

### Unit 5: Biological Screening of Herbal Drugs

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#### 5.1 Introduction and need for phytopharmacological screening

Biological screening is the systematic testing of plant materials in living or derived biological systems to discover whether they do anything, and if so, what. It is needed because of the arithmetic of the herbal world: there are far more plants, extracts and traditional claims than can ever be isolated, trialled or developed, so a rational order of testing must spend cheap, fast experiments first to decide where expensive, slow ones are deserved. Screening is that ordering system. It serves four purposes in pharmacognosy. It tests traditional claims at the first level, giving ethnopharmacology, Unit 3, its experimental verdicts. It guides chemistry, since bioassay guided fractionation follows activity through separation until the responsible constituents stand alone. It provides the first safety signals, because a screen that finds strong unwanted activity, above all cytotoxicity without selectivity, stops a line of work before animals or patients are exposed. And it standardises research materials biologically where chemistry is not yet able, an extract being describable by what it does as well as by what it contains. The rest of the unit teaches the standard instruments: in vitro techniques, which test effects in cells, microbes and chemical systems, and in vivo techniques, which test them in whole animals, ending with the toxicity studies by which safety is formally established.

#### 5.2 New strategies for evaluating natural products

The classical strategy, grind, extract, test one extract in one model, isolate if active, wasted most of its effort on rediscovery and on activities that never survived contact with a whole organism. The newer strategies named in the syllabus correct those weaknesses and should be known as a set. **Bioassay guided fractionation** remains the backbone, now accelerated: activity is tracked at every separation step so effort concentrates only on active fractions. **High throughput screening** tests extract libraries, of the kind bioprospecting builds, Section 3.4, against molecular and cellular targets in automated miniature formats, converting screening from a craft into a statistics problem. **Dereplication** identifies known compounds in active extracts at the start, by liquid chromatography with mass spectrometry and database matching, so that known chemistry is recognised before it is reisolated at full cost. **Metabolomic and fingerprint based approaches** compare the whole chemical profile of many samples with their measured activities and let statistics nominate the peaks responsible, reversing the classical order by proposing candidates from data rather than from fractions. **In silico screening** tests constituents computationally against target structures before any bench work. Running through all of them is the strategic discipline of the cascade: cheap and predictive before dear and definitive, in vitro before in vivo, general screens before specific models, with the ethical and cost logic made explicit at the end of this unit.

## The screening cascade



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Figure: The screening cascade. Cheap and predictive tests come first, dereplication removes the known, fractionation follows activity, and in vivo models with OECD toxicity studies decide what may proceed.

