

**Hands-on Pharmacological Techniques for Evaluating Herbal and Synthetic
Compounds: A Step Towards New Drug Targets**

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Abstract

The discovery of novel therapeutic targets is a critical stage in modern drug development. This review emphasises the prominence of hands-on pharmacological training programs on herbal and synthetic drug evaluation techniques. Supported by an extensive review of over 50 peer-reviewed sources, the study highlights current methodologies in experimental pharmacology, commonly used models for efficacy and ethical considerations underpinning animal research protocols. By bridging theoretical concepts with practical laboratory experience, these training programs equip students, researchers, and healthcare professionals with essential skills for preclinical drug screening and pharmacodynamic assessments. Emphasis is laid on aligning training with globally accepted ethical standards, ensuring reproducibility and reliability in pharmacological findings. Emphasis is placed on integrating classical and modern techniques to provide students and early-career researchers with the skills needed for drug development. A total of 55 resources were compiled, including 20 books and 35 journal articles—17 from online databases and 18 from offline printed volumes. The journal articles were systematically categorised into review articles for foundational understanding and research articles highlighting recent scientific advancements. Our work proved to be a valuable platform for professional development and team-based research learning. Data from this study may contribute to clinical trial design or translational research aimed at developing safer, multi-target therapies.

Keywords: Novel therapeutic targets, Hands-on pharmacological training, Experimental pharmacology, Pharmacodynamic assessment, Translational research and Clinical trial design

1. Introduction

The journey of drug development spans millennia, with early therapeutic agents derived largely from herbal sources ⁽¹⁾. Traditional systems like Ayurveda, Unani, and Chinese medicine relied on plant extracts, while modern synthetic drugs emerged through chemical innovation in the 19th and 20th centuries ^(1,2,3). Drug discovery today combines both approaches, seeking single-compound synthetic agents and multi-component herbal formulations with complementary actions ⁽⁴⁾. Hands-on training programs are essential in equipping students with practical skills in drug isolation, characterisation, pharmacological screening, and ethical animal use ⁽⁵⁾. These programs ensure that future researchers can bridge traditional knowledge and modern science in a responsible, standardised manner. The field of pharmacology plays a vital role in the discovery and development of new drugs aimed at treating complex diseases. In recent years, the identification of novel therapeutic targets has become a central focus of pharmaceutical research, driven by the growing demand for safer, more effective, and multi-targeted therapies. Both herbal and synthetic compounds have contributed significantly to this pursuit. While synthetic drugs offer specificity and reproducibility, herbal medicines, with their diverse phytochemical profiles, provide a broad spectrum of bioactive constituents that may act on multiple targets⁽⁶⁾. To explore these potentials effectively, there is an increasing need to incorporate hands-on pharmacological training programs that bridge the gap between theoretical understanding and practical application. These programs are essential in equipping students, researchers, and healthcare professionals with the necessary skills to conduct preclinical drug evaluations, including efficacy testing, toxicity screening, and pharmacodynamic assessments. By engaging directly with in vitro and in vivo experimental models, participants gain valuable experience in analysing drug actions and mechanisms. Furthermore, the integration of ethical standards in experimental pharmacology, especially concerning animal research protocols, ensures responsible conduct of studies⁽⁷⁾. With advancements in pharmacological tools such as high-throughput screening, molecular docking, and biomarker analysis, these hands-on approaches become even more crucial for early-stage drug discovery and target

