NUTRACEUTICALS

- ✓ General aspects, Market, growth, scope and types of products available in the market.
- ✓ Health benefits and role of Nutraceuticals in ailments like Diabetes, CVS diseases, Cancer, Irritable bowel syndrome and various Gastro intestinal diseases.
- ✓ Study of following herbs as health food: Alfalfa, Chicory, Ginger, Fenugreek, Garlic, Honey, Amla, Ginseng, Ashwagandha, Spirulina

The definition of nutraceutical is largely based on the alleged Hippocratic principle “let food be thy medicine and medicine be thy food”

The term "nutraceutical" was introduced in 1989 by Dr. Stephen De Felice MD, the founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ.

He defined “nutraceutical” as **“a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease”.**

Hence nutraceutical is any substance that may be considered as a food or part of a food which provides medical or health benefits, encompassing, prevention and treatment of disease.

CLASSIFICATION OF NUTRACEUTICALS

Nutraceutical can be classified based on;

1. Nutraceuticals based on natural source
2. Nutraceutical based on food source
3. Nutraceuticals based their mechanism of action
4. Nutraceuticals based on their chemical nature

1. Based on natural sources: On the basis of natural source, it can be classified as the products obtained from plants, animals, minerals, or microbial sources.

Plants: ascorbic acid, geraniol, allicin, pectin, curcumin

Animals: conjugated linoleic acid (CLA), docosahexaenoic acid (DHA), Calcium, selenium, zinc, choline, sphingolipids.

Microbial: *Lactobacillus acidophilus* (LCI), *Sacromyces cerevisiae* (yeast), *Bifidobacterium bifidum*

2. **Based on food sources it can be categorized as**

Traditional nutraceuticals: the category consists of the food which does not undergo any chemical changes. the components are natural and having potential health benefits. e.g., lycopene from tomato.

Non-traditional nutraceuticals: boosting of nutritional content by addition of nutrients. Eg: beta-carotene enriched rice

Fortified nutraceutical: fortification of food components with micronutrients (essential trace elements and vitamins) to food for enhancing the effectiveness and nutritional value eg: milk fortified with Cholecalciferol, fortified fruit juices.

Recombinant nutraceuticals: it involves application of biotechnology and genetic engineering in the production of energy providing foods, eg: gold kiwi fruit is genetically modified with high level of ascorbic acid, carotenoids etc.

Functional foods: functional foods are source of absolutely necessary nutrients providing more than the quantities for the maintenance, growth and development. It provides health beyond basic nutrition. The class of functional food includes cereals such as rice, corn, wheat, millets, sorghum and buckwheat and useful in many diseases like coronary heart disease, Diabetes, and hypertension.

3. Based on Mechanism of action

Anticancer: curcumin, ellagic acid, capsaicin, lutein

Antioxidants: ascorbic acid, b carotene, polyphenols, lycopene, catechins, tannins, tocopherols.

Anti-inflammatory: linoleic acid, eicosapentaenoic acid (EPA), Curcumin

hypolipidemic: resveratrol, B- glucan, mono unsaturated fatty acids (MUFA), quercetin, polyunsaturated fatty acids (PUFA)

4. Based on chemical nature of the products

- ✓ Enzymes: Prebiotics, probiotics
- ✓ Amino acid-based substances
- ✓ Carbohydrates and derivatives
- ✓ Fatty acids and structural lipids

- ✓ Isoprenoid derivatives
- ✓ phenolic substances,
- ✓ fibres, minerals, enzymes
- ✓ phenols, alkaloids,

Types of Nutraceutical products available in the market

1. Dietary supplements
2. Functional foods
3. Botanicals
4. Medicinal foods

1. DIETARY SUPPLEMENTS

A dietary supplement is a product that contains nutrients derived from food products that are concentrated in liquid or capsule form. It is a product taken by mouth and, contains a dietary ingredient proposed to supplement the diet. These products may include vitamins, minerals, herbs or other botanicals, dietary supplements do not have to be approved by U.S food and drug administration before marketing but include a label that says “these statement have not been evaluated by the food and drug administration” these includes,

- ✓ Probiotics and prebiotics
- ✓ dietary fibre
- ✓ omega 3 fatty acid
- ✓ antioxidants
- ✓ protein supplement
- ✓ vitamins and minerals supplements
- ✓ chyavanaprash

PROBIOTICS

Probiotics are according to the WHO and FAO joint statement-live microorganisms that when administered in adequate amounts confer a health benefit on the host. **A probiotic can be defined as the live microbial food supplement which when administered in adequate amounts beneficially affects the host animal by improving its intestinal microbial balance**. Their action includes adhesion to gastrointestinal tract at specific sites and their survival led to elimination of pathogens.

Probiotics are available in the form of powder form, liquid form, gel, paste or granule form, capsule form etc. Specific probiotics are generally used to treat gastrointestinal conditions such as lactose intolerance, acute diarrhoea and antibiotic associated GI side effects. Probiotic agents possess the properties of non-pathogenic, non-toxic, resistance to gastric acid. E.g. : lactobacillus such as L. acidophilus, bifidobacterium such as *B. bifidum*, *B.longum*.

Main indications are;

- ✓ Diarrhoea (Acute diarrhoea, Travelers diarhoea, Antibiotic-induced diarrhoea)
- ✓ Lactose intolerance
- ✓ IBS and IBD (ulcerative colitis, pouchitis, Crohn's disease)
- ✓ Helicobacter pylori infection
- ✓ Metabolic diseases (diabetes mellitus, dyslipidaemia, obesity)
- ✓ Immunity (allergic conditions), Respiratory tract infections
- ✓ Mental health and neurological conditions

Marketed Products:

Premium Probiotic, Primal Probiotic, Yakult, Bifilac, Depro, Lactibase

PREBIOTICS (fibre)

A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host health.

Non-digestible substance

- ✓ enhances probiotic growth
- ✓ serves as fermentation media for probiotics.
- ✓ The prebiotic consumption generally promotes the lactobacillus and bifidobacterial growth in the gut thus **helping in metabolism**

They are short chain polysaccharides that have unique chemical structure that are not digested by humans.

Eg: fructo - oligosaccharides in chicory roots, banana, tomato

Raffinose and stachylose found in beans and peas

Inulin, lactulose

They are useful in appetite reduction, in glucose intolerance, and in Hypercholesteremia. Beneficial in the prevention of colon cancer, relief from GI-inflammation, and lowering of risk for cardiovascular diseases.

Marketed Products: Prebiotin, Performance lab prebiotic, Dr formulated prebiotic fibre, Pinnaclife Prebiotic

DIETARY FIBRE

Dietary fibre (DF) consists of non-digestible carbohydrates and lignins, that are intrinsic and intact in plants. Dietary fibres present in various parts of plants i.e. leaves, stem, and seeds are not digested by human body. However, their high intake benefits for the human body. The adequate intake for fibre defined by the Dietary Reference Intake (DRI) is 38 grams/day for adult men and 25 grams/day for adult women.

Generally categorised into;

Water-soluble fibres: fibres present in oats, dried beans, legumes, chicory

Water insoluble fibres: mainly helping in bulking of stools and their quick passage through digestive tract. E.g.: fibres present in, Brown rice, bananas, cassavas, vegetables, wheat etc.

Dietary fibres are used to correct constipation irregularities, haemorrhoids

Marketed products: Metamucil powder Fibogel, Now Psyllium husk powder, Naturolax, Organic India Psylium, Fenulife (fenugreek dietary fibre containing galactomannan)

2. FUNCTIONAL FOODS:

Functional foods are source of absolutely necessary nutrients providing more than the quantities for the maintenance, growth and development. It provides health beyond basic nutrition The, class of functional food includes cereals such as rice, corn, wheat, millets, sorghum and buckwheat and useful in many diseases like coronary heart disease, Diabetes.

Oats, Barley, buckwheat, Shia seeds, flax seeds, pumpkin seeds, almonds, cashews, Brazil nut

3. BOTANICALS

A botanical is a plant or a plant part valued for its medicinal or therapeutic properties. Botanicals are dietary supplements from non-food origin (plants, algae, fungi, or lichens). A compendium of 900 botanicals has been reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. They are not

essential for human life. They can be also found in food preparations. For example: Ginseng, Gingko extracts, Teamax (Green tea extract), Moringa extract.

4. MEDICINAL FOODS

A medicinal food is formulated to be consumed or administered internally, under the supervision of a qualified physician. Its intended use is a specific dietary management of a disease or condition for which distinctive nutritional requirements are established by the medical evaluation. Eg: polyphenols, cholestaid (saponin), soylife (soyabean phytoestrogen).

GLOBAL MARKET AND OVERVIEW OF NUTRACEUTICAL SEGMENT

- ✓ The nutraceutical market is predicted to record revenue of USD671.30 million by 2020.
- ✓ The report, "2019 Nutraceuticals Market Assessment, "says consumers around the world are seeking alternatives to better health, suffer from pill fatigue, and look for more innovative foods and drinks that supply all their nutritional needs for exercise, weight management, digestive health, and general well-being.
- ✓ Global nutraceutical market is projected to grow at a CAGR of 7.5% during the forecast period (2019-2024).
- ✓ Developing countries have a high prevalence of non-communicable diseases like cancer, diabetes, CV ailments etc. therefore the demand for nutraceutical is expected to rise in these nations.
- ✓ Nutraceutical also gaining the global importance and have become a part of daily diet due to increased risk of diseases due to improper lifestyle and people consciously adapting preventive healthcare measures.
- ✓ The gradually increasing healthcare expenses are also stimulating the demand for nutraceutical.
- ✓ Developed countries like United States and Europe have seen a fast-emerging segment of customised products especially functional foods and beverages. These countries are discovering the untapped segment of customized products based on health claims. Functional food is the largest shareholding category of the studied market, followed by functional beverage and dietary supplement.
- ✓ Global Nutraceuticals market is segmented by Type as Functional Foods (Cereal, Bakery and Confectionery, Dairy, Snacks, Other Functional Foods), Functional Beverages Energy Drinks, Sports Drinks, Fortified Juice, Dairy and Dairy Alternative Beverages, and Other Functional Beverages), Dietary Supplements

(Vitamins, Minerals, Botanicals, Enzymes, Fatty Acids, Proteins, and Other Dietary Supplements), and by Geography as North America, South America, Europe, Asia-Pacific, and Middle East & Africa.

- ✓ Nutraceutical have become an opportunity for economic growth of many developing countries which have a rich source of medicinal herbs and traditional knowledge of such plants especially India, China, and South American countries.

Top manufacturers of Nutraceutical Products

- ✓ Nestlé
- ✓ Kraft Heinz Company
- ✓ Kelloggs
- ✓ Hero Group
- ✓ Raişio Group
- ✓ Amway
- ✓ General Mills
- ✓ The Hain Celestial Group
- ✓ Freedom Food group Limited
- ✓ Nature's Bounty
- ✓ Conagra
- ✓ Barilla Group

Indian nutraceutical market

- ✓ The nutraceuticals market in India is expected to grow from $ 4 billion in 2017 to $ 18 billion in 2025 in the backdrop of rising demand for dietary supplements from upper and middleclass.
- ✓ The nutraceuticals market in India can be further divided into functional food, beverages and dietary supplements. While functional food includes items such as breakfast cereals, and fortified flour, functional beverages include commodities like Sports drinks, fortified juices, and glucose.
- ✓ Dietary supplements, which constitute over 65 percent of the Indian nutraceuticals market, include commodities such as macronutrients, herbal and non-herbal extracts.
- ✓ The major manufactures are Amway, Himalaya, Dabur and Emami.
- ✓ This segment is growing at a rate of 17 percent, and hence, will drive the growth of the market.

Health benefits and role of Nutraceuticals in treatment of Diabetes

The most common type of diabetes is type 2 diabetes with 95% prevalence and is associated with obesity. In recent years a wide range of herbal dietary supplements and herbal medicines have scientifically proven to benefit type 2 diabetes in preclinical studies.

1. Omega 3-fatty acids

Ethyl esters of n-3 fatty acids may be beneficial in diabetic patients. Docosahexaenoic acid modulates insulin resistance and is also vital for neurovisual development.

2. Antioxidants

A plenty supply of dietary antioxidants may prevent, or delay diabetes complications including renal and neural dysfunction by providing protection against Oxidative stress.

3. Vitamin C

Vitamin C also called ascorbic acid is a chain breaking antioxidant. vitamin C (800mg/day) partially replenishes vitamin c levels in type 2 diabetic patients. Low vitamin level impair insulin resistance

4. Vitamin E

Vitamin E is an essential fat-soluble vitamin and functions mainly as antioxidants. Low levels of vitamin E produces increased frequency of diabetes. Dose of vitamin E up to 400 IU are generally safe to use.

5. Magnesium and chromium

chromium supplents and magnesium rich diet improves the insulin sensitivity and improves the glucose tolerance in type 2 diabetic patients.

6. α- Lipoic acid

It is an antioxidant, used for treatment of diabetic neuropathy and seems to be effective as a long-term dietary supplement for protection of diabetes from complications.

7. Dietary fibres

Fibres from Psyllium have been used for glucose control in diabetic patients and to reduce lipid levels in hyperlipidaemia. Foods rich in fibres like fruits, vegetables and specially food made of whole cereals also suggested.

HEALTH BENEFITS AND ROLE OF NUTRACEUTICALS IN TREATMENT OF CARDIOVASCULAR DISEASES (CVD)

1. Phytosterols

Plant sterols are known as phytosterols and are present in plant products including vegetables, cereals, seeds, and nuts. They are structurally and functionally similar to cholesterol. phytosterols are reported to possess antiatherogenic effect. Phytosterols inhibit the intestinal absorption of cholesterol. It improves the serum lipid profile lower-the LLDL levels and decrease the risk of cardiovascular disease. Eg: campesterol and sitosterol.

2. Polyphenols

Polyphenolics include flavonoids, phenolic acids and stilbenes. They are found in fruit, vegetables, cereal, legumes and beverages. Polyphenols exert antiatherosclerosis effect in initial stages of atherosclerosis development, improve endothelial function and increase nitric oxide release; which acts as a potent vasodilator, protect against myocardial ischemia and platelet aggregation.

3.Flavonoids

Plant derived flavonoids are present in vegetables and fruits, beverages like cocoa, tea and wine. Flavonoids protect LDL from oxidation and attenuate atherosclerosis. Flavonoids exert their effect due to their antioxidant activity and metal chelation property. E.g.; quercetin, rutin

4. Resveratrol

Resveratrol a polyphenolic compound notably present in grapes, red wine, peanuts, berries etc. In several animal models, resveratrol supplementation in endothelium-dependent effects, has been shown to improve flow mediated vasodilation-a surrogate for endothelial function (n to stimulating endothelium dependent vasodilation.

5. Anthocyanins

Anthocyanins are flavonoids that exist in various fruits and vegetables. They are present in purple grapes, figs, blueberries, and blackberries. Anthocyanins are widely known as nutraceuticals and are a group of soluble vacuole pigments, which are red, purple, or blue in color based on the pH of the micro environment Anthocyanins" is believed to support healthy blood pressure, reduces risk of heart disease.

6. Curcumin

Curcumin is an active biomarker of turmeric (Curcuma longa Fam: Zingiberaceae). Curcumin prevents cardiac hypertrophy and heart failure. Its long-term use modifies genetic expression involved in cholesterol homeostasis. The incorporation of curcumin into, food has been reported to reduce circulation of C-reactive protein levels. It also impedes proliferation of peripheral blood mononuclear cells; Besides, curcumin prevents the oxidation of LDL's. It also inhibits platelet aggregation and reduces the incidence of myocardial infarction.

7. Garlic

Garlic (Allium sativum) is another potential herb to be employed in fighting CVD disorder. Garlic has been shown to protect against myocardial infarction, doxorubicin-induced cardiotoxicity, viscous arrhythmias, internal organ hypertrophy, and ischemia-reperfusion injury. The sulphur components like allicin responsible for the medicinal uses. Totally different mechanisms, such as regulating ion channels, modulating AKT signalling pathways, protein deacetylase inhibition, and haemoprotein, P450 inhibition, can even be responsible for the cardioprotective results of garlic.

8. Omega-3-Fatty Acid

Omega-3 fatty acids are 'found in fish oils and in some plants, such as oilseed. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most important omega 3-fattyacids these fatty acids are renowned for lowering blood triglycerides and have a positive effect in patients suffering from cardiac illness (heart attack) or heart conditions.

9.Spirulina and Soy nutrients

Spirulina is a rich source of protein. Vitamin, Minerals, carotenoids and phycocyanins. spirulina supplementation alters blood lipid profiles. Soy products are rich in polyunsaturated fatty acids, fibre, vitamins, and minerals and contain low amount of saturated fatty acids.

