saponification test</i>	+	+	+++	+	+
4	Proteins and Amino Acids	<i>Biuret test</i>	++	+	+	+	+
5	Saponins	<i>Foam test</i>	+	+	+	+	+
6	Phenolic Comp. and Tannins	<i>FeCl₃ test</i>	++	+	+	+	++
7	Steroids	<i>Liebermann- buehard test</i>	+	+	+	+	+
8	Alkaloids	<i>Dragendorff's test</i>	+	+	-	+	+
		<i>Mayer's test</i>	-	+	+	+	+
		<i>Wagner's test</i>	+	+	+	+	+
9	Terpines		+	+	+	+	+++
10	Flavonoids	<i>Lead acetate test</i>	-	+	+	+	+
		<i>Con. H₂SO₄ test</i>	+	+	+	+	+++
		<i>FeCl₃ test</i>	+	+	+	+	+

Pharmacological evaluation viz. antifungal activity In-vitro

The antimicrobial activity of dried powder extracts were tested against human pathogenic fungi. The plants extract at different concentrations such as 25 mg/ml, 50 mg /ml and 100 mg /ml were taken for study against bacterial species *Streptococcus pneumonia* and *Mycoplasma pneumonia* for the determination of zone of inhibition. The minimum inhibitory concentration was determined by agar well diffusion method. Pure cultures of bacteria were obtained from Indore (M.P.).

Table no. 6.2 The zone of inhibition of different extracts

Zone of inhibition (in mm)			
Plants Extract	Concentration (mg/ml)	Streptococcus pneumonia	Mycoplasma pneumonia
Chloroform	25	10.5	11.5
	50	14.1	12.4
	100	16.8	13.8
Ethyl acetate	25	9.6	9.6
	50	10.1	9.9
	100	11.4	10.8
Ethanol	25	10.5	11.5
	50	12.1	12.7
	100	13.8	14.8
Water	25	9.5	11.8
	50	11.4	14.4
	100	12.8	16.8
Hydro-alcoholic (1:1)	25	14.5	13.5
	50	16.1	15.4
	100	20.8	18.8

Table no. 6.3 Minimum inhibitory concentration of different extracts

Minimum Inhibitory Concentration MIC (%w/v)			
Plants Extract	Concentration (mg/ml)	Streptococcus pneumonia	Mycoplasma pneumonia
Hydro-alcoholic (1:1)	0.1	N	N
	0.19	N	N
	0.39	N	N
	0.78	N	N
	1.56	N	N
	3.13	N	Y
	6.25	Y	Y
	12.5	Y	Y
	25	Y	Y

N= No inhibition, Y= inhibition found

PREFORMULATION STUDIES**Table no. 6.3 Results of Preformulation studies**

Parameters	Polyherbal formulation
Angle of repose	26.11±1.11
Loose bulk density (g/cm ³)	0.693±0.016
Tapped bulk density (g/cm ³)	0.911±0.022
Hausner ratio	1.17±0.032
Compressibility index (%)	13.11±2.21

Evaluation of polyherbal syrup**Table no. 6.4 Results of evaluation of Polyherbal syrup**

Sr. No.	Evaluation	F1	F2	F3	F4
1	Colour	Brown	Brown	Brown	Brown
	Taste	Sweet, mint	Sweet, mint	Sweet, mint	Sweet, mint
	Odor	Mint	Mint	Mint	Mint
2	pH	4.12	4.13	4.11	4.11
3	Viscosity	300 cP	320 cP	312 cP	315 cP
4	Specific Gravity	47.59	47.37	47.42	47.42
5	Density	47.44	47.22	47.27	47.27
6	Anti-microbial (Streptococcus pneumonia)	15 mm	20 mm	22 mm	22.5 mm
7	Anti-microbial (Mycoplasma pneumonia)	17 mm	19 mm	23 mm	23 mm

The polyherbal syrup formulations (F1 to F4) were evaluated for organoleptic, physicochemical, and antimicrobial properties. These evaluations are essential for determining the stability, quality, and therapeutic potential of the formulation.

1. Organoleptic Evaluation

All formulations showed consistent sensory characteristics:

- Colour: Brown in all four formulations, suggesting uniform composition and processing.
- Taste: Sweet with a minty note, which is likely to improve palatability and consumer acceptability.
- Odor: Minty aroma across all samples, indicating the presence of aromatic and volatile herbal constituents, possibly contributing to therapeutic effects like soothing respiratory passages.

Consistency in organoleptic properties indicates a standardized formulation process and stability of herbal components during production.

2. pH

The pH values of the syrups ranged from 4.11 to 4.13, reflecting a mildly acidic nature:

- This pH is appropriate for oral herbal syrups, helping to maintain chemical stability and minimize microbial growth during storage.

3. Viscosity

Viscosity values ranged from 300 to 320 cP, indicating suitable syrup consistency:

- A moderately viscous syrup ensures ease of swallowing, proper dosing, and adequate coating of the throat in case of respiratory use.
- Minor variations are acceptable and within the standard range for herbal syrups.