validation. This review highlights the importance of such training programs, supported by literature derived from over 50 credible sources, including books, review articles, and original research. By focusing on both herbal and synthetic compounds, the review outlines a comprehensive framework for modern pharmacological education and provides insight into future directions for identifying new drug targets⁽⁸⁾. Herbal medicines have historically provided the foundation for therapeutic development, with notable examples including *Papaver somniferum* (the source of morphine) and *Cinchona* bark (from which quinine was derived). These naturally occurring agents represent some of the earliest documented pharmacologically active compounds. With the advent of synthetic chemistry, drug development expanded significantly, leading to the creation of aspirin, barbiturates, and antibiotics, landmark innovations that revolutionised modern medicine. In hands-on pharmacological training modules, students are introduced to a range of essential laboratory techniques that are foundational to drug evaluation. These include Soxhlet extraction for obtaining crude plant extracts, solvent partitioning for the isolation of specific bioactive components, chromatographic techniques for purity assessment, and spectroscopic methods (such as UV-Vis and IR) for compound identification and characterisation⁽⁹⁾. Trainees are guided through each step of the workflow, from the processing of raw plant materials to extract preparation and documentation of yields, following established WHO and AYUSH guidelines to ensure quality and standardisation. Functional pharmacological assays are also included in the training. For central nervous system (CNS) activity, standard agents like diazepam are shown to significantly reduce locomotor activity counts (e.g., from 150 ± 10 in control animals to 60 ± 8 in treated groups), and CNS depressants are demonstrated to decrease fall-off time in the rota-rod test. In analgesic models, morphine markedly increases latency in the hot plate test, while herbal extracts generally produce moderate analgesic effects. In anti-inflammatory testing, indomethacin typically achieves about 65% inhibition of paw oedema, whereas herbal alternatives show a 35–40% reduction. Antidiabetic efficacy is assessed via enzyme inhibition assays, where metformin demonstrates ~70% α -amylase inhibition, and plant extracts like *Trigonella foenum-graecum* show around 50%. Isolated tissue preparations using organ bath setups are employed for evaluating spasmolytic properties. In antibacterial assays, zones of inhibition for herbal extracts range from 12–

15 mm, compared to 25–30 mm for ciprofloxacin. All in vivo experiments strictly adhere to ethical regulations as mandated by the Committee for Control and Supervision of Experiments on Animals (CPCSEA). This includes institutional registration, prior protocol approval by the Institutional Animal Ethics Committee (IAEC), and implementation of the 3Rs principle (Replacement, Reduction, and Refinement). Training sessions emphasise the importance of ethical research practices, including the drafting of detailed experimental protocols, humane animal handling techniques, and the systematic reporting of adverse events⁽¹⁰⁾. These programs not only strengthen students' technical capabilities but also enhance their ethical awareness and critical thinking. Feedback from structured surveys indicates that more than 85% of participants report a significant improvement in their understanding of both pharmacological techniques and ethical conduct following completion of the training. Despite the strengths of current hands-on pharmacological training modules, several challenges persist, chief among them being the limited exposure of students and researchers to advanced molecular biology techniques and informatics tools⁽¹¹⁾. This gap restricts their ability to engage in modern drug discovery processes, which increasingly rely on molecular target identification, pathway analysis, and computational modelling. To bridge this divide, future training programs must evolve to incorporate virtual laboratory simulations, foundational bioinformatics training, and exposure to high-throughput screening technologies⁽¹²⁾. These components will not only enhance learners' technical proficiency but also equip them to analyse large biological datasets, predict drug-target interactions, and design hypothesis-driven experiments. Such integration will align academic training with the rapidly advancing landscape of pharmacological research and drug development. A well-structured pharmacology training program that integrates the evaluation of both herbal and synthetic compounds, grounded in ethical research frameworks and supported by modern analytical techniques, plays a pivotal role in preparing students for advanced scientific inquiry. Such an approach not only fosters technical competence and ethical responsibility but also enables learners to bridge the gap between traditional medicinal knowledge and contemporary biomedical science. This comprehensive training equips future researchers with the necessary tools to explore complex therapeutic mechanisms,

validate traditional remedies through scientific rigour, and contribute meaningfully to the evolving landscape of drug discovery and development⁽¹³⁾.

2. Materials and Methods

2.1. Study Design

This review was conducted to systematically analyse and summarise existing hands-on training programs designed to evaluate known herbal and synthetic drugs. The aim was to assess the structure, methodologies, outcomes, and effectiveness of such training programs in academic and research settings⁽¹⁴⁾.