10. Tomato and lycopene

Lycopene is a carotenoid pigment found in red fruits and vegetables like papaya, tomatoes, red peppers, water melons etc. Tomatoes arc rich source of lycopene. The consumption of lycopene reduces myocardial infarction, coronary insufficiency and angina pectoris. Lycopene is a powerful antioxidant and plays an important role in preventing cardiovascular diseases.

HEALTH BENEFITS OF NUTRACEUTICAL IN CANCER

1. Polyphenols

Polyphenols are plant secondary metabolites that contain one or more hydroxyl group attached to a benzene ring in their structure. More than 8000 different, polyphenols found in food (mainly, wine, tea, coffee, cocoa, vegetables and cereals) are present in the human diet. They may be classified into different groups according to their number of phenol rings and the structure that links these rings. In this context, the groups of phenolic acids, flavonoids are the most important for their capacity for blocking initiation of carcinogenic process and to Suppress cancer progression

2. Epigallocatechin-3-gallate (from green tea)

Epigallocatechin-3-gallate EGCG (epigalfocatechin-3-gallate) is the major catechin found in green tea (*Camellia sinensis*). The frequent consumption of green tea in Asian countries has been related to several health Benefits and it is recognized as the most effective cancer-preventive beverage.

3. Grape seed

Grape seed is effective in the prevention of UV light induced cancer. grape seed extract also decreases the risk of cutaneous cell carcinoma as it contains polyphenols and proanthocyanidins which inhibit lung cancer. Grape seed also inhibit blood cancer and prostate cancer when taken as supplements.

4. Resveratrol

Resveratrol (found in grapes, blueberries, raspberries, mulberries, and peanuts.) is the most important stilbene related to cancer. It possesses a natural anti-proliferative activity due to its role as a phytoalexin (plant antibiotic). It is believed to have also multiple bioactivities including anti-cancer, anti-carcinogenesis and anti-inflammatory effects. The mechanisms by which resveratrol might produce these effects are not completely understood, but the man molecular mechanism seems to be the activation of sirtuin protein.

5. Flavonoids

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally n foods of plant origin. Tea polyphenols, quercetin, and genistein have been widely studied tor their

potential chemo preventive applications. Some flavonoids were first shown, to be apoptotic in human lymphoid leukemic cells and human carcinoma.

6. Lycopene and B-carotene

Tomato is rich in various carotenoids. Lycopene is the precursor of ß-carotene in tomato, which accumulates after the lycopene cyclase gene is down regulated during ripening. Lycopene and ß-carotene can induce apoptosis in prostate cancer cells and malignant lymphoblast cells.

7. Curcumin

Curcumin induces apoptosis in colon carcinoma cells, leukemic cells, prostate cancer cells, melanoma cells, and breast cancer cells

8. Garlic

The use of garlic as anticancer agent has long been established. The allyl sulfur compounds derived from garlic, have significant anti-proliferate activity against human cancers. Diallylsulfide and diallyldisulfide induce apoptosis in non-small cell lung cancer cells and in prostate cancer and breast cancer cells

9. Lentinan

Lentinan, B-1,3 beta-glucan with B-1,6 branching, is an edible mushroom that occurs naturally in Lentinula edodes. It is an adjuvant extracted from the fruit body of *L. edodes*. It has been reported to significantly reduce the formation of colon tumours in animal models. Orally active lentinan stimulates production of white blood cells in the human cell line.

10. Dietary fibres and cruciferous vegetables

Dietary fibres prevent constipation and reduce the risk of colon rectal cancer, Dietary fibre rich diet reduces the risk of breast cancer in postmenopausal women, fibre includes broccoli, cabbage, cauliflower, Brussel sprouts and greens. Raw cruciferous vegetables orc more efficient than cooked ones.

HEALTH BENEFITS OF NUTRACEUTICALS IN IRRITABLD BOWEL SYNDROME AND GASTRO INTESTINAL DISEASES

1. Curcumin

Curcumin is a phytochemical derived from rhizome of the Indian herb *Curcuma longa* and belongs to the family Zingiberaceae. It has been used in both ayurvedic and traditional Chinese medicine especially in abdominal pain as well in inflammatory diseases. Curcumin has anti-inflammatory properties and reduces the mucosal injuries.

2. Aloe vera

It contains several potentially bioactive compounds like salicylates, lupeol, campesterol, sitosterol, linolenic acid, aloctin A, acemannan, a component of aloe vera prevents stress induced gastric ulceration.

3. Bael (*Aegle marmelos*)

It grows in tropical and subtropical countries. luvaangettin a pyranocoumarin isolated from the seeds of *Aegle marmelos* protect the gastric mucosa in gastric ulceration through a non-identified prostaglandin independent pathway.

4. Fibre

An important step in controlling the symptoms of IBS is to increase dietary fibre from plants, which the human body cannot digest on it's on. Fibre can act as a bulking agent to improve intestinal transit and decrease constipation in a subgroup of IBS patients. Thus, dietary recommendations for IBS patients often include fibre supplementation, especially with soluble (psylium/ispaghula husk) rather than insoluble (bran) fibre.

5. Fennel

Anethole, the major component of fennel fruit, is chemically similar to the neurotransmitter dopamine and has a relaxant effect on intestinal smooth muscle reduced crampiform abdominal pain, a mechanism likely mediated by the anethole-dependent relaxation of intestinal smooth muscle. It improves symptoms and quality of life in IBS patients.

6. Natural honey and *Niglla sativa* (Black cumin)

Have been used as a natural remedy in various parts of the world. Honey has the capacity to stimulate the tissue growth, enhance re epithelisation and minimise scar formation Honey and

Nigella sativa seeds are equally effective in healing gastric ulcers induced by acetyl salicylic acid and honey inhibits the growth of H pylori, the main etiologic agent of gastritis, peptic ulcer and gastric adenocarcinoma.

7. Probiotics

Probiotics are live microorganisms with a vas, array of therapeutic potential for gastrointestinal disease Probiotics have a beneficial effect on intestinal mucosa via several proposed mechanisms that include suppression of the growth and binding of pathogenic bacteria, improvement of the barrier function of the epithelium, and alteration of the immune activity of the host. They are available in the form of foods like yogurt, fermented and unfermented milk and some juices and soy beverages.

Study of following herbs as health food: Alfalfa, Chicory, Ginger, Fenugreek, Garlic, Honey, Amla, Ginseng, Ashwagandha, Spirulina

ALFALFA

Common name : Lucerne, purple medic, Purple Medick,Buffalo Herb.

Botanical source : It is the dried whole herb, including blossoms of***Medicago sativa*** belongs to the family**Leguminosae**.

Geographical source: Alfalfa is grown in many other parts of the world, from China to Spain, Sweden to North Africa. Outside of cultivation alfalfa occurs as a weed throughout Asia, Europe and America. The largest producerof alfalfa today is North America, South America and Asia.

Chemicalconstituents: Alfalfa contain protein, minerals (Ca, Cu, Fe, Mg, Mn, P, Zn, Si), vitamins (A, B, C, D, E, K, U), phytochemical substances (carotene, chlorophyll, coumarins, isoflavones, alkaloids, saponins), contains secondarymetabolites of plants (phytoestrogens: isoflavones and coumestrol), and antinutritional components (phytates, L-canavanine, saponins).

Uses: Alfalfais used for kidneyconditions, bladderand prostate conditions,and to increase urine flow. It is also used forHigh cholesterol,asthma,osteoarthritis,rheumatoid arthritis,diabetes,upsetstomach,anda bleedingdisordercalled thrombocytopenicpurpura. Peoplealso take alfalfaas a source of vitamins A, C, E, and K4;and minerals calcium,potassium,phosphorous,and iron.

CHICORY

Common names: blue sailors, coffeeweed, cornflower, Italian dandelion, or succory.

Botanical source: Dried leaves and root of ***Chichorium intybus***, **Astraceae**.

Geographical source: Chicory is native to western Asia, North Africa, and Europe.It lives as a wild plant on roadsides in Europe. The plant was brought to North America by early European colonists.It is also common in China, and Australia, where it has become widely naturalized. It is more common in areas with abundant rain.

Chemical constituents: Substances which contribute to the plant's bitterness are primarily the *two sesquiterpene lactones, lactucin and lactucopicrin*. Other components are *aesculetin,*

aesculin, cichoriin, umbelliferone, scopoletin, 6,7-dihydrocoumarin, and further sesquiterpene lactones and their glycosides. It was discovered that the root contains up to 20% inulin, a polysaccharide similar to starch.Raw chicory leaves are 92% water, 5% carbohydrates, 2% protein, and contain negligible fat. In a 100-gram reference amount, raw chicory leaves provide 23 caloriesand significant amounts (more than 20% of the Daily Value) of vitamin K, vitamin A, vitamin C, some B vitamins, and manganese. Vitamin E and calcium are present in moderate amounts.

Uses: reduce inflammation, antimicrobial properties, antioxidant properties, manage blood glucose levels, reduce lipid levels, reduce the proliferation of cancer cells, lower uric acid, positively impact the lipid profile. Inulin fibre in chicory acts as a prebiotic and may help improve digestive health in children, adults and elderly. Additionally, the presence of this fibre stimulates gastric acid, improves bowel movements and may help relieve constipation.Chicory aids the regulation of ghrelin (the hunger hormone) and increases the feeling of satiety. Thus, the consumption of chicory increases feeling of fullness, reduces overeating and may help in weight loss.Chicory contains Manganese and Vitamin B6, both of which are needed for the formation of neurotransmitters (chemical messengers) in the brain and thus, it helps improve brain health.

GINGER

Synonyms: Rhizoma zingiberis, Zingibere.

Biological Source: Ginger consists of the dried rhizomes of the *Zingiber officinale* Roscoe, belonging to family Zingiberaceae.

Geographical Source: It is mainly cultivated in West Indies, Nigeria, Jamaica, India, Japan, and Africa.

Chemical Constituents: Ginger contains 1 to 2% volatile oil, 5 to 8% pungent resinous mass and starch. The volatile oil is responsible for the aromatic odour and the pungency of the drug is due to the yellowish oily body called *gingerol* which is odourless. Volatile oil is composed of sesquiterpene hydrocarbon like α-zingiberol; α-sesquiterpene alcohol α-bisabolene, α-farnesene, α-sesquiphellandrene. Less pungent components like gingerone and shogaol are also present. Shogal is formed by the dehydration of gingerol and is not present in fresh rhizome.

Uses: Ginger is used as an antiemetic, positive inotropic, spasmolytic, aromatic stimulant, carminative, condiment, and flavouring agent. It is prescribed in dyspepsia, flatulent colic, vomiting spasms, as an adjunct to many tonic and stimulating remedies, for painful affections of the stomach, cold, cough, and asthma. Sore throat, hoarseness, and loss of voice are benefited by chewing a piece of ginger.

FENUGREEK

Synonyms: Methi, Methika, Chandrika

Biological source: Fenugreek consists of dried ripe seeds of *Trigonella foenum-graecum* Family: Leguminosae

Geographical source: The plant grows wild in Northern India and is cultivated as a crop throughout India. It is also cultivated in Southern and Eastern Europe, Pakistan, France, Morocco and Egypt.

Chemical constituents: The seed is a good source of calcium, minerals, iron, β-carotene and several vitamins like vitamins A and D. It is rich source of dietary fibre. It is consisting of free amino acids; 4-hydroxyisoleucine, lysine, histidine, and arginine (25.8%), protein (20-30%), moisture (11.76%), fat (6.53%), crude fibre (6.28%), ash content (3.26%) and energy (394.46 Kcal/100 g seed). Fenugreek seeds also contains lecithin, choline, minerals, B. Complex, Phosphates, and Para-Amino Benzoic acid (PABA). In addition, the main chemical compounds in fenugreek are saponins, fenugreekine, trigonelline, coumarin, scopoletin, phytic acid and nicotinic acid.

Uses: Controls diabetes, Breast milk production, Cholesterol, Digestion, Weight management, Antioxidant activity, Inflammation, Fenugreek seeds treat dandruff helps in Hair growth, Anticancer effect, Bone health etc.

GARLIC

Common name: Stinking rose,Rashoon, Ajo,Allium.

Biological source: It is the dried bulb of *Allium Sativum* Linne. Family : Liliaceae.

Geographical source: Garlic is a native of WestAsiaand Mediterraneanarea. China, Korea, India,USA, Spain, Argentinaand Egyptare the major garlic growing countries.

Chemical constituents: Enzymes like-Allinase,peroxidases,catalases,superoxide dismutase, arginases,lipases.Volatile oils-alliin, allicin, allylpropyl disulfide,diallyldisulfide,terpenesinclude citral, geraniol,linalool, α and β-phellandrene.Besides,Proteins(e.g: glutamyl peptides),amino acids (e.g.,arginine, glutamic acid,asparagic acid,methionine,threonine),minerals,vitamins, trace elements.lipids, prostaglandins.

Uses: Lowercholesteroland triglycerides, Kill Worms, Hypertension, helps in blood circulation, Cancerprevention, Diabetes, Bronchitis, Asthma, Allergies, Antithrombotic, antimicrobial, fibrinolytic.

HONEY

Common name: Madhu, Madh, Mel.

Biological source: Honey is a viscid and sweet secretion stored in the honey comb*Apis dorsata, Apis florea, Apis indica, Apis mellifera* Linne. Family: Apideae.

Geographical source: Honey is available in abundance in Africa, India, Jamaica. Australia, California. Chili. Great Britain and New Zealand.

Chemical constituents: The average composition of honey ranges as follows: Moisture 14-24%, Dextrose 23-36%, Levulose (Fructose) 30-47%, Sucrose0.4-6%, Dextrin and Gums 0-7% andAsh 0.1-0.8%. Besides, it is found to contain small amounts of essential oil, beeswax, pollen grains, formic acid, acetic acid, succinic acid, maltose, dextrin, colouring pigments, vitamins and an admixture of enzymes e.g.; diastase, invertase and inulase.

Uses: It is used as a sweetening agent in confectionaries.Being a demulcent, it helps to relieve dryness and is, therefore, recommended for coughs, colds, sore-throats and constipation.Because of its natural content of easily assimilable simple sugars, it is globally employed as a good source of nutrient for infants, elderly persons and convalescing patients.

AMLA

Synonyms: Emblica, Indian goose berry, amla.

Biological Source: This consists of dried, as well as fresh fruits of the plant *Emblica officinalis* Gaerth (*Phyllanthus emblica* Linn.), belonging to family Euphorbiaceae.

Geographical Source: It is a small- or medium-sized tree found in all deciduous forests of India. It is also found in Sri Lanka and Myanmar.

Chemical Constituents: It is highly nutritious and is an important dietary source of vitamin C, minerals, and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains considerably higher concentration of most minerals and amino acids than apples. The pulpy portion of fruit, dried and freed from the nuts contains: gallic acid 1.32%, tannin, sugar 36%; gum 13%; albumin 12 %; crude cellulose 17%; mineral matter 4%; and moisture 3%. Tannins are the mixture of gallic acid, ellagic acid, and phyllembin. The alkaloidal constituents such as phyllantidine and phyllantine have also been reported in the fruits.

Uses: The fruits are diuretic, acrid, cooling, refrigerant, and laxative. Dried fruit is useful in haemorrhage, diarrhoea, diabetes, and dysentery. They are useful in the disorders associated with the digestive system and are also prescribed in the treatment of jaundice and coughs. It has antioxidant, antibacterial, antifungal, and. antiviral activities. Amla is one of the three ingredients of the famous ayurvedic preparation, triphala, which is given to treat chronic dysentery, and other disorders, and it is also an ingredient in chyavanprash.

GINSENG

Synonyms: Panax, Asiatic Ginseng, Chinese Ginseng, Ginseng Root, Pannag, Ninjin.

Biological Source: It consists of dried roots of *Panax ginseng* and other species of Panax like *Panax japonicus* (Japanese Ginseng), *Panax pseudoginseng* (Himalayan Ginseng), *Panax quinque-folius* (American Ginseng), *Panax trifolius* (Dwarf Ginseng) and *Panax vietnamensis*(Vietnamese Ginseng), belonging to family Araliaceae.

Geographical Source: It is mainly found in China, Russia, Korea, Japan, Canada and India.

Chemical Constituents: Several saponin glycosides belonging to triterpenoid group, ginsenoside, panxoside. More than 13 ginsenosides have been identified. Ginsenosides consists of aglycone dammarolwhereas panaxosides have oleanolic acid as aglycone. It also contains large amount of starch, gum, some resin and a very small amount of volatile oil.

Uses: The root is adaptogen, alterative, carminative, demulcent, emetic, expectorant, stimulant and tonic. The saponin glycosides, also known as ginsenosides or Panaxosides, are thought responsible for Panax ginseng's effects. Ginsenosides have both stimulatory and

inhibitory effects on the CNS, alter cardiovascular tone, increase humoral and cellular-dependent immunity, and may inhibit the growth of cancer in vitro. It encourages the secretion of hormones, improves stamina, lowers blood sugar and cholesterol levels. It is used internally in the treatment of debility associated with old age or illness, lack of appetite, insomnia, stress, shock and chronic illness.