The cascade in working order runs as follows.

```
Extract library or traditional preparation
|
Primary in vitro screens: antioxidant, antimicrobial, cytotoxicity
|
Active hits confirmed, dereplication applied
|
Bioassay guided fractionation to active constituents
|
Specific in vivo models for the claimed activity
|
Safety pharmacology and OECD toxicity studies
|
Candidate for validation and clinical study (Unit 1)
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### 5.3 In vitro evaluation techniques for antioxidants

Antioxidant screening is the commonest first screen applied to herbal extracts, and its standard methods are chemical assays with biological meaning rather than biological systems in the strict sense. The **DPPH assay** measures the ability of an extract to quench a stable free radical, followed by the fading of the radical's colour; the **ABTS assay** applies the same logic to a different radical cation; the **FRAP assay** measures reducing power, the conversion of ferric to ferrous iron; and scavenging assays against superoxide, hydroxyl radical and hydrogen peroxide, with lipid peroxidation inhibition in membrane or oil systems, complete the usual panel. Results are expressed in comparison with standard antioxidants and as the concentration producing a stated degree of effect, and extracts are ranked rather than certified. The interpretation discipline is the examinable core: these assays measure electron and hydrogen donation in a test system, they do not prove that an extract protects a tissue in a body, where absorption, metabolism and distribution intervene, and many polyphenol rich extracts score highly in vitro while doing little in vivo. Antioxidant screening is therefore a legitimate first filter and a chemical characterisation, valuable for ranking and for the fingerprint work of Unit 4, and a claim of therapeutic antioxidant action requires the in vivo work of the later sections before it is made.

## 5.4 In vitro evaluation techniques for antimicrobial activity

Antimicrobial screening tests extracts against panels of bacteria and fungi under controlled conditions, and its standard methods descend from the clinical laboratory. **Disc and well diffusion** methods place the extract on or in inoculated agar and measure the zone in which growth is inhibited: fast, cheap and suitable for first screening, though diffusion through agar biases the result against large and poorly soluble molecules. **Dilution methods**, broth microdilution above all, determine the minimum inhibitory concentration, the lowest concentration preventing visible growth, and from it the minimum bactericidal or fungicidal concentration, converting the screen from a zone into a number that can be compared across extracts and against standard antibiotics run in parallel. Test panels are chosen to represent Gram positive and Gram negative bacteria and pathogenic fungi, with standard strains from culture collections so that results are reproducible between laboratories. The cautions parallel Section 5.3 and are regularly examined: plant extracts carry colour, precipitate in media, and contain tannins that inhibit microbes nonspecifically, so controls for solvent, colour and tannin effects are part of the method, and an in vitro minimum inhibitory concentration is a screening result, not a promise of clinical usefulness, which must survive the pharmacokinetic realities of Unit 1.

## 5.5 In vitro evaluation techniques for anticancer activity

Anticancer screening in vitro is cytotoxicity screening conducted with discipline: the question is not merely whether an extract kills cultured cells, which strong detergents also do, but whether it kills cancer cells preferentially, at concentrations that spare normal cells, through a mechanism worth pursuing. The standard viability assays measure living cell mass or metabolism after exposure: the **MTT assay**, in which living cells convert a tetrazolium dye to a coloured product; the **sulforhodamine B assay**, which stains cellular protein and is the assay of the well known national screening programmes; and dye exclusion and clonogenic methods in supporting roles. Panels of human cancer cell lines, representing different tissues of origin, are screened first, and an extract or compound earns progression by potency against the panel together with a selectivity margin against normal cells, the single most important interpretive rule of the section. Positive screening results then direct the fractionation of Section 5.2, and historically this screen has earned its keep: the vinca alkaloids and taxol reached medicine through plant screening programmes of exactly this architecture, the standing demonstration that a disciplined in vitro sieve, followed honestly, finds real drugs. Results are still screening results: potency in a dish must be rewon in the animal models of Section 5.7 before the word anticancer is used of a product.

## 5.6 In vivo evaluation techniques: anti-inflammatory and antiulcer

In vivo evaluation moves the question into the whole animal, where absorption, metabolism, distribution and integrated physiology decide whether the in vitro promise was real. The anti-inflammatory models are the most taught in pharmacognosy and should be known with their logic. **Carrageenan induced paw oedema** in the rat, in which an irritant injection produces a measurable, time phased swelling that test extracts are given against, is the standard acute inflammation screen, sensitive to the prostaglandin mediated phase of inflammation and therefore to the cyclooxygenase type activity of many plant constituents. The **cotton pellet granuloma** model, in which implanted pellets acquire a proliferative tissue mass over days, represents the chronic, proliferative side of inflammation that the acute model cannot reach. Antiulcer evaluation uses models that ulcerate by different mechanisms so that the mode of protection can be read from the pattern: **pylorus ligation** collects acid and pepsin against the mucosa and measures secretion and damage together; **ethanol induced** ulceration attacks the mucosal barrier directly and tests cytoprotection; and **NSAID induced** models imitate the commonest clinical cause. Endpoints are ulcer number and severity scored as an ulcer index, with gastric secretion parameters in the ligation model. The teaching point general to this unit applies here first: an in vivo model is an argument, not a verdict, and a

herbal claim is strengthened when activity appears in mechanistically distinct models and weakens when it appears in only one.