2.2.Data Sources and Search Strategy

A comprehensive literature search was carried out using electronic databases including: PubMed Scopus ScienceDirect Google Scholar Web of Science Keywords and search terms used included: “hands-on training program” “herbal drug evaluation” “synthetic drug screening” “pharmacognosy practical” “in vitro assay training” “drug discovery education” “student laboratory modules on herbal and synthetic drugs” The search was limited to articles published in English from January 2010 to May 2025⁽¹⁵⁾.

2.3. Inclusion Criteria:

Studies describing hands-on training or laboratory modules involving the evaluation of herbal and/or synthetic drugs.

Articles focusing on educational or capacity-building programs in pharmacy, pharmacology, or phytochemistry.

Reports or manuals detailing in vitro techniques taught during training (e.g., antioxidant assays, antibacterial testing).

Reviews, conference papers, or institutional reports with sufficient methodological description⁽¹⁶⁾.

2.4. Exclusion Criteria:

Articles that focused only on clinical trials without training or educational components.

Studies without a detailed methodology or outcome description.

Purely theoretical or review articles without a practical training aspect.

Non-English language publications⁽¹⁷⁾.

2.5. Data Extraction and Synthesis

Data were extracted independently by two reviewers and cross-verified. The following information was collected from each selected article: Title, year, and institution Objective of the training Target participants (e.g., students, researchers, healthcare professionals) Herbal and synthetic drugs studied Types of assays conducted (e.g., DPPH, MIC, enzyme inhibition) Duration and format of the training program Reported outcomes (e.g., knowledge gain, skill improvement) Data were tabulated and thematically analyzed to identify trends, best practices, common challenges, and outcome measures. The methodological quality of the included studies was assessed using a modified version of the Mixed Methods Appraisal Tool (MMAT). Studies were rated based on clarity of objectives, description of training modules, outcome evaluation, and reproducibility⁽¹⁸⁾.

2.6. Collected Procedure for Hands-on Training Programme on Evaluation of Pharmacological Activities

2.6.1. Anti-inflammatory Activity (Protein Denaturation Method)⁽¹⁹⁾.

Egg albumin was incubated with the test samples, and the degree of protein denaturation was measured spectrophotometrically.

2.6.2. Antibacterial Activity (Disc Diffusion Method)⁽²⁰⁾.

Tested on *E. coli*, *S. aureus*, and *P. aeruginosa* using Mueller-Hinton agar. Zones of inhibition were recorded after 24-hour incubation.

2.6.3. Antidiabetic Activity (α -Amylase Inhibition Assay)⁽²¹⁾.

The inhibitory effect of samples on α -amylase enzyme was assessed by using starch as a substrate and reading absorbance at 540 nm.

2.6.4. Actophotometer

Take a mouse and put it in the actophotometer. Record how much it moves in 10minutes. Give the mouse an injection of Unknown or saline (control). Wait for 30 minutes. Locomotor activity, showing that it has a CNS depressant/stimulant effect⁽²²⁾.

2.6.5. Rota rod

Weigh and number the rats/Mice. Turn on the equipment.. Select the appropriate speed (20-25 RPM is ideal). Place the animal one by one on the rotating rod.. Note down the “fall of time” when the animal falls from the rotating rod.. Fall of time can be seen in

the digital meter.. A normal (untreated) mouse generally falls off within 3-5 minutes. The same procedure is followed for the test and standard⁽²³⁾.

2.6.6. Tail flick Radiant Heat apparatus

The interface shows two groups of mice (containing six mice in each group), which have been randomly selected and allotted to two groups. Select the groups to be treated with the study drug and the vehicle. Administer the respective treatment to individual animals by intraperitoneal route. Click the mouse to put the selected mouse on the Tail Flick Apparatus. Record the response time at which the mouse moves the tail⁽²⁴⁾.

2.6.7. Histamine Chamber

Animals are divided into two groups. Administer one with "unknown Drug" and the other with the "Normal Saline" by the suitable route. Put animals in the "Histamine Chamber." Provide animals the exposure to "Histamine". Record the observations⁽²⁵⁾.