ASHWAGANDHA

Synonyms: Withania root. Ashwagandha, Clustered Wintercherry.

Biological Source: It consists of the dried roots and stem bases of *Withania somnifera* Dunal, belonging to family Solanaceae.

Geographical Source: Withania is widely distributed from southern Europe to India and Africa.

Chemical Constituents: The plants contain the alkaloid withanine as the main constituent and somniferine, pseudowithanine, tropine and pseudotropine, hygrine, isopellederine, anaferine, anahygrine and steroid lactones. The leaves contain steroid lactone, commonly known as withanolides.

Uses: All plant parts are used including the roots, bark, leaves, fruit and seed are used to treat nervous disorders, intestinal infections and leprosy. Ashwagandha is one of the most widespread tranquillizers used in India, where it holds a position of importance similar to ginseng in China. It acts mainly on the reproductive and nervous systems, having a rejuvenate effect on the body, and is used to improve vitality and aid recovery after chronic illness. It is also used to treat nervous exhaustion, debility, insomnia, wasting diseases, failure to thrive in children, impotence, infertility; multiple sclerosis, etc.

SPIRULINA

Common name: Blue-green algae.

Botanical source: Spirulina is a cyanobacterium that can be consumed by humans and other animals and is made primarily from two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima* Linne. Family: Oscillatoriaceae

Geographical source: Spirulina is grown in Hawai, United States, India, Thailand, and Taiwan.

Chemical constituents: It contains 65% Protein and Amino Acids,20% Carbohydrates,7% Minerals, 5% Fats, 3% moisture, vitamins B1, B2, B3, B6, B12 and E.

Uses: Antioxidant, Immunomodulation, appetite stimulant andAnti-viral effects.

PROCESSING OF HERBAL MATERIALS

Processing of herbal material is done to maintain the quality of the herbal raw materials which are to be further used for the preparation of herbal medicinal products. Processing of herbal materials include

- Primary processing
- Secondary processing
- Special processing

Primary processing:

- ✓ Harvested or collected raw medicinal plant materials should be promptly unloaded and unpackedupon arrival at the processing facility.
- ✓ Prior to processing, the medicinal plant materials shouldbe protected from rain, moisture and any other conditions that might cause deterioration.
- ✓ Medicinal plant materials should be exposed to direct sunlight only where there is a specific needfor this mode of drying.
- ✓ Medicinal plant materials that are to be used in the fresh state should be harvested/collected anddelivered as quickly as possible to the processing facility in order to prevent microbialfermentation and thermal degradation.
- ✓ The use of preservatives should be avoided if used, they should conformto national and/or regional regulations for growers/collectors and end-users.
- ✓ Medicinal plant materials that are to be employed fresh should be stored under refrigeration, injars, in sandboxes, or using enzymatic or other appropriate

conservation measures, andtransported to the end-user in the most expeditious manner possible.

- ✓ All medicinal plant materials should be inspected during the primary-processing stages ofproduction, and any substandard products or foreign matter should be eliminated mechanically orby hand.
- ✓ For example, dried medicinal plant materials should be inspected, sieved or winnowed to removediscoloured, mouldy or damaged materials, as well as soil, stones and other foreign matter.Mechanical devices such as sieves should be regularly cleaned and maintained.

- ✓ All processed medicinal plant materials should be protected from contamination anddecomposition from insects, rodents, birds and other pests, and from livestock anddomestic animals.

1. **PRIMARY PROCESSING:** (It is the basic or initial processing)

It includes **instant post-harvest treatments** performed to herbal raw materials obtained from collection either from wild or from cultivated varieties and includes simple procedure by which the herbs are prepared like **Garbling/sorting** of different parts, **washing, parboiling, leaching/cleaning, drying** etc.

- **Garbling (Sorting/ Dressing):**This process helps in ensuring the purity and cleanliness of the harvested materials. Dirt like soil, dust, impurities like insects, dead tissues and residual non-medicinal plants are separated from the raw material. The process depends on the part of the plant to be prepared. The process may involve procedures such as removing dirt, foreign substances, discarding damaged parts, peeling of barks, sieving, trimming, removal of hair from roots, removal of seeds from fruits, stripping of leaves

from stems.This may be done by mechanical means but in some cases, it is usually performed manually by hands.

- **Washing:**After garbing the herbal raw materials **should be cleaned well to remove the traces of remaining soil, dirt and other impurities from the surface.**The roots, rhizomes and tubers are washes with clean water. **During the washing process, scrapping and brushing may be necessary.**
- **Parboiling (Blanching):**After washing, **certain herbal raw materials** need to undergo **parboiling process in which they are put in boiling water for short period**.This may help in improving the storage life of the raw materialsand preventing insert/mould contamination.It may also facilitate in further processing such as removal of stubborn impurities as well as outer coats/covering of raw materials
- **Leaching:Some impurities can be removed by subjecting the plant material under running waterknown as leaching.The duration of leaching should be controlled to prevent the loss of chemical constituents present in the drug**
- **Drying:Drying may have considerable impact on the quality of the herbal raw materials.** When medicinal plant materials are prepared for use in dry form, they must be dried as soon as possible to maintain the **moisture content of thematerial. The moisture should be kept as low as possible in order to reduce damage from mould and othermicrobial infestation.**
- **In some cases, the plant material should be thoroughly dried after washing in order to prevent the deterioration and degradation of active constituents.**When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents.
- **The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials.**

Advantages:

- Drying prevents the activation of certain enzymes which may otherwise degrade or decompose the active ingredients.
- It also facilitates grinding and milling of the raw material.

1. Natural drying:

a)Sun drying:

- Most herbal raw material can be dried in open air under direct sunshine provided the climate is suitable. The duration of drying process depends on the physical structure of the plant material and weather conditions.
- The plant materials should be spread out in thin layers, care should be taken to prevent contamination by dirt impurities.
- In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials and so avoid mould formation.
- Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, medicinal plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting.
- Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites.

b)Shade drying/open air drying:

- Some medicinal plants cannot be directly exposed to sunlight, hence need to be dried under shade.Shade drying is slow but usually preferred to maintain or minimize loss of

colour of leaves and flowers and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances and aromatic components.

- For e.g. **leaves and flowers, barks, stems, roots etc of plants with any volatile ingredients such as essential oils are placed in thin layers on drying frames, and turned or stirred frequently in wire screened rooms, or buildings.**
- The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently in wire screened rooms or buildings.
- For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils.
- If possible, the source of heat for direct drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 60°C. If other sources of fire are used, contact between those materials, smoke and medicinal plant material should be avoided.

2. Artificial drying:

- **Drying by artificial heat is** more rapid than open air drying and is **necessary in rainy seasons and regions where there is high humidity.**
- The temperature and equipment used for drying depends on the physical and chemical nature of the drug and its constituents. **various equipment such as tray dryers, spray dryers, vacuum dryers are used**
- **Overheating may lead to excessive loss of volatile components** as well as decomposition of chemical constituents. **The temperature should be kept maintained below 60°C wherever possible.**

a)Tray dryers (Oven)

- **The drugs which do not contain volatile oils and are quite stable to heat or which need deactivation of enzymes are dried in tray dryers.**
- In the process, hot air of the desired temperature is circulated through the dryers and this facilitates the removal of water content of the drug (**belladonna roots, cinchona bark, tea and raspberry leaves and gums are dried by this method**)

b) Vacuum dryers:

The drugs which are sensitive to higher temperature are dried by this process. E.g., Tannic acid and digitalis leaves.

Advantages of vacuum drying:

(i) Rapid drying.

(ii) Relatively low temperature.

(iii) Cleanliness and freedom from odour and dust.

(iv) Independence of climate conditions.

(v) Control of temperature.

(vi) Elimination, of risk of fire.

(vii) Compactness.

c) Spray dryers:

Few drugs which are **highly sensitive to atmospheric conditions and also to temperature of vacuum-drying are dried by spray-drying** method. The technique is **followed for quick**

drying of economically important plant or animal constituents, rather than the crude drugs

2. SECONDARY PROCESSING

- **Cutting/sectioning**
- **Aging/sweating**
- **Baking/Roasting**
- **Boiling/Streaming**
- **Stir frying Fumigation Extraction**

1. <u>Secondary processing:</u>

SECONDARY PROCESSING: (It includes advanced techniques or done post primary processing). The secondary processing differs from one herbs to another depending upon the natureof active ingredients as well as therapeutic properties. Secondary processing includes techniques such as removal of foreign substances, prevention of microbial/infestation, enhancing the efficacy of drugs, reducing the toxicity, extraction using suitable solvents, conc. & drying of extracts

These are further standardized by different methods.

Cutting, Sectioning and communition:

•After thoroughly drying, the herbal materials are processed by cutting and sectioning into smaller sizes which are convenient for storage as well as extraction

•Various sizes can be obtained depending on the part of herb and extraction methods used. It may be small particles, coarse powder or fine powder

Aging/Sweating:

•Aging refers to storing the raw material for a specified time after harvesting

•It is generally done under sun or in shade for up to a year

•During the process of aging excessive water is evaporated & enzymatic reactions may occur to alter the chemical composition of herbal materials

•E.g., Cascara bark should be aged for at least one year prior to use in medicinal preparations to reduce its irritant effects.

•Sweating is done by subjecting the herbal materials at a temperature between 45 to 65°C with high humidity for a period ranging from one week to few months. The herbal materials are stacked between woolen blankets or other kind of cloth

•The sweating process is considered a hydrolytic and oxidative process in which some of the chemical ingredients of the herbs are hydrolyzed or oxidized

Baking/Roasting:

•It is a process of drug heating where the herbal materials is heated in ovens. Thetemperature of heating and duration of baking /roasting vary from one herbal material to another until the drug develops a specific colour

•E.g., Nutmeg is roasted till they turn to yellowish brown colour

Boiling/Streaming:

•In the boiling process the drug is cooked in water or any other liquid solvent such as vinegar, wine, milk or animal urine.

•E.g. Acorus calamus rhizome is boiled in cow's urine with stream using a streamer resulting in development of moist texture

Stir frying:

•In the process in which the herbal material are put in spot of frying pan and continuously stirring or tossed for a specific period under heat until the external color changes, charred or even carbonized. To facilitate uniform heating, the drug material can be admixed with sand, talc or clay.

•E.g., Liquorice roots and rhizomes are stir fried with honey

Fumigation:

- Sometimes the harvesting raw materials are subjected to fumes. Fumigation with Sulphur-dioxide is commonly employed for some medicinal herbs for the purpose of preserving, colour, improved appearance, bleaching and preventing the growth of inserts and moulds.
- It is the process converting the primary processed herb into herbal materials by a variety of material by a variety of additional procedures including aging or sweating baking or roasting, boiling or steaming and sir frying.

2. **Special processing:**

It is the extension of secondary process usually performed to treat selected herbs to reduce their toxicity and modify their therapeutic activity.

Specific Processing:

Some medicinal plant materials require specific processing to: improve the purity of the plant part being employed; reduce drying time; prevent damage from mould, other microorganisms

and insects; detoxify indigenous toxic ingredients; and enhance therapeutic efficacy.

Common specific processing practices include pre selection, peeling the skins of roots and rhizomes, boiling in water, steaming, soaking, pickling, distillation, fumigation, roasting, natural fermentation, treatment with lime and chopping. Processing procedures involving the formation of certain shapes, bundling and special drying may also have an impact on the quality of the medicinal plant materials.

Antimicrobial treatments of medicinal plant materials (raw or processed) by various methods, including irradiation, must be declared and the materials must be labelled as required.

Only suitably trained staff using approved equipment should carry out such applications, and They should be conducted in accordance with standard operating procedures and national and/or regional regulations in both the grower/collector country and the end-user country. Maximum residue limits, as stipulated by national and/or regional authorities, should be respected.

- By direct fire, baking, lyophilisation, microwave or infrared devices

Bio-pesticides/ Bioinsecticides

- Conventional pesticides are synthetic materials that can directly kill or inactivate the pest and have numerous harmful effects to the ecosystem.

- Bio-pesticides /Bioinsecticides are those biological agents that are used for control of weeds, insects and pathogens.

- They are the compounds that are used to control the agricultural pests by using biological agents like natural organisms or substances derived from natural materials.

Types of Biological Insecticides

There are three main categories of biopesticides.

1. **Biochemical pesticides**: These are substances that are naturally found in nature. biochemical pesticides control pests through non-toxic mechanisms.
2. **Microbial pesticides**: These include microorganisms as the active ingredient. These microorganisms are usually bacteria or fungi but can also be viruses, protozoa, or oomycetes.
3. **Plant-Incorporated Protectants (PIPs)**: These are substances that are produced by plants after genetic material, such as genes or proteins, has been added to the plant by humans. Plants are modified in this way so that they are naturally resistant to insect pests, and different types of genes and proteins can enhance a plant's resistance to different types of pests.

The micro-organisms used as bio-pesticides are **viruses, bacteria, protozoa, fungi and mites.** Most important examples: **soil bacterium, Bacillus thuringiensis (Bt).**

Spores of this bacterium possess the insecticidal Cry protein which kill larvae of certain insects. (The commercial preparations of B. thuringiensis contain a mixture of spores, Cry protein and an inert carrier).

This bacterium was the first bio-pesticide to be used on a commercial scale in the world, and is the first bio-pesticide being produced on a commercial scale in India.

Uses and Advantages

- Biopesticides are used to control or kill some type of pest.
- Biopesticides are made from naturally occurring substances
- Biopesticides can control insect pests by disrupting their mating patterns, attracting them to traps, or being sprayed on the crop as a pest repellent.
- Most biopesticides are used as one component of an integrated pest management approach that enables the gradual control of an insect pest over time.
- Bio pesticides are controlling the pest through indirect mechanisms instead of by directly killing the insect.
- Biopesticides are usually less toxic than conventional pesticides.
- Most are capable of affecting only the target species or closely related species instead of broadly killing all organisms that come into contact with them.
- Biopesticides are often effective at low doses, and they decompose quickly. Hence exposure time is lowered and less pollution is produced.
- Biopesticides are often approved for use faster than conventional pesticides because they pose fewer safety risks.

conventional pesticides are made from synthetic chemicals or agrochemicals. conventional pesticides are used to directly kill pests rather than control them.

Bio-pesticides are of two types: bio-herbicides and bio-insecticides.

(i) Bio-herbicides:

Herbicides are chemicals that are used for inhibiting the growth of plants in unwanted places. Herbicides used for controlling weeds in the cultivated areas are called weedicides. A number of risks are involved in the use of chemical herbicides. This can be avoided if herbicide resistance can be introduced in the crop plants. It is possible through genetic engineering or recombinant DNA technology. Transgenic Tomato and Tobacco plants have been developed which show tolerance to specific herbicides.

Certain crop plants do not allow the weeds to grow nearby. They are called smoother crops, e.g., Barley, Rye, Sorghum, Millet, Sweet clover, Alfalfa, Soybean, and Sunflower. Smoother

crops eliminate weeds through chemicals. Crop rotation with these crops will naturally reduce the incidence of weeds.

Another way of weed control is the introduction of specific insects which feed on the weeds. Extensive growth of Opuntia in India and Australia was checked through the introduction of its natural herbivore, cochineal insect (Cactoblastis cactorum). Similarly, growth of Hypericum perforatum or Klamath weed was checked by U.S.A. through the introduction of Chrysolina beetles.

An organism which controls or destroys unwanted plant growth without harming the useful plant is called bioherbicide. The first bioherbicide happened to be mycoherbicide. It was put to use in 1981. The herbicide is Phytophthora palmivora. The fungus does not allow the Milkweed Vine to grow in Citrus orchards. Growth of Eichhornia crassipes (Water Hyacinth) is being controlled by Cercosporarodmanii in USA and Alternaria eichhorniae in India.

Puccinia chondrilla has controlled the growth of skeleton weed, Chondrillajuncea in Australia. Fungal spores are now available to be sprayed over weeds for their elimination. Two of them are 'Devine' and 'Collego'. The spores are ideal for marketing because they can tolerate adverse conditions and can remain viable for long periods.

(ii) Bio-insecticides:

Bio-insecticides are those biological agents that are used to control harmful insects. They include the following.

(a) Predators:

Destructive insects or plant pests can be brought under control through introduction of their natural predators. The predators should be specific and unable to harm the useful insects. Introduction of ladybugs (Lady Bird Beetles) and Praying Mantis has been successful in combating scale insects or aphids which feed on plant sap.