4. Specific Gravity and Density

- Specific Gravity ranged from 47.37 to 47.59

- Density ranged from 47.22 to 47.44

These values indicate consistent formulation and solute concentration across batches. Uniform density and specific gravity help ensure dose uniformity and stable shelf life.

5. Antimicrobial Activity

The antimicrobial activity was tested against two respiratory pathogens:

- *Streptococcus pneumoniae* (a Gram-positive bacterium): Zone of inhibition increased from 15 mm (F1) to 22.5 mm (F4).
- *Mycoplasma pneumoniae* (a cell wall-less respiratory pathogen): Activity ranged from 17 mm (F1) to 23 mm (F4).

These results suggest that:

- All formulations exhibit antimicrobial properties.
- F3 and F4 demonstrate the strongest activity, indicating a possibly higher concentration or synergy of active herbal components.
- The activity against both bacterial types reflects the broad-spectrum potential of the formulation, particularly in treating respiratory infections.

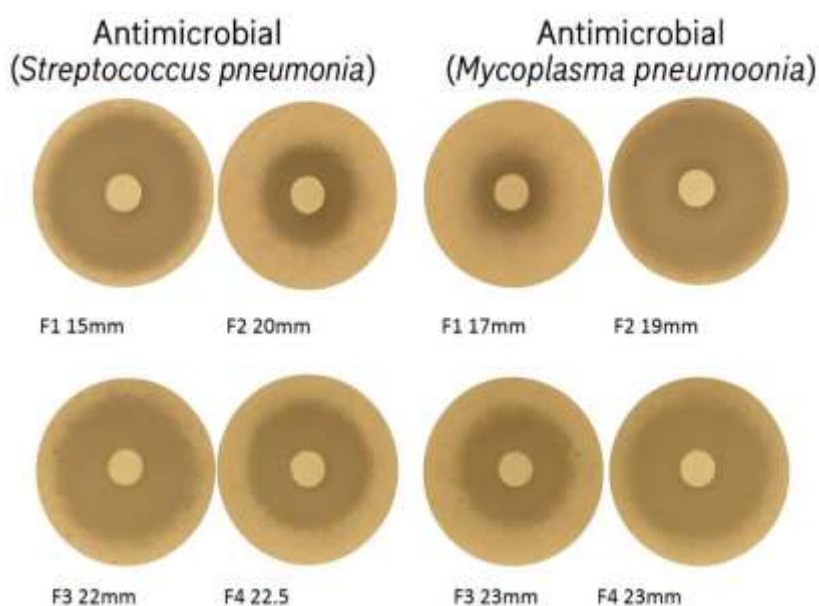


Figure 6.1: Results of antimicrobial activity of polherbal syrups formulations

REFERENCES

1. Acharya A, Ghimire G, Shakya P. Medicinal Herbs. Herbs and Spices—New Perspectives in Human Health and Food Industry; IntechOpen: Rijeka, Croatia. 2024 Nov 13;1:161-86.
2. Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA. Studies on phytochemical screening and antimicrobial potentials of *Phyllanthusamarus* against multiple antibiotic resistant bacteria. Int. J. App. Res. Nat. Prod., 2010;3 (3):6–12.
3. Alamgir, A.N.M., 2017. Medicinal, non-medicinal, biopesticides, color-and dye- yielding plants; secondary metabolites and drug principles; significance of medicinal plants; use of medicinal plants in the systems of traditional and complementary and alternative medicines (CAMs). In *Therapeutic use of medicinal plants and their extracts: Volume 1: Pharmacognosy* (pp. 61-104). Cham: Springer International Publishing.
4. Boxi M, Rajesh Y, Kumar VR, Praveen B, Mangamma K. Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of *Commicarpuschinesis* (aqueous leaf extract). Int. J. Pharm. Bios., 2010; 1:537– 47.
5. Brooks SM. Perspective on the human cough reflex. Cough. 2011 Nov 10;7(1):10.
6. Canter PH, Thomas H, Ernst E. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. TRENDS in Biotechnology. 2005 Apr 1;23(4):180-5.
7. Husen A, editor. Medicinal Spice and Condiment Crops. CRC Press; 2024 Apr 22.
8. Jadhaio AG, Sanap MJ, Patil PA. Formulation and evaluation of herbal syrup. Asian Journal of Pharmaceutical Research and Development. 2021 Jun 15;9(3):16-22.