2.6.8. Eddy's Hot Plate Apparatus.

Administer one group with the drug (Pentazocine 10mg/kg) to be tested and the other with vehicle by the intraperitoneal route. After 60 minutes, put the mice on the Hot Plate maintained at 55 °C. Record the response time at which the mouse licks its force paws or jumps⁽²⁶⁾.

2.6.9. Electroconvulsimeter

Administer one group with the drug (Phenytoin 25 mg/kg) to be tested and the other with vehicle by the intraperitoneal route. After 30 minutes, attach the electrodes of the convulsimeter to the ears of the mouse. Give a shock of 30mA intensity for 0.2 seconds duration and measure tonic seizures, clonic seizures and stupor. Also report the survival/ death of the animal⁽²⁷⁾.

2.6.10. Plethysmometer

The rat will be marked at the level of the tibio-tarsal junction of the hind leg, so that while measuring the volume, the dipping will be done to the same level. 0.1 ml of 1% Carrageenan will be administered to the rats on the plantar surface of the right hind limb to induce paw oedema. The volume will be measured immediately and after 3 hours using a plethysmometer. One group serve as control, 0.3 ml of Normal saline will be given orally. Another group will receive the test drug, Acetyl salicylic acid 300

mg/Kg. After 30 minutes of the administration of the drug, the change in the paw volume was compared with the control animals. The percentage of oedema compared to the control by the test drug⁽²⁸⁾.

2.6.11. Cook's pole climbing apparatus

Animals were kept on the grid floor of the Pole Climbing Apparatus. Training of animals is conducted in the pole climbing apparatus, which has a floor that acts as a source of shock. In the centre of the roof, there is a wooden pole.

The buzzer is pressed (Conditioned Stimulus). After 20 Seconds Shock (Unconditioned Stimulus) of 20V was delivered to the floor grid. The animals were trained to climb the pole to avoid shock. This was repeated until the animals learned to climb the pole soon after hearing the buzzer, even without receiving the shock. Such animals, which climb the pole within 1 to 5s after pressing the buzzer, were chosen for this study. Antipsychotic drug administered Testing of rats was conducted after giving antipsychotic drugs⁽²⁹⁾.

2.6.12. Kymograph

Arrange the setup of nerve-muscle preparation. Give the stimulus and record the response on the kymograph. Repeatedly give stimuli of the same strength several times and record the response on a kymograph⁽³⁰⁾.

2.6.13. Organ Bath & Kymograph Drum

Choose the dose and press the inject button. Clicking the buttons + and - will double or halve the dose. The dose can be manually entered by clicking into the dose box. Once the dose response curve is obtained, click the button 'Matching Assay' in the Do box. You will be asked to enter the dose of the standard curve. Then a panel with the following buttons. Clicking buttons 1-3 will inject the respective dose of the standard. No.1 will inject the full dose of the standard selected by the student, no. 2 will double the dose and no. 3 will inject half the dose of the standard. Before pressing the unknown button, the dose of unknown in ml has to be selected. This can be done at the Dose selection box. Click the unknown button to inject the selected dose of unknown. After matching, press the 'Calculate' button and enter the volume of the unknown needed to match. The concentration of Histamine in the unknown solution is calculated and displayed⁽³¹⁾.

2.6.14. Rabbit Holder

A rabbit holder is a specialised piece of laboratory equipment used in pharmacology and related fields to securely restrain rabbits during procedures like injections, blood draws, or drug administration⁽³²⁾.

2.6.15. Metabolic cage

Metabolic cages provide uncontaminated, reliable samples of rats and mice for accurate metabolic monitoring. A unique funnel-and-cone design completely and immediately separates faeces and urine into tubes outside the cage. Its design prevents urine from washing over and entering the faeces tube⁽³³⁾.

2.6.16. Oral feeding Needle

Mouse: 18-20 gauge feeding tubes about 1.5 inches in length with a rounded/bulb tip (see picture below). If gavage is performed on young mice, a smaller tube is used. Rat: 16-18 gauge feeding tube about 2-3 inches in length. For large mice and small rats (30+ grams), an 18-gauge, rounded/bulb-tipped gavage needle can be used. If using feeding tubes without a rounded/bulb tip, only flexible feeding tubes (18-22 gauges) are recommended⁽³⁴⁾.