(b) Parasites and Pathogens:

This is alternate biological control of plant pests through the search of their natural parasites and pathogens. They include viruses, bacteria, fungi and insect parasitoids. Parasitoids are organisms that live as parasites for some time (as early or larval stage) and free living at other times, e.g., Trichogramma. Nucleopolyhedrovirus (NPV) are species specific.

For example, Baculovirus heliothis (a virus) can control Cotton bollworm (HeliothisZea). Similarly, Bacillus thuringenesis (a bacterium) is effective against the cabbage looper (Trichoplausiani) and Entomophthoraignobilis (a fungus) the green peach aphid of Potato (Myzuspersicae). In U.S.S.R. the fungus Beauveria bassiana has been successfully employed in controlling Potato beetle and Codling moth.

(c) Natural Insecticides:

They are insecticides and related pesticides which are obtained from microbes and plants. A number of natural insecticides are available.

The common ones include

(i) **Azadirachtin from Margosa or Neem (Azadirachta indica).** It occurs in Margosa extract. Spray of the same keeps away the Japanese beetles and other leaf eating pests because of the antifeedant property of azadirachtin.

(ii) **Rotenones**. They are powerful insecticides which are harmless to warm blooded animals. Chinese are believed to be first to discover their insecticidal properties. Rotenones are obtained from the roots of Derris elliptica and Lonchocarpusnicou.

(iii) **Squill.** The red variety of Sea Onion (Red Squill, Ureginea maritima) produces a radicide which does not have any harmful effect on other animals,

(iv) **Nicotine.** It is obtained from Nicotiana species. The purified chemical is highly poisonous. Nicotine sulphate is one of the most toxic insecticides,

(v) **Pyrethrum**.It is an insecticide which is obtained from the inflorescence of Chrysanthemum cinerarifolium (Dalmation Pyrethrum), C. coccineum and C. marshallii. The active compounds are pyrethrin and cinerin. Pyrethrin is also used in fly sprays, aerosols, mosquito coils, etc.

(vi) **Thurioside.** It is a toxin produced by bacterium Bacillus thuringenesis. The toxin is highly effective against different groups of insects like moths, flies, mosquitoes and beetles. It does not cause any adverse environmental pollution or disturbance.

Thurioside occurs as crystals in the bacterium. It kills the susceptible insects through inhibiting ion transport in the midgut, formation of pores in gut epithelium, swelling and bursting of cells,

(vii) **Transgenic Plants.** They are crop plants which are modified through genetic engineering to develop natural resistance to insects by inserting cry genes of Bacillus thuringenesis into them, e.g., Bt Cotton. Similarly, transgenic Tomato has been developed which is resistant to homworm larvae.

Selection of Herbal materials:

- The species selected should be the same as that of specified in national pharmacopoeias or recommended by other authoritative national documents of the end user's country.
- In the absence of such national documents, the selection of species or botanical varieties
- Specified in the pharmacopoeia or authoritative national documents of other countries should be considered.
- In the case of newly introduced medicinal plants, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine of the original country.

Identification and Authentication of Herbal Materials:

A. Identification of herbal material:

- The botanical identity includes scientific name (genus, species, subspecies/ variety, author and family) of each of medicinal plant under cultivation should be verified and recorded. If available, the local and English common names should also be recorded.
- Other relevant information, such as cultivar name, ecotype, chemotype or phenotype may also be provided.
- For commercially available cultivars, the name of the cultivar and the name of the supplier should be provided.
- In case of landraces collected, propagated, disseminated and grown in a specified region, records should be kept of the local named line, including the origin of the source seeds, plant or propagation material.
- Herbal material includes whole or fragmented/ cut (including chopped) plants, parts of plants (including leaves, roots flowers, seeds, barks) etc in an unprocessed state usually in dried form are usually used for identification of herbal material.
- Use scientific literature including current editions of National Pharmacopoeias to choose an authoritative literature source.
- If the specimen monograph is available in the official Pharmacopoeia, compliance with all identification tests in the relevant monograph including organoleptic, microscopic, chemical tests etc is required to be carried out.
- Identification tests should be specific for the herbal material and Comparison must include usually a combination of three or more of the following:
    - ❖ Macroscopic characters
    - ❖ Microscopic characters

- ❖ Chromatographic procedures
- ❖ Chemical reactions

- If there is no Pharmacopoeial monograph, manufacturers should use the scientific literature (including current editions of other National Pharmacopoeias like European, United States, Chinese, German, Indian, Japanese Etc) to choose an authoritative source.Other literature sources are not usually acceptable
- A suitable qualified person must perform a comparative analysis between all consignments of herbal sample and the authenticated reference specimen.All morphological testing must be done by a person suitably qualified in the field of botanical authentication is necessary for the morphological examination of samples. The qualified person may or may not be independent of the manufacturer).

B. Authentication of Herbal materials:

- Authenticated reference specimen is a specimen that has been certified by a person suitably qualified in the field of botanical authentication. If the reference specimen is not the whole plant it must contain the same part of the plant as that requiring identification. (Eg: Root compared to root and leaf compared to leaf)
- Herb authentication is a quality assurance process that ensures the correct plant species and plant parts are used as raw materials for herbal medicines. The proper authentication of herbal raw materials is critically important to the safety and efficacy of herbal medicines. Authenticated raw material is the basic starting point in developing a botanical product.
- Authentication is especially useful in cases of drugs that are frequently substituted or adulterated with other varieties which are morphologically and chemically indistinguishable. Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics.
- Evaluation has become even more difficult when several different individual species were powdered and mixed together in a proprietary medicine.

There are different methods for authentication

1. Taxonomic method: This is the initial step in the identification and authentication of herbal materials.The botanical origin of the drug is identified and its scientific Latin binomial (i.e., genus species) name is determined based on this method. Information such as botanical

name, vernacular names, site of collection of plant material, details of collector, habitat, season of collection, altitude and part collected etc. are the essential prerequisites even before authentication.

Herbarium voucher sample: The sample of collected material should be kept as a voucher sample inherbarium or a research institute for future references.

2. Macroscopic method: Macroscopic identity of botanical materials is based on parameters like shape, size, colour, texture, surface characteristics, fracture characteristics, odour, taste and such organoleptic properties that are compared to a standard reference material.
3. Microscopic method: Microscopy is used to determine the structural, cellular and internal tissue features of botanicals. It is usually used to identify and differentiate two herbals that are similar. This is the commonly used technique, convenient, quick and can be applied to proprietary medicines too. Use of polarised and fluorescence Microscope along with ordinary light microscope, expands the number of features available for use in identification. For example, it has been found that starch grains, crystals of calcium oxalate, stone cells, vessels and fibres have stable and special polariscopic characteristics, which has enhanced the accuracy of authentication.
4. Physicochemical methods: Physicochemical parameters include total ash, water soluble ash, acid insoluble ash and sulphated ash. These values of the individual drugs or the proprietary medicines can be compared with the standard values of Indian pharmacopoeia and thus the identity can be ascertained.
5. Chemometric and Spectral methods: Initially the use of infrared (IR) spectroscopic method is restricted only for structural elucidation of isolated compounds from the herbal matrices. It is also found useful in phytochemical studies as a “fingerprinting” device, for comparing a natural with synthetic sample.
6. Chromatographic methods: High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE) and Thin Layer Chromatography (TLC) are the most commonly used analytical methods for herbal products.The analysis of volatile compounds by gas chromatography is very important in chemical analysis of herbal medicines.
7. Chemical Fingerprinting: A chemical fingerprinting is a unique pattern that indicates the multiple chemical markers within a sample.The European Medicines Agency (EMEA) defines chemical markers as chemically defined constituents, or group of constituents of herbal medicinal product which are of interest, regardless whether they possess any therapeutic activity.

8. Molecular markers: DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors. DNA can be extracted from fresh or dried organic tissue of the botanical material and hence the physiological form of the sample for assessment does not restrict detection. Various types of DNA based molecular techniques are utilized to evaluate DNA polymorphisms. These are hybridization-based methods, polymerase chain reaction (PCR) based methods and sequencing based methods

Study of following herbs as health food: Alfalfa, Chicory, Ginger, Fenugreek, Garlic, Honey, Amla, Ginseng, Ashwagandha, Spirulina

ALFALFA

Common name : Lucerne, purple medic, Purple Medick,Buffalo Herb.

Botanical source : It is the dried whole herb, including blossoms of***Medicago sativa*** belongs to the family**Leguminosae**.

Geographical source: Alfalfa is grown in many other parts of the world, from China to Spain, Sweden to North Africa. Outside of cultivation alfalfa occurs as a weed throughout Asia, Europe and America. The largest producerof alfalfa today is North America, South America and Asia.

Chemicalconstituents: Alfalfa contain protein, minerals (Ca, Cu, Fe, Mg, Mn, P, Zn, Si), vitamins (A, B, C, D, E, K, U), phytochemical substances (carotene, chlorophyll, coumarins, isoflavones, alkaloids, saponins), contains secondarymetabolites of plants (phytoestrogens: isoflavones and coumestrol), and antinutritional components (phytates, L-canavanine, saponins).

Uses: Alfalfais used for kidneyconditions, bladderand prostate conditions,and to increase urine flow. It is also used forHigh cholesterol,asthma,osteoarthritis,rheumatoid arthritis,diabetes,upsetstomach,anda bleedingdisordercalled thrombocytopenicpurpura. Peoplealso take alfalfaas a source of vitamins A, C, E, and K4;and minerals calcium,potassium,phosphorous,and iron.

CHICORY

Common names: blue sailors, coffeeweed, cornflower, Italian dandelion, or succory.

Botanical source: Dried leaves and root of ***Chichorium intybus***, **Astraceae**.

Geographical source: Chicory is native to western Asia, North Africa, and Europe.It lives as a wild plant on roadsides in Europe. The plant was brought to North America by early European colonists.It is also common in China, and Australia, where it has become widely naturalized. It is more common in areas with abundant rain.

Chemical constituents: Substances which contribute to the plant's bitterness are primarily the *two sesquiterpene lactones, lactucin and lactucopicrin*. Other components are *aesculetin,*

aesculin, cichoriin, umbelliferone, scopoletin, 6,7-dihydrocoumarin, and further sesquiterpene lactones and their glycosides. It was discovered that the root contains up to 20% inulin, a polysaccharide similar to starch.Raw chicory leaves are 92% water, 5% carbohydrates, 2% protein, and contain negligible fat. In a 100-gram reference amount, raw chicory leaves provide 23 caloriesand significant amounts (more than 20% of the Daily Value) of vitamin K, vitamin A, vitamin C, some B vitamins, and manganese. Vitamin E and calcium are present in moderate amounts.

Uses: reduce inflammation, antimicrobial properties, antioxidant properties, manage blood glucose levels, reduce lipid levels, reduce the proliferation of cancer cells, lower uric acid, positively impact the lipid profile. Inulin fibre in chicory acts as a prebiotic and may help improve digestive health in children, adults and elderly. Additionally, the presence of this fibre stimulates gastric acid, improves bowel movements and may help relieve constipation.Chicory aids the regulation of ghrelin (the hunger hormone) and increases the feeling of satiety. Thus, the consumption of chicory increases feeling of fullness, reduces overeating and may help in weight loss.Chicory contains Manganese and Vitamin B6, both of which are needed for the formation of neurotransmitters (chemical messengers) in the brain and thus, it helps improve brain health.

GINGER

Synonyms: Rhizoma zingiberis, Zingibere.

Biological Source: Ginger consists of the dried rhizomes of the *Zingiber officinale* Roscoe, belonging to family Zingiberaceae.

Geographical Source: It is mainly cultivated in West Indies, Nigeria, Jamaica, India, Japan, and Africa.

Chemical Constituents: Ginger contains 1 to 2% volatile oil, 5 to 8% pungent resinous mass and starch. The volatile oil is responsible for the aromatic odour and the pungency of the drug is due to the yellowish oily body called *gingerol* which is odourless. Volatile oil is composed of sesquiterpene hydrocarbon like α-zingiberol; α-sesquiterpene alcohol α-bisabolene, α-farnesene, α-sesquiphellandrene. Less pungent components like gingerone and shogaol are also present. Shogal is formed by the dehydration of gingerol and is not present in fresh rhizome.

Uses: Ginger is used as an antiemetic, positive inotropic, spasmolytic, aromatic stimulant, carminative, condiment, and flavouring agent. It is prescribed in dyspepsia, flatulent colic, vomiting spasms, as an adjunct to many tonic and stimulating remedies, for painful affections of the stomach, cold, cough, and asthma. Sore throat, hoarseness, and loss of voice are benefited by chewing a piece of ginger.

FENUGREEK

Synonyms: Methi, Methika, Chandrika

Biological source: Fenugreek consists of dried ripe seeds of *Trigonella foenum-graecum* Family: Leguminosae

Geographical source: The plant grows wild in Northern India and is cultivated as a crop throughout India. It is also cultivated in Southern and Eastern Europe, Pakistan, France, Morocco and Egypt.

Chemical constituents: The seed is a good source of calcium, minerals, iron, β-carotene and several vitamins like vitamins A and D. It is rich source of dietary fibre. It is consisting of free amino acids; 4-hydroxyisoleucine, lysine, histidine, and arginine (25.8%), protein (20-30%), moisture (11.76%), fat (6.53%), crude fibre (6.28%), ash content (3.26%) and energy (394.46 Kcal/100 g seed). Fenugreek seeds also contains lecithin, choline, minerals, B. Complex, Phosphates, and Para-Amino Benzoic acid (PABA). In addition, the main chemical compounds in fenugreek are saponins, fenugreekine, trigonelline, coumarin, scopoletin, phytic acid and nicotinic acid.

Uses: Controls diabetes, Breast milk production, Cholesterol, Digestion, Weight management, Antioxidant activity, Inflammation, Fenugreek seeds treat dandruff helps in Hair growth, Anticancer effect, Bone health etc.

GARLIC

Common name: Stinking rose,Rashoon, Ajo,Allium.

Biological source: It is the dried bulb of *Allium Sativum* Linne. Family : Liliaceae.

Geographical source: Garlic is a native of WestAsiaand Mediterraneanarea. China, Korea, India,USA, Spain, Argentinaand Egyptare the major garlic growing countries.

Chemical constituents: Enzymes like-Allinase,peroxidases,catalases,superoxide dismutase, arginases,lipases.Volatile oils-alliin, allicin, allylpropyl disulfide,diallyldisulfide,terpenesinclude citral, geraniol,linalool, α and β-phellandrene.Besides,Proteins(e.g: glutamyl peptides),amino acids (e.g.,arginine, glutamic acid,asparagic acid,methionine,threonine),minerals,vitamins, trace elements.lipids, prostaglandins.

Uses: Lowercholesteroland triglycerides, Kill Worms, Hypertension, helps in blood circulation, Cancerprevention, Diabetes, Bronchitis, Asthma, Allergies, Antithrombotic, antimicrobial, fibrinolytic.

HONEY

Common name: Madhu, Madh, Mel.

Biological source: Honey is a viscid and sweet secretion stored in the honey comb*Apis dorsata, Apis florea, Apis indica, Apis mellifera* Linne. Family: Apideae.

Geographical source: Honey is available in abundance in Africa, India, Jamaica. Australia, California. Chili. Great Britain and New Zealand.

Chemical constituents: The average composition of honey ranges as follows: Moisture 14-24%, Dextrose 23-36%, Levulose (Fructose) 30-47%, Sucrose0.4-6%, Dextrin and Gums 0-7% andAsh 0.1-0.8%. Besides, it is found to contain small amounts of essential oil, beeswax, pollen grains, formic acid, acetic acid, succinic acid, maltose, dextrin, colouring pigments, vitamins and an admixture of enzymes e.g.; diastase, invertase and inulase.

Uses: It is used as a sweetening agent in confectionaries.Being a demulcent, it helps to relieve dryness and is, therefore, recommended for coughs, colds, sore-throats and constipation.Because of its natural content of easily assimilable simple sugars, it is globally employed as a good source of nutrient for infants, elderly persons and convalescing patients.

AMLA

Synonyms: Emblica, Indian goose berry, amla.

Biological Source: This consists of dried, as well as fresh fruits of the plant *Emblica officinalis* Gaerth (*Phyllanthus emblica* Linn.), belonging to family Euphorbiaceae.

Geographical Source: It is a small- or medium-sized tree found in all deciduous forests of India. It is also found in Sri Lanka and Myanmar.

Chemical Constituents: It is highly nutritious and is an important dietary source of vitamin C, minerals, and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains considerably higher concentration of most minerals and amino acids than apples. The pulpy portion of fruit, dried and freed from the nuts contains: gallic acid 1.32%, tannin, sugar 36%; gum 13%; albumin 12 %; crude cellulose 17%; mineral matter 4%; and moisture 3%. Tannins are the mixture of gallic acid, ellagic acid, and phyllembin. The alkaloidal constituents such as phyllantidine and phyllantine have also been reported in the fruits.