### 5.7 In vivo evaluation techniques: anticancer and wound healing

Anticancer evaluation in vivo historically rests on transplantable tumour models in rodents, of which the ascites tumours are the pharmacognosy standards: **Ehrlich ascites carcinoma** and **Dalton's lymphoma ascites**, grown in the peritoneal cavity of mice, where a test extract is judged by increase in life span of treated animals, reduction in tumour cell count and volume, and restoration of haematological parameters disturbed by the tumour. Solid tumour versions of the same lines, and sarcoma models, test growth of a measurable mass rather than survival in ascites. The models are screens with known limits: they reward cytotoxicity reachable in the peritoneum and miss much human cancer biology, which is why Section 5.5's selectivity rule and honest interpretation govern their use. Wound healing evaluation divides by wound type, each model measuring a different repair property. The **excision wound** model, a standard area of skin removed, is followed by wound contraction and time to complete epithelialisation. The **incision wound** model measures the tensile, breaking strength of the healed wound, the mechanical truth of repair. The **dead space wound** model grows granulation tissue around implanted material, allowing its weight and its hydroxyproline content, the chemical index of collagen, to be measured. An extract claiming wound healing must show itself across these complementary measures, and the models connect directly to the traditional wound applications of drugs such as turmeric profiled in Unit 4.

### 5.8 In vivo evaluation techniques: antidiabetic and hepatoprotective

Antidiabetic screening is built on chemically induced diabetes in rodents: **alloxan** and **streptozotocin** each destroy pancreatic beta cells by related chemistry and produce a stable hyperglycaemia against which extracts are tested, in acute experiments for immediate glucose lowering and in repeated dosing studies for sustained control, with oral glucose tolerance tests in normal and diabetic animals distinguishing effects on absorption and tolerance from effects on established diabetes. Body weight, food and water intake and, in longer work, lipid and complication markers accompany the glucose readings. Interpretation carries the unit's usual discipline: many extracts lower glucose modestly in these models, and the claim matures only with dose response, comparison against a standard drug, and safety over the treatment period.

Hepatoprotective evaluation induces a defined liver injury and measures its prevention or reversal: **carbon tetrachloride** produces a radical mediated centrilobular injury, **paracetamol** overdose produces the clinically familiar hepatocellular damage, and other agents model cholestatic and alcoholic patterns. Endpoints are the serum enzymes of hepatocellular injury, bilirubin, and histopathology of the liver as the confirming evidence, with silymarin, itself a plant product from milk thistle, standing as the conventional standard of comparison in these models, a neat closing of the circle in which a validated herbal becomes the yardstick for screening the next.

### 5.9 In vivo evaluation techniques: cardioprotective, diuretic and antifertility

The remaining in vivo techniques of the syllabus are grouped here as they are grouped in teaching.

**Cardioprotective** evaluation centres on the **isoproterenol induced myocardial injury** model in rats, in which high doses of the catecholamine produce a reproducible necrosis resembling infarction; protection is read from cardiac marker enzymes in serum, lipid and antioxidant status, electrocardiographic changes and histology of the heart muscle. **Diuretic** evaluation follows the **Lipschitz test** design: rats are given a standard fluid load, urine is collected over a defined period in metabolic cages, and the volume and the sodium, potassium and chloride content of the urine of extract treated animals are compared with control and with a standard diuretic, distinguishing mere water excretion from the saluretic pattern of useful diuretics. **Antifertility** evaluation is a family of screens rather than one model: in females, anti implantation

activity is tested by dosing in early pregnancy and counting implantation sites, and oestrogenic and anti oestrogenic activity by uterotrophic assays in immature or ovariectomised animals; in males, effects on sperm count, motility and reproductive organ weights are measured. These screens carry an obvious ethical and interpretive weight beyond their technique, since an antifertility finding in a crude drug taken for other purposes is a safety signal, while the same finding in a plant traditionally used for fertility control is a validation lead: the model is identical, and the reading of it depends on the claim under test, which is the last and most general lesson of the screening unit.

## 5.10 Toxicity studies as per OECD guidelines

Screening finds activity; formal toxicity studies establish the safety frame within which activity may be used, and for herbal drugs entering regulated development those studies follow the test guidelines of the Organisation for Economic Co-operation and Development, the OECD, whose protocols are the international common language of toxicology, run under good laboratory practice. The architecture should be known by tier. **Acute toxicity** studies estimate the effect of a single exposure: the acute oral toxicity guidelines, of which the acute toxic class method, Test Guideline 423, and the up and down procedure, Test Guideline 425, are the modern animal sparing forms, classify a substance by stepwise dosing in few animals with observation for mortality and signs, replacing the classical lethal dose determination in large groups. **Repeated dose studies** imitate real exposure: the 28 day oral study, Test Guideline 407, and the 90 day study, Test Guideline 408, dose animals daily and examine clinical signs, body weight, food intake, blood and clinical chemistry, organ weights and histopathology, together identifying target organs and the no observed adverse effect level from which safe human exposure is reasoned. Supporting families complete the frame: skin and eye irritation and sensitisation studies for topical products, genotoxicity testing, of which the bacterial reverse mutation, Ames, test, Test Guideline 471, is the first screen, and reproductive and developmental studies where exposure of the population requires them.