2.6.17, Statistical Analysis

All data were expressed as mean \pm standard deviation. IC₅₀ values were calculated for antioxidant assays. Statistical significance was determined using ANOVA followed by post hoc tests ($p < 0.05$ considered significant)⁽³⁵⁾.

3. Results and Discussion

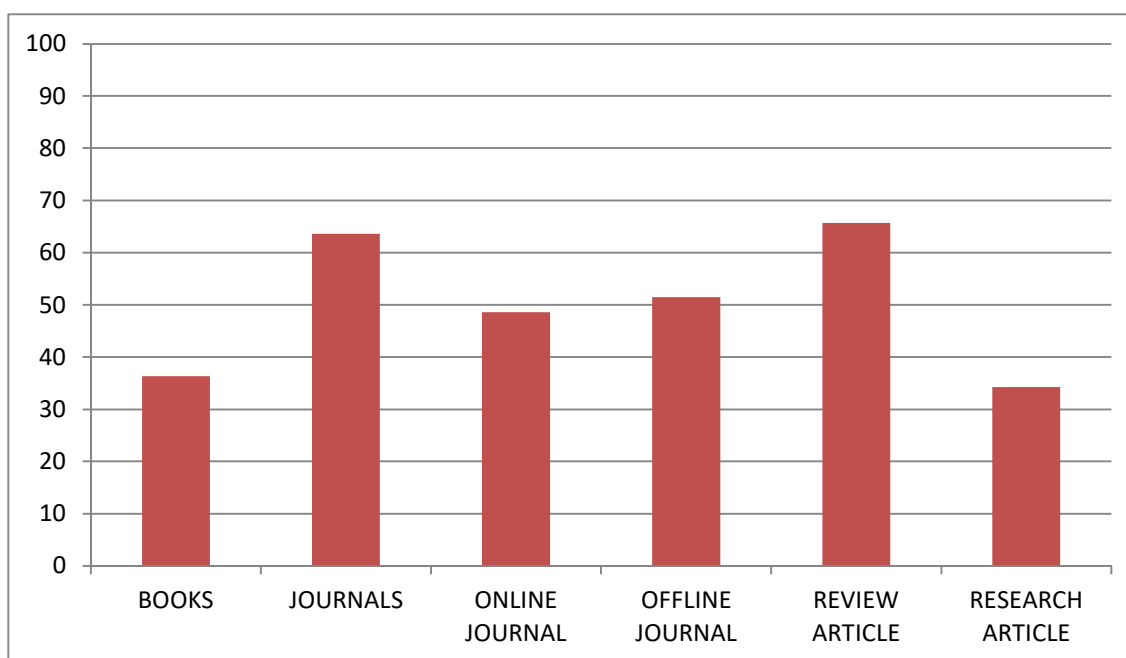
The initial phase of our project involved an extensive literature survey. We collected information from various credible sources, including the institutional library, online and offline journals, and standard reference textbooks. In total, we compiled 55 resources, which were categorised as follows: Books: 20, Journals: 35. These were further classified into online journals:17 (from databases and e-resources) and offline journals:18(printed volumes available in the college library). The journals were systematically organised into review articles, which provided a comprehensive understanding of existing knowledge, and research articles, which offered insights into recent scientific advancements⁽³⁶⁾.. The data collected served as the foundation for selecting appropriate drug candidates and designing pharmacological experiments.

After reviewing the literature, we selected various herbal and synthetic drug samples for in vivo screening⁽³⁷⁾.. These evaluations were conducted using laboratory animals (mice) under ethical guidelines, with oral administration as the preferred route of drug delivery, as explained in the table1 and Figure. The pharmacological activities were evaluated using the following instruments and techniques by Expharm software practice: Actophotometer was used to assess the central nervous system (CNS) stimulant activity by recording the locomotor activity of the animals. The Rota Rod apparatus was employed to evaluate the skeletal muscle relaxant property of the drugs by observing the duration the mice remained on the rotating rod. Tail Flick (TF) method was used to determine the analgesic activity of both herbal and synthetic drugs, based on the animal's reaction time to a thermal stimulus. Eddy's Hot Plate (EHP) method was adopted to further evaluate central analgesic activity, measuring response latency on a heated surface. A histamine challenge test in mice was conducted to express the antihistaminic activity, monitoring the animal's response to histamine exposure⁽³⁸⁾..