Uses: The fruits are diuretic, acrid, cooling, refrigerant, and laxative. Dried fruit is useful in haemorrhage, diarrhoea, diabetes, and dysentery. They are useful in the disorders associated with the digestive system and are also prescribed in the treatment of jaundice and coughs. It has antioxidant, antibacterial, antifungal, and. antiviral activities. Amla is one of the three ingredients of the famous ayurvedic preparation, triphala, which is given to treat chronic dysentery, and other disorders, and it is also an ingredient in chyavanprash.

GINSENG

Synonyms: Panax, Asiatic Ginseng, Chinese Ginseng, Ginseng Root, Pannag, Ninjin.

Biological Source: It consists of dried roots of *Panax ginseng* and other species of Panax like *Panax japonicus* (Japanese Ginseng), *Panax pseudoginseng* (Himalayan Ginseng), *Panax quinque-folius* (American Ginseng), *Panax trifolius* (Dwarf Ginseng) and *Panax vietnamensis*(Vietnamese Ginseng), belonging to family Araliaceae.

Geographical Source: It is mainly found in China, Russia, Korea, Japan, Canada and India.

Chemical Constituents: Several saponin glycosides belonging to triterpenoid group, ginsenoside, panxoside. More than 13 ginsenosides have been identified. Ginsenosides consists of aglycone dammarolwhereas panaxosides have oleanolic acid as aglycone. It also contains large amount of starch, gum, some resin and a very small amount of volatile oil.

Uses: The root is adaptogen, alterative, carminative, demulcent, emetic, expectorant, stimulant and tonic. The saponin glycosides, also known as ginsenosides or Panaxosides, are thought responsible for Panax ginseng's effects. Ginsenosides have both stimulatory and

inhibitory effects on the CNS, alter cardiovascular tone, increase humoral and cellular-dependent immunity, and may inhibit the growth of cancer in vitro. It encourages the secretion of hormones, improves stamina, lowers blood sugar and cholesterol levels. It is used internally in the treatment of debility associated with old age or illness, lack of appetite, insomnia, stress, shock and chronic illness.

ASHWAGANDHA

Synonyms: Withania root. Ashwagandha, Clustered Wintercherry.

Biological Source: It consists of the dried roots and stem bases of *Withania somnifera* Dunal, belonging to family Solanaceae.

Geographical Source: Withania is widely distributed from southern Europe to India and Africa.

Chemical Constituents: The plants contain the alkaloid withanine as the main constituent and somniferine, pseudowithanine, tropine and pseudotropine, hygrine, isopellederine, anaferine, anahygrine and steroid lactones. The leaves contain steroid lactone, commonly known as withanolides.

Uses: All plant parts are used including the roots, bark, leaves, fruit and seed are used to treat nervous disorders, intestinal infections and leprosy. Ashwagandha is one of the most widespread tranquillizers used in India, where it holds a position of importance similar to ginseng in China. It acts mainly on the reproductive and nervous systems, having a rejuvenate effect on the body, and is used to improve vitality and aid recovery after chronic illness. It is also used to treat nervous exhaustion, debility, insomnia, wasting diseases, failure to thrive in children, impotence, infertility; multiple sclerosis, etc.

SPIRULINA

Common name: Blue-green algae.

Botanical source: Spirulina is a cyanobacterium that can be consumed by humans and other animals and is made primarily from two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima* Linne. Family: Oscillatoriaceae

Geographical source: Spirulina is grown in Hawai, United States, India, Thailand, and Taiwan.

Chemical constituents: It contains 65% Protein and Amino Acids,20% Carbohydrates,7% Minerals, 5% Fats, 3% moisture, vitamins B1, B2, B3, B6, B12 and E.

Uses: Antioxidant, Immunomodulation, appetite stimulant andAnti-viral effects.

EVALUATION OF CRUDE DRUGS

❖***Drug evaluation may be defined as the determination of a drug's identity, purity and quality.***

✓***Identity*** – Identification of biological source of the drug.

✓***Quality*** – quality of the active constituents present.

✓***Purity*** – the extent of foreign organic material present in a crude drug.

Importance of evaluation of crude drugs:

✓Determination of Biochemical variation in the drugs

✓Identification of deterioration due to treatment and storage

✓Reporting Substitution and adulteration, as a result of carelessness, ignorance, and fraud

METHODS OF DRUG EVALUATION

- ***Morphological evaluation***
- ***Microscopical evaluation***
- ***Physical evaluation***
- ***Chemical evaluation***
- ***Biological evaluation***

MORPHOLOGICAL EVALUATION

- Organoleptic evaluation, study of morphology and other sensory characters.
- Based on color, odour, taste, size, shape, texture etc.

Part of drug	Examples
Bark	Cinnamon
Underground part	Ginger, turmeric
Leaves	Digitalis
Flowers	Saffron
Fruits	Fennel
Seeds	Nuxvomica
Entire plant	Vinca

Character	Example
Aromatic odour	Umbelliferous fruits
Sweet taste	Liquorice
Fractured surface	Cinchona
Pungent taste	Ginger
Brown colour	Cinnamon
Wavy shape	Rauwolfia
7-8 mm width, 25-60 mm length	Senna leaf

Study of sensory characters

Flowers: Floral parts: stigmas, corollas, anther, ovary, receptacle.

Leaves and leaflets: Length, width, apex, margin, base, venation, the texture of the leaf and the hairs in upper and lower surface. The feel of the surface described as soft, hairy smooth.

Bark: Flat or curved pieces, Single quill, Double quills, Barks have two surfaces, an outer and inner, The inner surface is usually lighter in color than the outer surface

- **Odor and taste:** distinct, indistinct, aromatic, balsamic, spicy
- **Taste:** Acidic (sour), Saccharine (sweet), Saline (salty), Alkaline, Bitter, Tasteless, Distinctive sensations to the tongue like Mucilaginous and oily (soft feeling), Astringent, Pungent (warm biting sensation), Acrid (irritant sensation), Nauseous (those tending to excite vomiting).

COLOUR AND EXTERNAL MARKINGS	
White	Starch
Pale yellow	Ginger, Squill, White pepper
Deep yellow	Peeled liquorice
Pale green	Lobelia
Greenish brown	Most of the leaf herbs
Light brown	Nuxvomica, fennel
Dark brown	Clove buds
Dark reddish brown	Cinchona
Brick red	Cinnamon bark inner

MICROSCOPIC OR ANATOMICAL EVALUATION

- This method allows a more detailed examination of a drug and it can be used to identify organized drugs by their known histological characters.
- Before examination through a microscope the material must be suitably prepared.
- This can be done by powdering, cutting thin sections of the drug or preparing a macerate.
- This method is used to identify organized drug by their known histological characters through Transverse section (T.S.) or Longitudinal Section (L.S.) or Radial Longitudinal Section (R.L.S.) or Tangential Longitudinal Section (T.L.S.).

LEAF CONSTANTS

- Palisade Ratio
- Stomatal Number
- Stomatal Index
- Vein- islet number
- Vein termination number
- Trichomes
- Calcium oxalate crystals
- Lycopodium spore method

Examples:

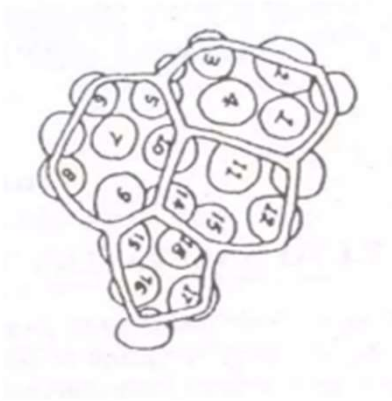
- ✓ Powder of clove stalk contain sclereids and calcium oxalate crystals, but cloves does not.
- ✓ Sclerenchyma absent in *Rauwolfia Serpentina* root but present in *Rauwofia micratha*, *R. densiflora*.
- ✓ Stone cell present in frangula bark and absent in cascara bark.
- ✓ Lignified Trichome and plasmodesmeta in nux-vomica.

1. Palisade ratio

- The average number of palisade cells beneath each epidermal cell is known as the palisade ratio.
- It is an important parameter for determination and characterization of leafy drugs.
- Determined using Camera lucida.

Eg;

- *Atropa belladonna*: 5-10
- *Adhatoda vasica*: 5.5-6.5
- *Cassia angustifolia*: 5.5-10
- *Digitalis lanata*: 2.5-6.5



2. Stomatal number

- It is defined as average number of stomata per square millimeter area of epidermis.

Eg;

- *Atropa belladonna*: UE: 7-10, LE: 77-115
- *Datura metel*: UE:147-160, LE: 200-209
- *Ocimum sanctum*: UE: 64-72, LE: 175-250

Stomata

- Stomata are minute pores or openings in the epidermis layer of the leaves, young stems, and some other parts of green plants which form an important constituent of their functioning.
- There are several types of stomata, distinguished by the forms and arrangement of the Surrounding cells.

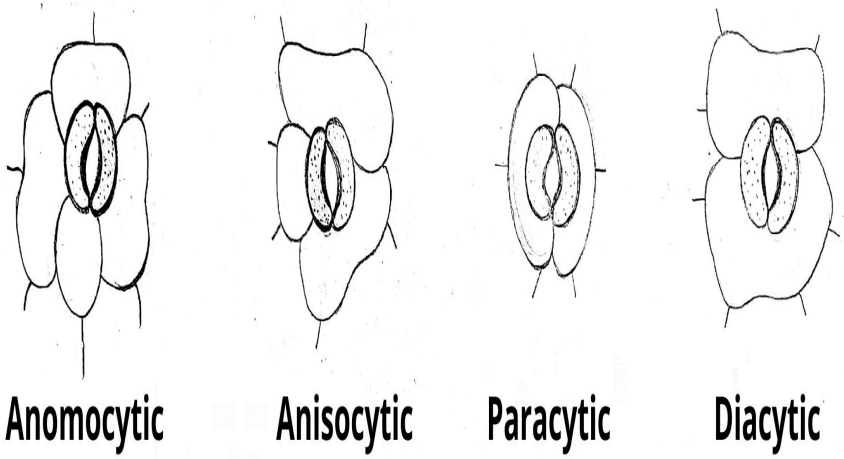
e.g.:

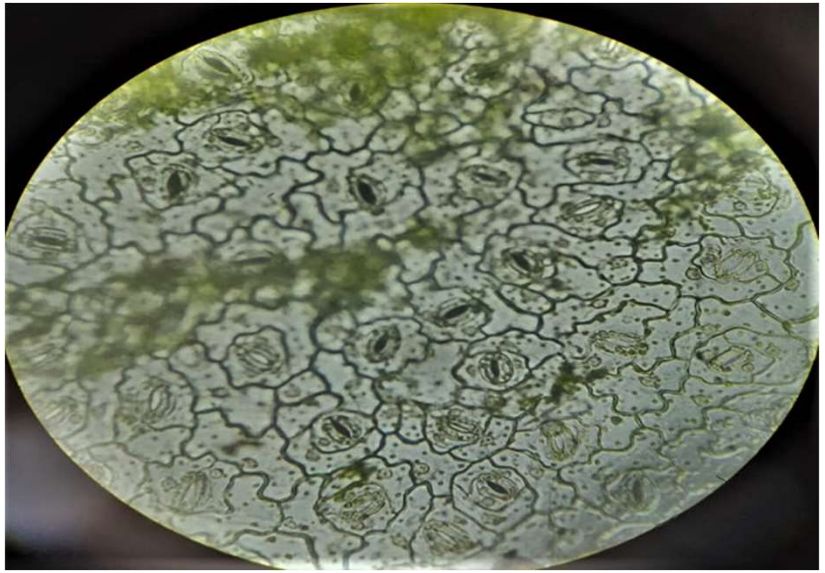
✓ **Anomocytic** (Ranunculaceous) irregular celled: Digitalis

✓ **Anisoytic** (Cruciferous) unequal – celled: Datura

✓ **Diacytic** (Caryophyllaceous) cross – celled: Mentha

✓ **Paracytic** (Rubiaceous) parallel celled: Senna





3. Stomatal index

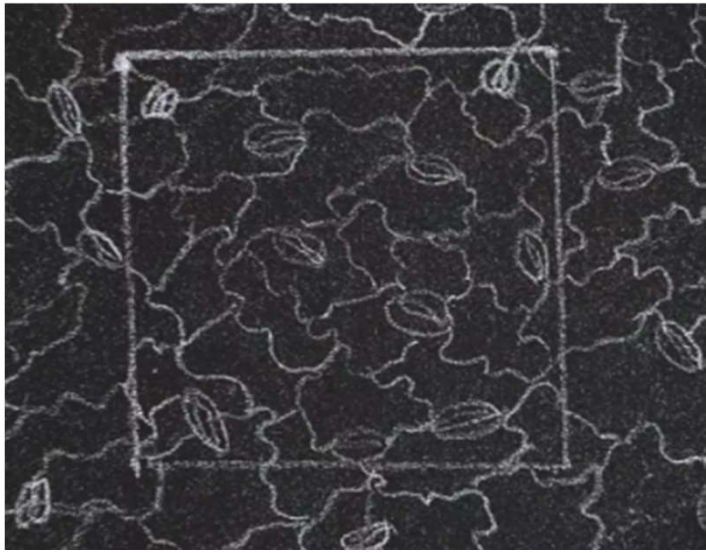
- Stomatal index (I) is the average number of stomata cells present per square millimeter of the epidermis of the leaf.
- It is the percentage proportion of the number of stomata to the total number of epidermal cells.
- Stomatal index can be calculated by using the following equation:

$$S.I = \frac{S}{E+S} * 100$$

- S= Number of stomata per unit area
- E= Number of epidermal cells in the same unit area.

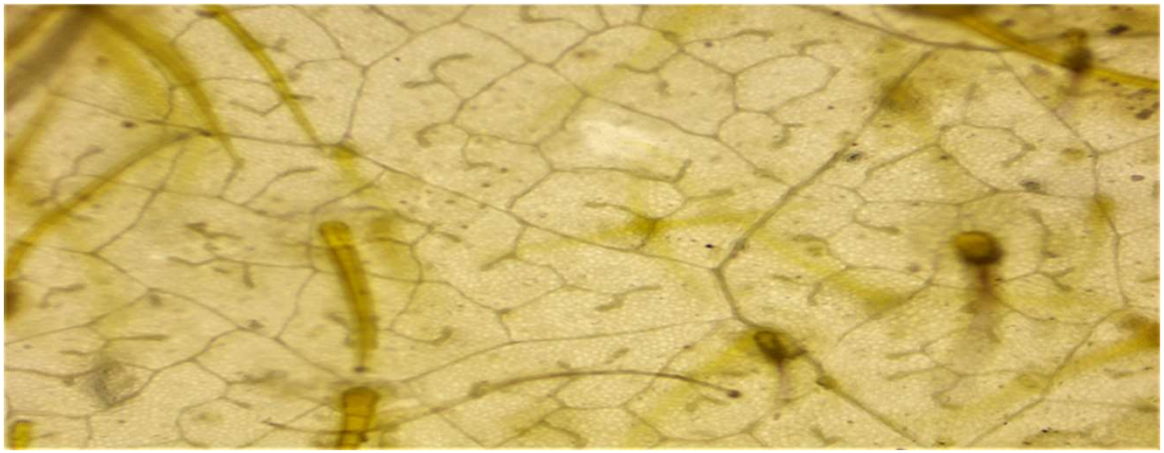
Examples:

- *Atropa belladonna*:-2.3-3.9 to 10.5 (Upper Surface), 20.2 to 23.0 (Lower Surface)
- *Digitalis Purpurea*- 2.7 to 4.0 (Upper Surface), 19.2- 25.2 (lower Surface).



4. Vein- islet number

- It is defined as average number of Vein Islet per square millimeter of the leaf surface midway between midrib and the margin.
- It is constant for a given species of the plant and is used as a character for the identification of the allied species.