9. Jadhav VB, Musale MA, Musale MD. Formulation and evaluation of herbal syrup for treatment of cough and asthma.
10. Joshee N, Dhekney SA, Parajuli P. Medicinal plants. Switzerland: Springer, Cham. 2019:1-427.
11. Kataria RK, Sharma M, Mittal A, Tyagi V. The Formulation and evaluation of herbal cough syrup. Journal of Applied Pharmaceutical Sciences and Research. 2024;7(4):8-12.
12. Lau E. Preformulation studies. In Separation science and technology 2001 Jan 1 (Vol. 3, pp. 173-233). Academic Press.
13. Manandhar S, Luitel S, Dahal RK. In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. Journal of tropical medicine. 2019;2019(1):1895340.
14. Mittal J, Kaushik K, Pathak D, Chaudhary K., Kumar N. Extraction, isolation, characterization, semisynthesis and biological evaluation of berberine from roots of *Berberis aristata*. Ind. Drug., 2013;50(9):18-24.
15. Nemati E, Rahman MM, Nathan V, Vatanparvar K, Kuang J. A comprehensive approach for classification of the cough type. In 2020 42nd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC) 2020 Jul 20 (pp. 208-212). IEEE.
16. Nerkar AG, Dumbre RK, Badar S. Formulation and evaluation of herbal syrup of Arjuna extract. Curr Trends Pharm Pharm Chem. 2023;5(2):75-8.
17. Obianime AW, Uche FI. The phytochemical screening and effects of methanolic extract of *Phyllanthus amarus* leaf on the biochemical parameters of male guinea pigs. J. App. Sci. Env. Manag., 2008;12(4):73-77.
18. Panda P, Sahu A. Formulation and evaluation of herbal cough syrup. Asian Journal of Pharmaceutical Research and Development. 2023 Apr 14;11(2):28-33.
19. Panda P, Sahu A. Formulation and evaluation of herbal cough syrup. Asian Journal of Pharmaceutical Research and Development. 2023 Apr 14;11(2):28-33.
20. Pardhi A, Patil R, Mahajan MS, Chopde MS, Bramhane MS. Formulation and evaluation of herbal cough syrup. Int. J Med. Pharm. Res. 2025;6(1):282-92.
21. Pastorino G, Comara L, Soares S, Rodrigues F, Oliveira MB. Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review. Phytotherapy research. 2018 Dec;32(12):2323-39.
22. Patil JK, Mali DR, More KR, Jain SM. Formulation and evaluation of herbal syrup. World journal of pharmaceutical research. 2019 Mar 11;8(6):604-13.
23. Pushpangadan P, George V. Basil. In Handbook of herbs and spices 2012 Jan 1 (pp. 55-72). Woodhead publishing.
24. Rohokale MT, Gavande MK, Khedkar AN, Ghadge MM. Review on Formulation and Evaluation of Herbal Cough Syrup.
25. Sachan NK, Singh D. Evaluation of dried pulp from *Carica papaya* fruits as
26. Disintegrating Agents in the formulation of metformin hydrochloride dispersible tablets. J. Assa. Sci. Soci., 2006;46:20-22. Sawant S, Gaikwad T. Formulation and Evaluation of Ginger Herbal Cough Syrup. International Journal of Scientific Research and Technology. 2025 Nov 4.
27. Sen T, Samanta SK. Medicinal plants, human health and biodiversity: a broad review. Biotechnological applications of biodiversity. 2014 Jul 8:59-110.
28. Seydi F, Emadi F, Iranzadasl M, Hashem-Dabaghian F, Salehi M, Gholami- Fesharaki M. Types of Cough and Therapeutic Recommendations from the Perspective of Persian Medicine. Complementary Medicine Journal. 2023 Aug 10;13(2):61-72.
29. Shahid-Ud-Duaula AFM and Anwarul BM. Phytochemical screening, plant growth inhibition, and antimicrobial activity studies of *Xylocarpus granatum* Malaysian. J. Pharm. Sci., 2009;7(1):9-21.
30. Sharma B, Bagchi A, Bhutia S, Sarkar BR, Pal P. Formulation and evaluation of expectorant activity of poly herbal cough syrup from traditional medicinal plant extracts of north East India. Research Journal of Pharmacy and Technology. 2022;15(3):949-53.
31. Shroff E. MAGIC SPICES: Ayurvedic Medicine and the Heart.
32. Srivastava AK. Significance of medicinal plants in human life. In Synthesis of medicinal agents from plants 2018 Jan 1 (pp. 1-24). Elsevier.
33. Stahl-Biskup E, Venskutonis RP. Thyme. In Handbook of herbs and spices 2012 Jan 1 (pp. 499-525). Woodhead Publishing.
34. Tiwari B, Mashale SR, Korke AG, Kulkarni SG. Formulation and evaluation of herbal cough syrup.
35. Turke V, Kothe M, Chincholkar P, Anap P, Darunde D. Formulation and evaluation of herbal cough syrup using jaggery base
36. Van Wyk BE, Wink M. Medicinal plants of the world. Cabi; 2018 Oct 31.

-
37. Wang K, Zeng R. Cough and expectoration. In Handbook of Clinical Diagnostics 2019 Aug 27 (pp. 27-29). Singapore: Springer Singapore.
 38. White B. Ginger: an overview. American family physician. 2007 Jun 1;75(11):1689-91.
 39. Zagade KA, Ingole RD. Formulation and evaluation of polyherbal cough syrup. International Journal of Research in Pharmacy and Allied Science. 2025 May 31;4(5):76-91