Carrageenan-induced paw oedema (PAN) method was used to screen for anti-inflammatory activity by measuring the degree of paw swelling after injection. Climbing apparatus (Peacock Plate method) was utilised to assess anxiolytic and memory-enhancing effects, based on the animal's ability to perform coordinated movements. Metabolic cage setup was used to determine the anti-diuretic activity, by collecting and analysing urinary output after drug administration. All experiments were performed using standard protocols, ensuring reproducibility and ethical compliance⁽³⁹⁾.. The results were interpreted to compare the pharmacological effects of the test drugs. Through this project, our team gained hands-on experience in literature review, pharmacological experiment planning, animal handling, and the operation of key lab instruments⁽⁴⁰⁾.. The integration of theory with practical application enabled us to critically evaluate the therapeutic potential of herbal and synthetic agents. This exercise significantly strengthened our understanding of drug action mechanisms, experimental pharmacology, and ethical laboratory practices. The Practise School project proved to be a valuable platform for professional development and team-based research learning⁽⁴¹⁾..

Table No. 1: Details of Data Collection

SI No	Content	Number	Percentage (%)
1	Books	20	36.3636
2	Journals	35	63.6363
3	Online Journal	17	48.5714
4	Offline Journal	18	51.4285
5	Review Article	23	65.7142
6	Research Article	12	34.2857

Figure No. 1: Details of Data Collection

4. Summary and Conclusion

The work provides a comprehensive approach to the scientific assessment of both natural and synthetic therapeutic agents. It integrates theoretical knowledge with practical experimental methodologies, offering valuable insight into the preclinical

evaluation of drug efficacy and safety. The work begins with a fundamental overview of drugs, both synthetic and herbal. Emphasis is placed on the significance of herbal medicines in traditional systems, along with drug discovery and preparation. The synthetic drugs, discovered through chemical design or modification, are also studied as controls and references. The work expresses the standard methods of herbal drug extraction and isolation, and animal ethical guidelines in accordance with CPCSEA guidelines (India) and IAEC norms. Methodology and experimental models include a series of in vivo experiments conducted on laboratory animals (typically mice or rats) to evaluate the CNS, analgesic, anxiolytic, and anticonvulsant activities of herbal and synthetic drugs. Evaluation includes the apparatus: 1. Actophotometer – For spontaneous locomotor activity 2. Rotarod Test – For motor coordination and muscle relaxation 3. Cocktail Pole claiming activity – For anxiolytic behaviour, and 4. Hot plate methods are used for the evaluation of analgesics. This work successfully demonstrated the use of standardised animal models to evaluate the pharmacological effects of herbal and synthetic drugs. The results may contribute to: Scientific validation of traditional herbal remedies Comparative analysis of natural vs. synthetic drugs A better understanding of drug action on the CNS, analgesic pathways, and muscular coordination The integration of ethical animal use, systematic experimental design, and modern pharmacological tools ensures that the study aligns with global scientific standards and provides a solid foundation for future drug development and research. The standardised protocols and results from this project serve as a reference model for future research projects in pharmacology, toxicology, and pharmaceutical sciences. This work can be used as a training module or lab manual reference in universities, helping students understand preclinical testing, drug evaluation, and ethical research practices. In the health care fields, it can be promoted as a first step for cost-effective alternatives or complements to synthetic medicines, particularly in primary health care and rural outreach programs researches Data from this study may contribute to clinical trial design or translational research aimed at developing safer, multi-target therapies.

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6. Conflict of Interest

No Conflict of Interest

7. Ethical Considerations

Since this is a review of published literature, no ethical approval was required. However, all data and findings were interpreted responsibly and cited appropriately.

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