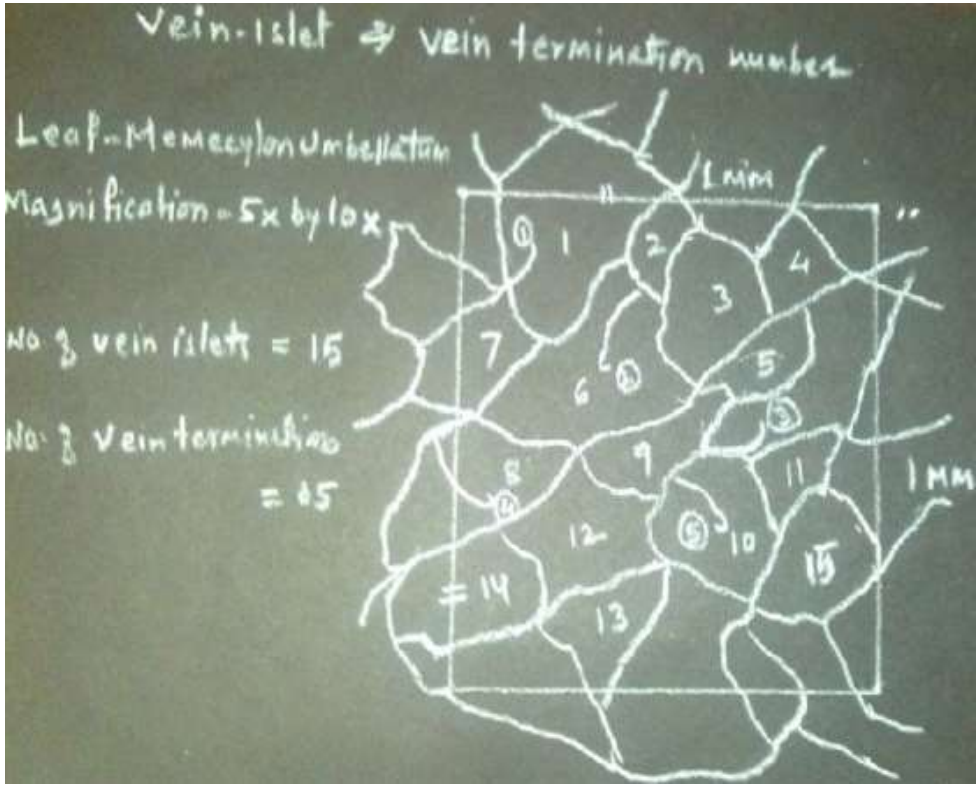


Examples:

NAME OF DRUG	Vein-islet Range
Andrograohis paniculata	9-12
Bacopa monniera	6-13
Cannabis sativa	18-24
Digitalis purpurea	2.5-3
Eucalpytus globules	8-13.5

5. Vein- termination number

- It is defined as average number of Vein terminations per square millimeter of the leaf surface midway between midrib and the margin.

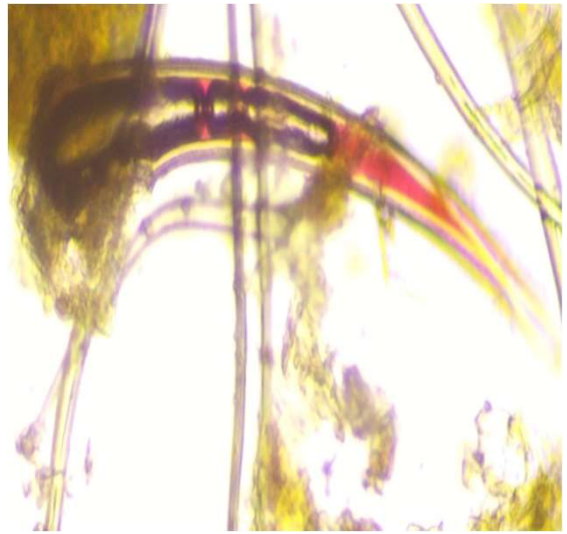


Examples:

- *Atropa belladonna* — 6.3-10.3
- *Atropa acuminate* — 1.4-3.5

6. Trichomes

- Trichomes are the tubular elongated or glandular outgrowth of the epidermal cells. Trichomes are also called as plant hairs. Trichomes consists of two parts root and body. Trichomes present in most of plant parts and are function less but some times perform secretory function.

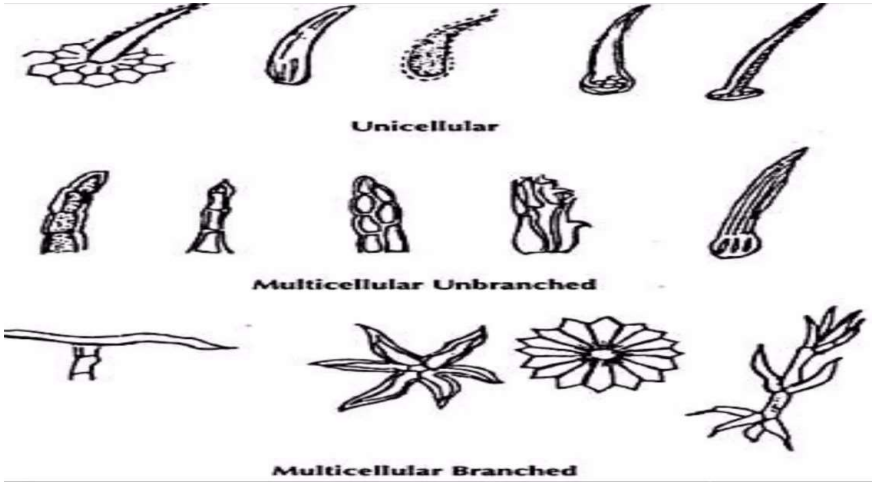


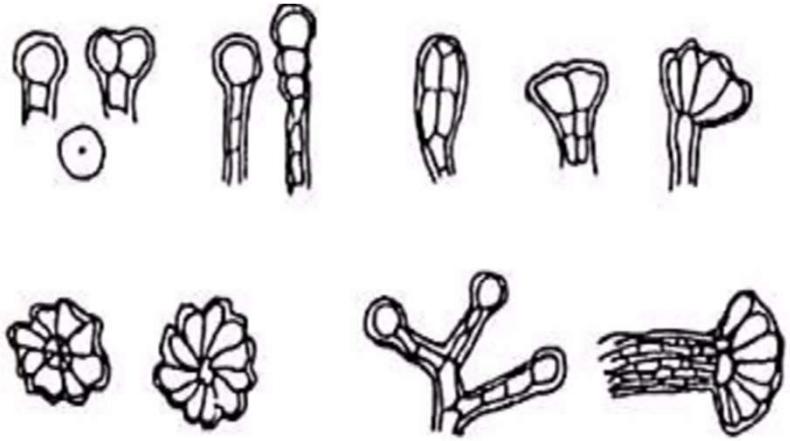
- Trichomes are divided and subdivided as follows;
- **Covering Trichomes:**
  - ✓ Unicellular Trichomes: Nux vomica, Cannabis
  - ✓ Uniseriate Multicellular Unbranched Trichomes: Datura
  - ✓ Biseriate Multicellular unbranched Trichomes: *Calendula officinalis*
  - ✓ Multiseriate Multicellular unbranched Trichomes: Male fern
  - ✓ Multicellular branched Trichomes: *Verbascum Thapsus*

- **Glandular Trichomes:**
  - ✓ Unicellular Glandular Trichomes: Vasaka
  - ✓ Multicellular Glandular Trichomes: *Digitalis purpurea*

Other types are;

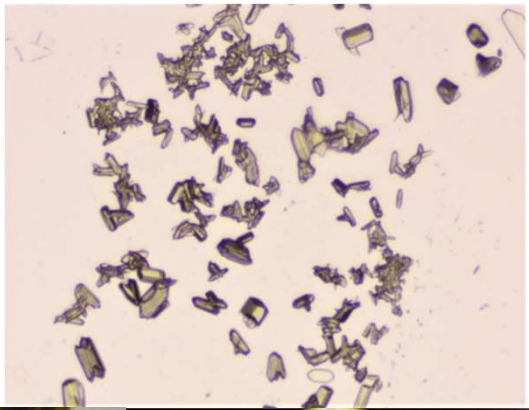
Glabrous, glabrate, hirsute, hispid, articulate, downy, pilose, puberulent, pubescent, strigillose, strigose, tomentellous, tomentose, villosulous, villous.





7. Calcium oxalate crystals

- The inorganic crystalline compounds by virtue of their specific shapes can be utilized for the identification of herbal drugs.
- Due to this reason they are called as **diagnostic characters of the plant**.



✓ **Cubical** (cube shape): Senna, Glycyrrhiza.

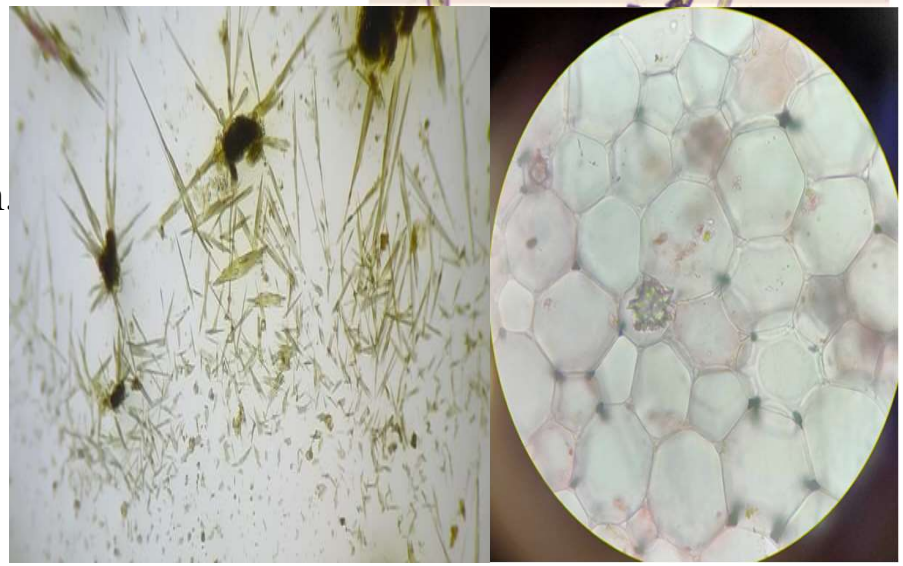
✓ **Rhombic** (diamond shape)

✓ **Tetragonal**: onion.

✓ **Acicular** (long slender, pointed, bundles): Squill, Cinnamon.

✓ **Rosettes** –clusters (aggregation of crystals): Clove, Arjuna.

✓ **Microsphenoidal** (minute in structures): Henbane.

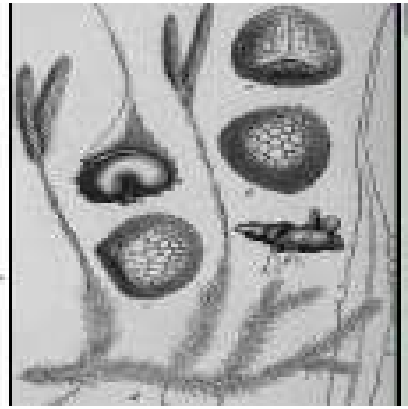
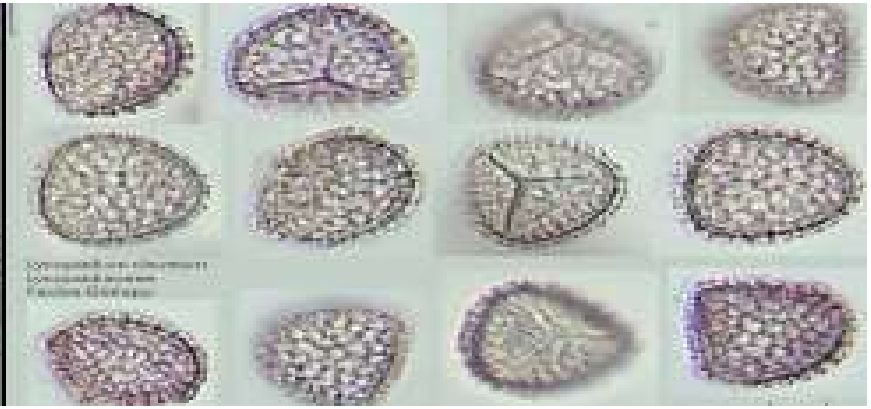


8. LYCOPODIUM SPORE METHOD

- Quantitative method
- It is used when especially chemical and other methods of evaluation of drugs fail to determine quality.
- Lycopodium spores are obtained from club moss, *Lycopodium clavatum* Linn., belonging to family Lycopodiaceae.
- The spores are yellow in colour, spheroidal, tetrahedral in shape with reticulate surface.
- They have uniform average diameter of 25 microns. One milligram contains average 94000 spores.
- They have uniform moisture content, hence the weight remains the same.
- This is the reason, why these spores are used to evaluate powdered drugs by comparison. The spores are also resistant to pressure.

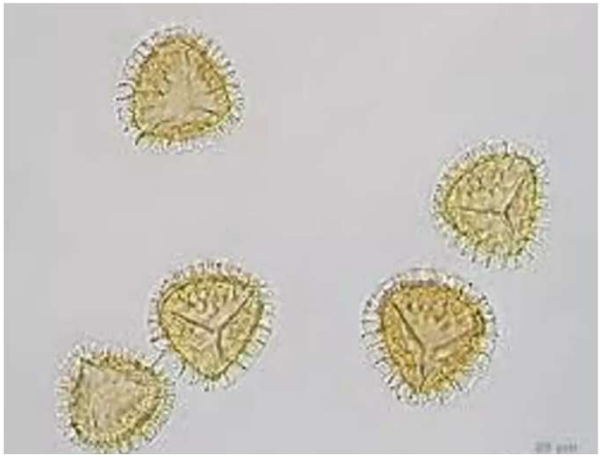
A powdered drug can be evaluated by comparison with lycopodium spores if it contains;

1. Well defined characteristic particles which can be counted (starch grains, pollen grains etc.)
2. Single layered cells or tissues, the area of which may be traced under suitable magnification and actual area calculated (eg: epidermal area of senna leaf).
3. The objects of uniform thickness, the length of which can be measured under suitable magnification and actual area calculated (eg: trichomes).

PROCEDURE

- Dry the powdered drug at 105^0 C and determine its steady weight at room temperature.
- Weigh accurately 200 mg of test material and 100 mg of lycopodium spore powder and mix with a glass rod or spatula uniformly.
- Make a thin smooth paste by adding 5 ml suspending medium (oil).
- Place a drop of above suspension on a glass slide, spread with a needle and put coverslip and observe under microscope in 10X.
- Count the characteristic particles of test and spores in the field.
- Take a count of 25 fields.
- Repeat the step in four times to get an average set of counts.



- Calculate the percentage purity using the formula;

$$\%\ purity = \frac{n * w * 94000 * 100}{s * m * p}$$

n= number of characteristic particles in 25 fields

s= number of lycopodium spores in the same 25 fields

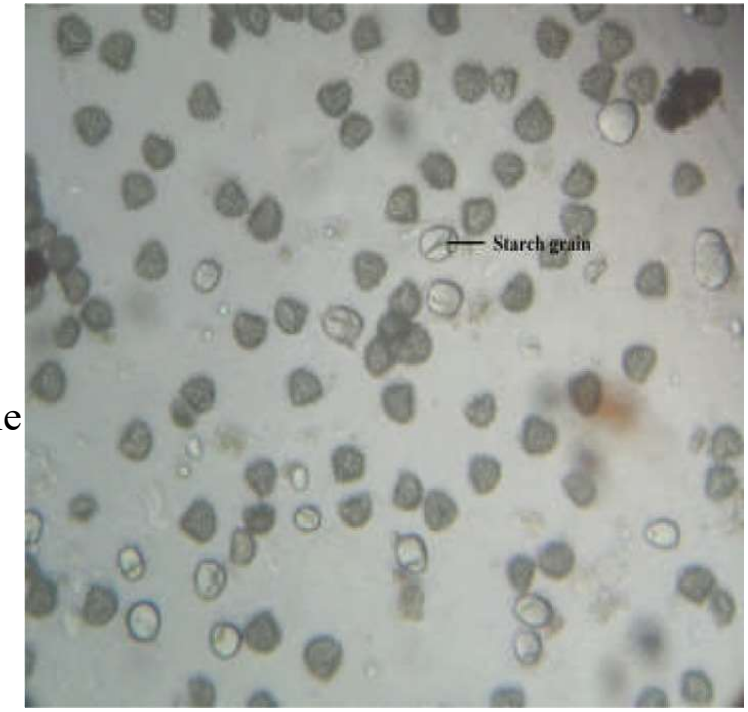
w= weight in mg of lycopodium spore powder taken

m= weight of the test sample taken in mg

p= standard value for number of characteristic particles per mg of pure sample

Eg: in case of starch grain it is 286000

94000= number of spores per mg of lycopodium spore powder



Applications

- Determine the size of the particles in powder such as fragments of leaves, isolated fibers.
- Lycopodium spore method can be used to evaluate powdered clove, ginger, cardamom, nutmeg, umbelliferous fruits etc.
- Determine the percentage of each powder particles in a mixture
- Determine the length of lignified ribs of trichomes in Nuxvomica
- Determine the Nuxvomica content of two veterinary medicines.

PHYSICAL EVALUATION

- For determining physical standards of a crude drug.
- In crude plant evaluation, physical methods are often used to determine the ***solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, degree of fibre elasticity***, and other physical characteristics of the herb material.