| Study purpose              | OECD guideline family | What it establishes                          |
|----------------------------|-----------------------|----------------------------------------------|
| Acute oral toxicity        | TG 423, TG 425        | Hazard class from a single dose, few animals |
| Repeated dose, 28 days     | TG 407                | Target organs, first no effect level         |
| Repeated dose, 90 days     | TG 408                | Subchronic safety for continued exposure     |
| Genotoxicity, first screen | TG 471 (Ames)         | Gene mutation potential in bacteria          |

Two closing connections give the section its place in the subject. First, these studies are where Unit 1's toxicity discussion becomes numbers and organs: the pyrrolizidine type liver injury or the aristolochic acid kidney injury, found in a guideline study, is a development stopping fact, not a traditional footnote. Second, herbal status earns no exemption and asks no special pleading: a traditional record may justify the order and emphasis of testing, and may support traditional use registration without the full programme, Section 1.5, but a product seeking full drug claims submits to the same OECD frame as any synthetic candidate. That equality of standard, reached here at the end, is the thesis of Advanced Pharmacognosy-II: the herbal drug is honoured not by lowering the tests but by passing them.

## Frequently Asked Questions

**Q: What is MPG202T in M.Pharm Pharmacognosy?** MPG202T is Advanced Pharmacognosy-II, the second theory subject of M.Pharm Pharmacognosy Semester 2 under the PCI syllabus. It carries 4 credits and 60 hours, taught at 4 hours per week for 100 marks, in five units of 12 hours each: herbal remedies, toxicity and regulations; adulteration and deterioration; ethnobotany and ethnopharmacology; analytical profiles of six named herbal drugs; and biological screening of herbal drugs.

**Q: How do herbal drugs differ from conventional drugs?** A conventional drug is normally a single defined chemical entity, dosed exactly and assayed directly. A herbal drug is a plant part or extract containing many constituents in proportions set by genetics, season and processing, standardised by marker content and fingerprint rather than by assay of one active. The questions asked of both are the same, what it is, how strong it is, whether it works and whether it is safe, but the methods are adapted to a mixture.

**Q: What are the main types of adulteration of herbal drugs?** The standard types are substitution with inferior commercial varieties, substitution with superficially similar drugs, artificially manufactured substitutes, exhausted drugs from which the active has been removed, vegetative parts of the same plant added as bulk, dilution of powders, and addition of toxic materials. Unintentional substitution through mistaken identity in collection produces the same defects without fraud.

**Q: What is DNA fingerprinting used for in pharmacognosy?** DNA fingerprinting identifies a drug at the level of its genome, using techniques such as RAPD, AFLP, ISSR and SCAR markers, together with DNA barcoding of standard loci such as *rbcL*, *matK* and *ITS2*. It works on powders and fragments where morphology and microscopy fail. It proves species identity only; it cannot measure strength or detect chemical contamination, so it stands beside chemical fingerprinting and assay, not instead of them.

**Q: Which contaminants are tested in herbal drugs and how?** The contaminant panel covers heavy metals such as lead, cadmium, arsenic and mercury, measured by atomic absorption and inductively coupled plasma spectrometry after digestion; pesticide residues, measured by gas and liquid chromatography with mass spectrometry after extraction and clean up; phytotoxins such as pyrrolizidine alkaloids and aristolochic acids, measured chromatographically; and microbial contamination, assessed by microbial limit testing with specified pathogen tests and mycotoxin analysis.

**Q: What is reverse pharmacology?** Reverse pharmacology is a drug discovery path that starts from documented clinical and traditional use and works backward into the laboratory, through experiential, exploratory and experimental stages, to find mechanism, active fractions and constituents. It reverses the conventional order, in which a molecule is developed in the laboratory first and tested in patients last. It suits herbal formulations because their human use record exists before their laboratory record does.

**Q: Which markers are used for the six profiled drugs in Unit 4?** *Andrographis paniculata* is standardised by andrographolide; *Boswellia serrata* by the boswellic acids, including KBA and AKBA; *Coleus forskohlii* by forskolin; *Curcuma longa* by the curcuminoids, chiefly curcumin; *Emblica officinalis* by its tannin markers emblicanin A and B with gallic and ellagic acids; and *Psoralea corylifolia* by psoralen, with bakuchiol as a supporting constituent.

**Q: What is the difference between in vitro and in vivo screening?** In vitro screening tests effects in chemical systems, microbes, enzymes and cultured cells: antioxidant, antimicrobial and cytotoxicity assays are the syllabus examples. In vivo screening tests effects in whole animals, where absorption, metabolism and integrated physiology operate: the syllabus models cover anti-inflammatory, antiulcer, anticancer, wound healing, antidiabetic, hepatoprotective, cardioprotective, diuretic and antifertility activities. In vitro results rank candidates; in vivo results test whether the promise survives the body.

**Q: Which OECD guidelines are used for toxicity studies of herbal drugs?** Acute oral toxicity is classified by the animal sparing stepwise guidelines TG 423 and TG 425. Repeated dose toxicity is studied over 28 days under TG 407 and 90 days under TG 408, identifying target organs and the no observed adverse effect level. Genotoxicity screening begins with the Ames bacterial reverse mutation test, TG 471. Herbal products seeking full drug claims follow the same OECD frame as synthetic drugs.

**Q: Are the MPG202T unit wise PDFs free to download on PharmacoLearn?** Yes. PharmacoLearn provides the MPG202T notes as free unit wise PDF downloads, one PDF for each of the five units, with the frequently asked questions appended to the Unit 5 PDF. The same page carries the complete notes for M.Pharm Pharmacognosy Semester 2 students.

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