Foreign matter	**Loss on drying**	Refractive Index	Optical Rotation
Solubility	Specific Gravity	Moisture Content	Viscosity
Ash values	Ultraviolet Light	Melting Point	**Extractive values**

1. Solubility

- Drugs-specific behavior towards solvents is taken into consideration. This is useful for the examination of many oils, oleoresins, etc.

DRUG	SOLUBILITY
Castor oil	Soluble in 3 volumes of alcohol
Balsam of Peru	Soluble in chloral hydrate solution
Asafoetida	Soluble in carbon disulphide
Alkaloid bases	Soluble in chloroform
colophony	Soluble in light petroleum

2. Optical Rotation

- Many substances of biological origin, having a chiral centre, can rotate the plane of polarised light either to right (dextro rotatory)or to the left (laevo).
- The extent of rotation is expressed in degrees, plus(+) indicating rotation to the right and minus(-) indication rotation in the left. Such compound are optically active and hence called optical rotation.

Drugs	Angles of Optical Rotation
Caraway oil	+ 75° to +80°
Clove oil	0° to +6.0°
Honey	+3° to -15°

3. Refractive Index

- When a ray of light passes from one medium to another medium of different density, it is bent from its original path.
- Thus, the ratio of velocity of light in vaccum to its velocity in the substance is said to the Refractive index of the second medium.
- It is measured by means of refractometer. RI of a compound varies with the wavelength of the incident light, temperature and pressure.

Eg: **Arachis oil: 1.4678- 1.470**

Clove oil: 1.527-1.535

4. Specific Gravity

- It is also known as relative density.
- The ratio of the mass of a solid or liquid to the mass of an equal volume of distilled water at 4°C (39°F) or of a gas to an equal volume of air or hydrogen under prescribed conditions of temperature and pressure.
    - Eg: **cottonseed oil: 0.88–0.93**

Coconut oil: 0.925

Castor oil: 0.95

5. Viscosity

- Viscosity is the resistance of a fluid to flow.
- The viscosity of a liquid is constant at a given temperature and is an index of its composition.
- Hence, it is used as a means of standardizing liquid drugs.
    - Eg: **Liquid paraffin: not less than 64 centistokes**

      Pyroxylin: 1100-2450 centistokes

6. Melting Point

- The melting point of a solid is the temperature at which it changes state from solid to liquid.
- Plant constituents have very sharp and constant melting points.
- It is one of the parameters to judge the purity of crude drugs containing lipids as constituents.
- The purity of the crude drugs can be ascertained by determining their melting points in the range shown against each of them.

Eg: **Beeswax: 62–65°C**

Wool fat: 34–44°C

Agar: 85°C

7. Moisture content

- The moisture content of a drug will be responsible for the decomposition of crude drugs either producing chemical change or microbial growth.
- So the moisture content of a drug should be determined and controlled.
- The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.

Eg: **Digitalis: 5% w/w**

Ergot: 8% w/w

8. Foreign Organic Matter

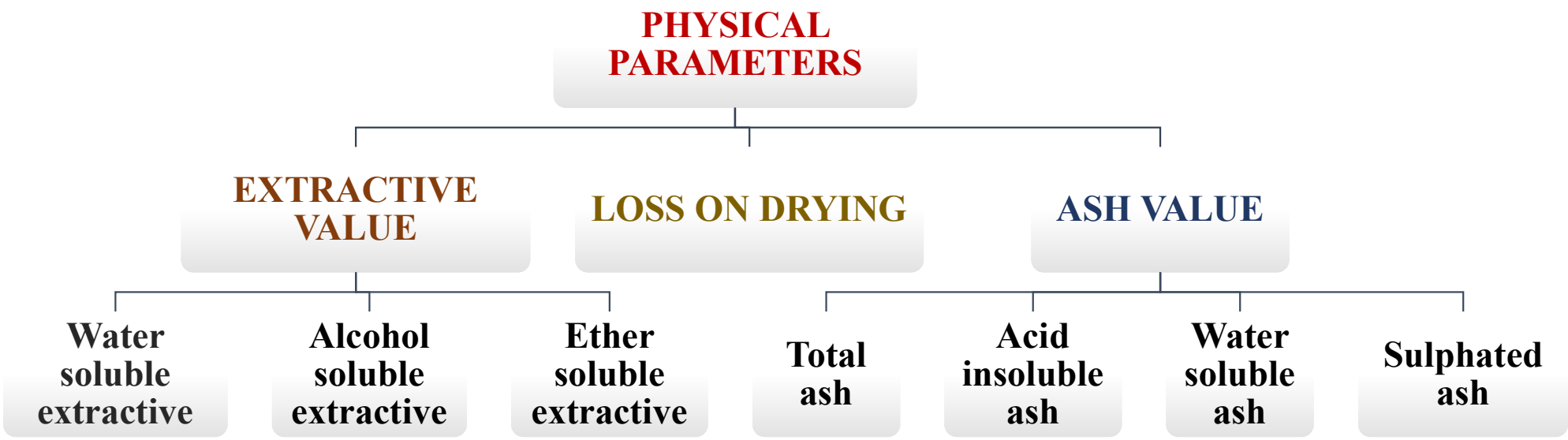
- The parts of the organ or organs other than those parts of drugs mentioned in the definition and description of the drug are known as foreign organic matters.
- They may be insect, molds, earthy material, animal excreta, etc.
- Each and every vegetable drug has its own limits.

Eg: **Garlic should not contain more than 2%**

Saffron should not contain more than 2%

Satavari should not contain more than 1%, etc.

IMPORTANT PHYSICAL PARAMETERS



ASH VALUE

- The residue left after incineration of the drug contains inorganic elements present in the plant is called as physiological ash.
- The determination of ash is useful for detecting low-grade products, exhausted drugs, and excess sandy or earthy matter.
- Ash values are helpful in determining the quality as well as purity of a crude drug, especially when the drug is present in powdered form.
- The object of ashing crude drugs is to remove the traces of organic matter which may interfere in an analytical determination.
- On incineration, the crude drugs normally produce ash which is usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.
- The total ash of a crude drug reveals the care taken during its preparation. A higher limit of acid-insoluble ash is incorporated especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.
- Ash value is a criterion to judge the identity or purity of the crude drug.
- The ash value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs.

1. Total Ash value

- Organic and carbon matter present in the drug is converted to ash at temperature of 450°C or above.
- It mostly contains carbonates, Phosphate, Silicate and Silica.
- Total ash value can be used further study water soluble and acid insoluble ash.

Procedure

Take about 2 or 3 g, accurately weighed, of the ground drug in a tarred platinum or silica dish previously ignited and weighed.

Incinerate by gradually increasing the heat until free from carbon, cool, and weigh.

If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water

Calculate the percentage of ash with reference to the air dried drug.

2. Water-soluble ash value

- It is introduced by separating the **water soluble material** which is direct to yield water soluble ash.
- In this case most of water insoluble salt may contribute in total ash, or remove to find out the water soluble contents.

Procedure

The total ash obtained was boiled for 5 minutes with 25ml of water.

↓

Collected the insoluble matter on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°c.

↓

Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash.

↓

Calculated the percentage of water-soluble ash with reference to the air-dried drug.

3. Acid insoluble ash value

- Total ash may be treated with **dilute hydrochloric acid** which removes any inorganic salts to yield many silica in the residue at acid-insoluble ash.

Procedure

The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid.

Collected the insoluble matter on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°c.

Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the acid insoluble ash.

Calculated the percentage of acid insoluble ash with reference to the air-dried drug.

4. Sulphated ash value

- The crude drug is incinerated at a temperature about 600°C with dilute sulphuric acid before ignition. This process converts all amides and carbonates to sulphate salt.

Procedure

Heated a platinum crucible to redness for 10 minutes, allowed to cool in a desiccator and weighed.

Put 3g of the powdered drug, accurately weighed, into the crucible, ignited gently at first, until the substance is thoroughly charred..

Cooled and moistened the residue with 1 ml sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800±25 ^{o} C until all black particles have disappeared.

The crucible was cooled, few drops of sulphuric acid was added and again heated.

The ignition was carried out as before, cooled and weighed to get a constant weight. Calculated the percentage of sulphated ash with reference to the air-dried drug

EXTRACTIVE VALUE

- Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method.
- Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs.
- Crude drugs contain a number of constituents and these have a selective solubility in different solvents.
- ***Alcohol, water and ether are used as solvents to prepare ethanol-soluble extractive, water soluble extractive (chloroform water), ether soluble extractive*** respectively.
- Extractive values indicate the presence of different constituents and TLC fingerprints can be developed for identification and semi-quantitative analysis from these extracts.

Procedure

5 g of accurately weight powdered air-dried drug was macerated with 100 ml of alcohol/water/ether in a closed flask for twenty-four hours, shaking frequently during six hours and allow standing for eighteen hours

It was then filtered rapidly, taking precautions against loss of solvent.

25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish and dried at 105°C to a constant weight and weighed.

The percentage of alcohol/water/ether soluble extractive was calculated with reference to the air-dried drug.

Significance

- ✓ Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents.
- ✓ Extractive values are primarily useful for the determination of exhausted or adulterated drugs and determines the quality as well as purity of the drug.
- ✓ Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating.

LOSS ON DRYING

- It is the loss of weight expressed as percentage w/w. It determines the amount of volatile matter of any kind (including water) that can be driven off under the conditions specified.

$$\text{Loss on drying (\%)} = \frac{\text{initial weight of sample - weight of sample after drying x 100}}{\text{Initial weight of sample}}$$

PROCEDURE

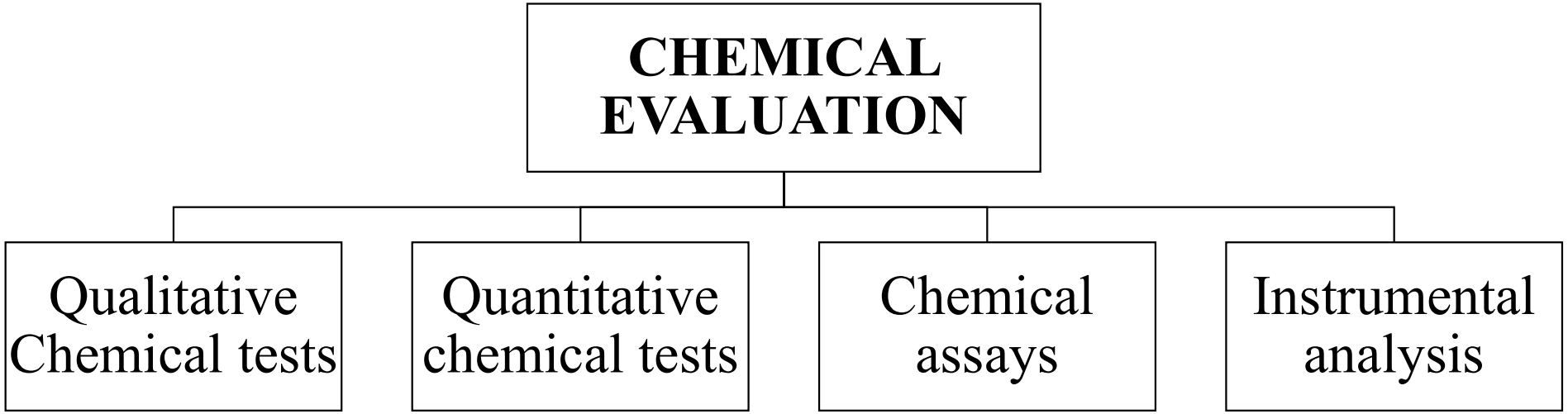
Accurately weighed about 1.5 g of powdered drug in a tarred porcelain dish and dried in an oven at 105°C to constant weight, cooled in desiccator and weighed. From the difference in weights, percentage loss on drying was calculated.

SIGNIFICANCE

Loss on drying is a widely used test method to determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample.

CHEMICAL EVALUATION

- Evaluation by chemical means;



2. Quantitative chemical tests

a) Acid value
b) Saponification value
c) Ester value
d) Acetyl value
e) Peroxide value
f) Hydroxyl value

These tests are helpful in the evaluation of drugs like volatile oil (acetyl and ester value), balsams (acid, esters, saponification value), resins (acid value) etc.

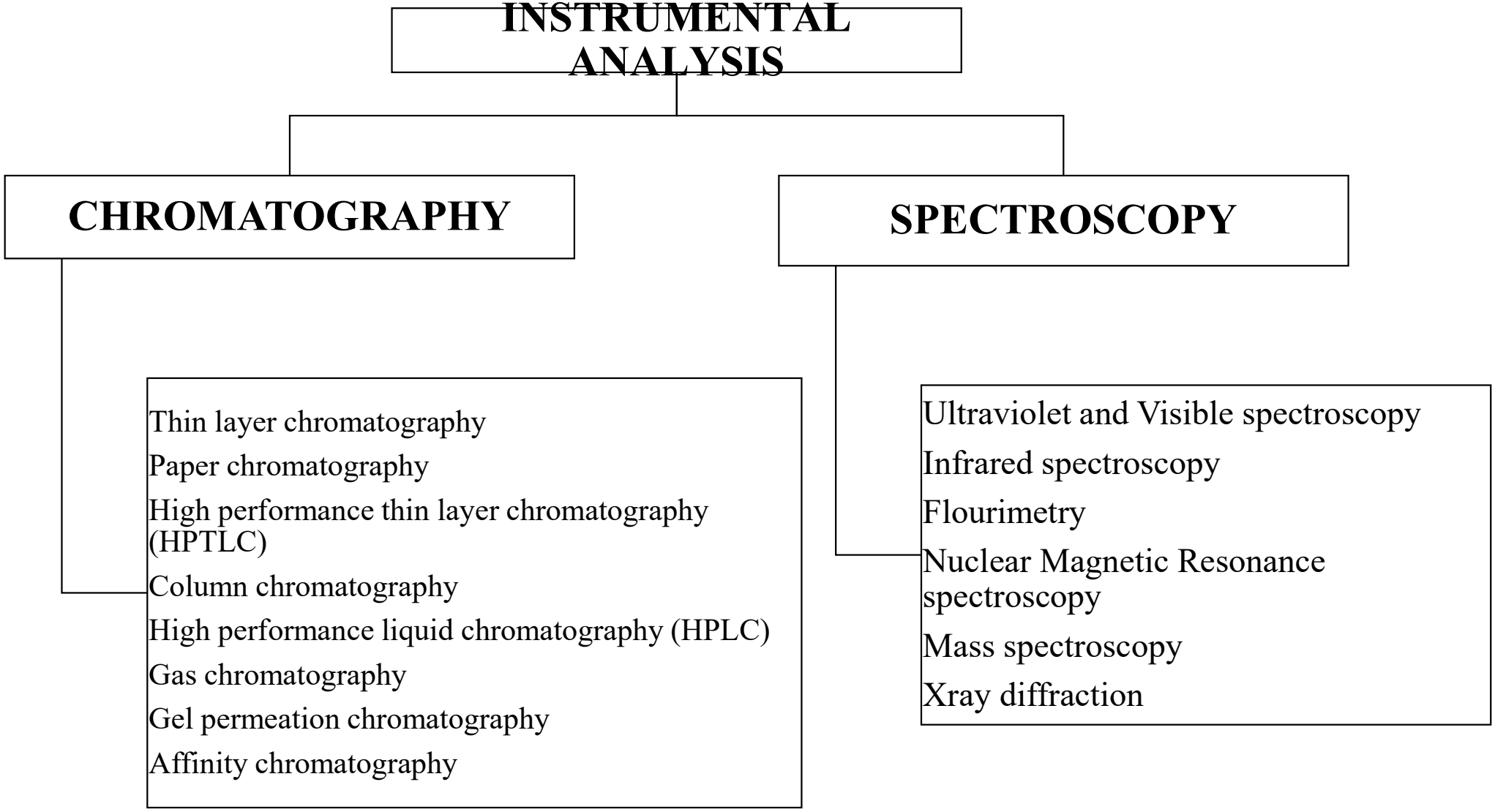
3. Chemical assays

- Assayed for a particular group of constituents.
- Commonly used techniques are **titrimetric and gravimetric methods**.

By titrimetric methods;

✓ Alkaloids can be estimated from alkaloidal drugs; eg: quinine from cinchona, reserpine from rauwolfia, atropine from belladonna
✓ Anthraquinone and cardiac glycosides.
✓ Estimation of carvone in dill oil, cineole in eucalyptus oil, aldehydes in lemon oil.

4. Instrumental analysis



BIOLOGICAL EVALUATION

- ✓ For the drugs cannot be evaluated using chemical & physical methods.
- ✓ Response produced on a test drug is compared with standard drug solution.
- ✓ Performed on living animals, isolated organ and tissue, animal preparations & micro-organisms.

Indication of Biological Evaluation:

- ✓ When the chemical nature of the drug is not known but is has an biological action.
- ✓ When chemical methods are not available.
- ✓ When the quantity of the drug is small, it cannot be evaluated chemically.
- ✓ Drugs which have different chemical composition but same biological activity. Example: Cardiac glycosides are evaluated by this method on cats, frogs or pigeons.

Examples;

- Antipyretic activity
- Anti-inflammatory activity
- Hypoglycaemic activity
- Antiulcer activity
- Analgesic activity
- Microbiological assays
- Anthelminthic activity

PHARMACOLOGICAL EVALUATION

- **Bitterness value**: Units equal to bitterness of std solution of quinine hydrochloride.
- **Heamolytic activity**: Ox blood comparison with std saponin.
- **Swelling index**: In water.
- **Foaming index**: Foam height produced by 14 gm material under std. conditions.

TOXICOLOGICAL EVALUATION

- **Pesticide residue**: estimation of chloride, phosphorus, lead, inorganic pesticides.
- **Arsenic & heavy metals**: limit tests
  - **Arsenic**: Stain produced in the $HgBr_2$ paper compared to std. stain.
  - **Heavy metals**: Atomic emission spectroscopy.
- **Microbial contamination**: maximum possible limits are determined. (E.coli, Salmonella, Enterobacteria)
- **Aflatoxins**: naturally occurring mycotoxins produced by Aspergillus species, determined by chromatographic methods using std aflatoxins B_1, B_2, G_1, G_2 mixtures.

11. Determination of bitterness value

Medicinal plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice.

Bitter substances can be determined chemically. However, since they are mostly composed of two or more constituents with various degrees of bitterness, it is first necessary to measure total bitterness by taste.

The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride R. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml.

Safe drinking-water should be used as a vehicle for the extraction of plant materials and for the mouth-wash after each tasting. Taste buds dull quickly if distilled water is used. The hardness of water rarely has any significant influence on bitterness.

Sensitivity to bitterness varies from person to person, and even for the same person it may be different at different times (because of fatigue, smoking, or after eating strongly flavoured food). Therefore, the same person should taste both the material to be tested and the quinine hydrochloride solution within a short space of time. The bitter sensation is not felt by the whole surface of the tongue, but is limited to the middle section of the upper surface of the tongue. A certain amount of training is required to perform this test. A person who does not appreciate a bitter sensation when tasting a solution of 0.058 mg of quinine hydrochloride R in 10 ml of water is not suitable to undertake this determination.

The preparation of the stock solution of each individual plant material (S_T) should be specified in the test procedure. In each test series, unless otherwise indicated, the determination should start with the lowest concentration in order to retain sufficient sensitivity of the taste buds.

Recommended procedure

Caution: This test should not be carried out until the identity of the plant material has been confirmed.

Preparation of solutions

Stock and diluted quinine hydrochloride solutions
Dissolve 0.100g of quinine hydrochloride R in sufficient safe drinking-water to produce 100 ml. Further dilute 5 ml of this solution to 500 ml with safe drinking-

Table 1
Determination of bitterness value: serial dilution for the initial test

	Tube no.								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
S_q (ml)	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Safe drinking-water (ml)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine hydro-chloride in 10 ml of solution *(= c)* (mg)	0.042	0.044	0.046	0.048	0.050	0.052	0.054	0.056	0.058

S_q, stock solution of quinine hydrochloride.

Table 2
Determination of bitterness value: serial dilution for the second test

	Tube no.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
S_T (ml)	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.0
Safe drinking-water (ml)	9.00	8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	-

S_T, stock solution of the plant material being examined.

water. This stock solution of quinine hydrochloride (S_q) contains 0.01 mg/ml. Use nine test-tubes for the serial dilution for the initial test as indicated in Table 1.

Stock and diluted solutions of the plant material
Prepare the solution as specified in the test procedure for the given plant material (S_T). Use 10 test-tubes for the serial dilution for the test as indicated in Table 2.

Method
After rinsing the mouth with safe drinking-water, taste l0ml of the most dilute solution swirling it in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds,

spit out the solution and wait for 1 minute to ascertain whether this is due to delayed sensitivity. Then rinse with safe drinking-water. The next highest concentration should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, rinse the mouth thoroughly with safe drinking-water until no bitter sensation remains. Wait for at least 10 minutes before carrying out the second test.

In order to save time in the second test, it is advisable to ascertain first whether the solution in tube no. 5 (containing 5 ml of S_T in 10 ml) gives a bitter sensation. If so, find the threshold bitter concentration of the material by tasting the dilutions in tubes 1-4. If the solution in tube no. 5 does not give a bitter sensation, find the threshold bitter concentration by tasting the dilutions in tubes 6-10.

All solutions and the safe drinking-water for mouthwashing should be at 20-25 °C.

Calculate the bitterness value in units per g using the following formula:
2000 x c

$$\frac{2000 \text{ x } c}{\boldsymbol{a} \text{ x } \boldsymbol{b}}$$

where a = the concentration of the stock solution (S_T) (mg/ml),
b = the volume of S_T (in ml) in the tube with the threshold bitter concentration,
c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

12. Determination of haemolytic activity

Many medicinal plant materials, especially those derived from the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae, and Dioscoreaceae contain saponins. The most characteristic property of saponins is their ability to cause haemolysis: when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.

The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin R, which has a haemolytic activity of 1000 units per g. A suspension of erythrocytes is mixed with equal volumes of a serial dilution of the plant material extract. The lowest concentration to effect complete haemolysis is determined after allowing the mixtures to stand for a given period of time. A similar test is carried out simultaneously with saponin R.

Procedures proposed for the determination of the haemolytic activity of saponaceous medicinal plant material are all based on the same principle although the details may vary, e.g. the source of erythrocytes, methods for the preparation of the erythrocyte suspension and the plant material extract, the defined haemolytic activity of the reference material of saponin, and the experimental method. In order to obtain reliable results, it is essential to standardize the experimental conditions, and especially to determine the haemolytic activity by comparison with that of saponin R.

Recommended procedure

To prepare the erythrocyte suspension fill a glass-stoppered flask to one-tenth of its volume with sodium citrate (36.5 g/l) TS, swirling to ensure that the inside of the flask is thoroughly moistened. Introduce a sufficient volume of blood freshly collected from a healthy ox and shake immediately. Citrated blood prepared in this way can be stored for about 8 days at 2-4°C. Place 1 ml of citrated blood in a 50-ml volumetric flask with phosphate buffer pH 7.4 TS and carefully dilute to volume. This diluted blood suspension (2% solution) can be used as long as the supernatant fluid remains clear and colourless. It must be stored at a cool temperature.

To prepare the reference solution, transfer about 10 mg of saponin R, accurately weighed, to a volumetric flask and add sufficient phosphate buffer pH 7.4 TS to make 100ml. This solution should be freshly prepared.

The extract of plant material and dilutions should be prepared as specified in the test procedure for the plant material concerned, using phosphate buffer pH 7.4 TS.

Table 3
Determination of haemolytic activity: serial dilution for the preliminary test

	Tube no.			
	1	2	3	4
Plant material extract (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 TS (ml)	0.90	0.80	0.50	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

Preliminary test
Prepare a serial dilution of the plant material extract with phosphate buffer pH 7.4 TS and blood suspension (2%) using four test-tubes as shown in Table 3.

As soon as the tubes have been prepared, gently invert them to mix, avoiding the formation of foam. Shake again after a 30-minute interval and allow to stand for 6 hours at room temperature. Examine the tubes and record the dilution at which total haemolysis has occurred, indicated by a clear, red solution without any deposit of erythrocytes. Proceed as follows.

- If total haemolysis is observed only in tube no. 4, use the original plant material extract directly for the main test.
- If total haemolysis is observed in tubes 3 and 4, prepare a two-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS.
- If total haemolysis is observed in tubes 2, 3 and 4, prepare a five-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS.
- If, after 6 hours, all four tubes contain a clear, red solution, prepare a ten-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS and carry out the preliminary test again as described above.
- If total haemolysis is not observed in any of the tubes, repeat the preliminary test using a more concentrated plant material extract.

Main test
Prepare a serial dilution of the plant material extract, undiluted or diluted as deter-mined by the preliminary test, with phosphate buffer pH 7.4 TS and blood suspension (2%) using 13 test-tubes as shown in Table 4.

Carry out the dilutions and evaluations as in the preliminary test but observe the results after 24 hours. Calculate the amount of medicinal plant material in g, or of the preparation in g or ml, that produces total haemolysis.

To eliminate the effect of individual variations in resistance of the erythrocyte suspension to saponin solutions, prepare a series of dilutions of saponin R in the same manner as described above for the plant material extract. Calculate the quantity of saponin R in g that produces total haemolysis.

Table 4
Determination of haemolytic activity: serial dilution for the main test

	Tube no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Plant material extract (diluted if necessary) (ml)	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
Phosphate buffer pH 7.4 TS (ml)	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05	
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Calculate the haemolytic activity of the medicinal plant material using the following formula:

$$1000 \times \frac{a}{b}$$

where 1000 = the defined haemolytic activity of saponin R in relation to ox blood,
a = quantity of saponin R that produces total haemolysis (g),
b = quantity of plant material that produces total haemolysis (g).

13. Determination of tannins

Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes. This process, when applied to living tissue, is known as an "astringent" action and is the reason for the therapeutic application of tannins.

Chemically, tannins are complex substances; they usually occur as mixtures of polyphenols that are difficult to separate and crystallize. They are easily oxidized and polymerized in solution; if this happens they lose much of their astringent effect and are therefore of little therapeutic value.

Recommended procedure

To prepare the plant material extract, introduce the quantity specified in the test procedure for the plant material concerned, previously powdered to a known fineness and weighed accurately, into a conical flask. Add 150ml of water and heat over a boiling water-bath for 30 minutes. Cool, transfer the mixture to a 250-ml volumetric flask and dilute to volume with water. Allow the solid material to settle and filter the liquid through a filter-paper, diameter 12cm, discarding the first 50ml of the filtrate.

To determine the total amount of material that is extractable into water, evaporate 50.0ml of the plant material extract to dryness, dry the residue in an oven at 105°C for 4 hours and weigh (T_1).

To determine the amount of plant material not bound to hide powder that is extractable into water, take 80.0ml of the plant material extract, add 6.0g of hide powder R and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_2).

To determine the solubility of hide powder, take 6.0g of hide powder R, add 80.0 ml of water and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_0). Calculate the quantity of tannins as a percentage using the following formula:

$$\frac{[T_1 - (T_2 - T_0)]x500}{w}$$

where w = the weight of the plant material in grams.

14. Determination of swelling index

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose.

The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized). Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

The mixing of whole plant material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

Recommended procedure

Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125 mm, marked in 0.2-ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material.

15. Determination of foaming index

Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Recommended procedure

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume.

Pour the decoction into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows.

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.
- If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

Calculate the foaming index using the following formula:

$$\frac{1000}{a}$$

where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

16. Determination of pesticide residues

Medicinal plant materials are liable to contain pesticide residues which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administration of fumigants during storage. It is therefore recommended that every country producing medicinal plant materials (naturally grown or cultivated) should have at least one control laboratory capable of performing the determination of pesticides in accordance with the procedure outlined below.

Since many medicinal preparations of plant origin are taken over long periods of time, limits for pesticide residues should be established following the recommendations of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) which have already been established for food and animal feed (9). These recommendations include the analytical methodology for the assessment of specific pesticide residues.

Classification of pesticides
Different classifications of pesticides exist (*10, 11*). A classification based on the chemical composition or structure of the pesticide is most useful for analytical chemists, for example:

- chlorinated hydrocarbons and related pesticides: aldrin, HCH (hexachlorocy-clohexane or benzene hexachloride, BHC), hexachlorobenzene (HCB), chlordane, DDT (clofenotane), dieldrin, endrin, heptachlor, lindane, methoxychlor, camphechlor (toxaphene);
- chlorinated phenoxyalkanoic acid herbicides: 2,4-D; 2,4,5-T;
- organophosphorus pesticides: carbophenothion (carbofenotion), coumaphos (coumafos), demeton, dichlorvos, dimethoate, ethion, fenchlorphos (fenclofos), malathion, methyl parathion, parathion;
- carbamate insecticides: carbaryl (carbaril);
- dithiocarbamate fungicides: ferbam, maneb, nabam, thiram, zineb, ziram;
- inorganic pesticides: aluminium phosphide, calcium arsenate, lead arsenate;
- miscellaneous: bromopropylate, chloropicrin, ethylene dibromide, ethylene oxide, methyl bromide;
- pesticides of plant origin: tobacco leaf and nicotine; pyrethrum flower, pyrethrum extract and pyrethroids; derris root and rotenoids.

Only the chlorinated hydrocarbons and related pesticides (e.g. aldrin, chlordane, DDT, dieldrin, HCH) and a few organophosphorus pesticides (e.g. carbophenothion) have a long residual action. Most other pesticides have very short residual actions. Therefore it is suggested that, where the length of exposure to pesticides is unknown, the medicinal plant material should be tested for the presence of organically bound chlorine and phosphorus, or the content of these two substances should be determined. Alternative classifications of pesticides may be based on their intended use, for example:

- insecticides;
- fungicides and nematocides;

- herbicides;
- other pesticides (e.g. acaricides, molluscicides, rodenticides);
- fumigants (e.g. ethylene oxide, ethylene chlorohydrin, methyl bromide).

Methods for the determination of pesticide residues

Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues. Samples are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and the presence of a moderately broad spectrum of pesticides is measured in a single determination. However, these techniques are not universally applicable. Some pesticides are satisfactorily carried through the extraction and clean-up procedures, others are recovered with a poor yield, and some are lost entirely. In chromatography, the separations may not always be complete, pesticides may decompose or metabolize, and many of the metabolic products are still unknown. Consequently, as a result of limitations in the analytical technique and incomplete knowledge of pesticide interaction with the environment, it is not yet possible to apply an integrated set of methods that will be satisfactory in all situations.

It is therefore desirable to test plant materials of unknown history for broad groups of compounds rather than for individual pesticides. A variety of methods meet these requirements. Chlorinated hydrocarbons and other pesticides containing chlorine in the molecule, for example, can be detected by the measurement of total organic chlorine; insecticides containing phosphate can be measured by analysis for total organic phosphorus, while pesticides containing arsenic and lead can be detected by measurement of total arsenic or total lead, respectively. Similarly, the measurement of total bound carbon disulfide in a sample will provide information on whether residues of the dithiocarbamate family of fungicides are present.

If the pesticide to which the plant material has been exposed is known or can be identified by suitable means, an established method for the determination of that particular pesticide residue should be employed.

General aspects of analytical methodology

The samples should be tested as quickly as possible after collection, before any physical or chemical changes occur. If prolonged storage is envisaged, the samples should preferably be stored in air-tight containers under refrigeration.

Light can cause degradation of many pesticides, and it is therefore advisable to protect the samples and any extracts or solutions from undue exposure.

The type of container or wrapping material used should not interfere with the sample or affect the analytical results.

Solvents and reagents used in the analytical method should be free from substances that may interfere with the reaction, alter the results or provoke degradation of the pesticide residue in the sample. It is usually necessary to employ specially purified solvents or to distil them freshly in an all-glass

apparatus. Blank determinations with the solvents should be carried out, concentrating and testing them as specified in the test procedure for the plant material concerned.

The simplest and quickest procedure should be used to separate unwanted material from the sample (clean-up procedure) in order to save time when many samples have to be tested.

The process of concentrating solutions should be undertaken with great care, especially during the evaporation of the last traces of solvent, in order to avoid losses of pesticide residues. For this reason, it is often not advisable to remove the last traces of solvent. Agents, such as mineral oil or other oils of low volatility, that may help to preserve the solution could be added to retard the loss of the relatively volatile pesticides, especially when the last traces of solvent are being evaporated. However, these agents, while satisfactory in colorimetric procedures, are usually not desirable in gas chromatographic methods. It may be necessary to evaporate heat-labile compounds using a rotary vacuum apparatus.

Maximum limit of pesticide residues for medicinal plant materials

The toxicological evaluation of pesticide residues in medicinal plant materials should be based on the likely intake of the material by patients. In general, the intake of residues from medicinal plant materials should account for no more than 1% of total intake from all sources, including food and drinking-water. Certain plant materials may contain extremely high levels of pesticide residues, but the levels remaining after extraction are usually much lower, because of the low solubility in water or ethanol. It is therefore important to determine the actual quantity of residues consumed in the final dosage form.

Where the nature of the pesticide to which the plant material has been exposed is unknown, it is sufficient to determine the content of total chlorine and to base the calculation on the acceptable residue level (ARL) of the most toxic chlorine-containing pesticide (e.g. aldrin or dieldrin).

An ARL (in mg of pesticide per kg of plant material) can be calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as, recommended by FAO and WHO, and the mean daily intake (MDI) of the medicinal plant material.

Some countries have established national requirements for residue limits in plant materials. Where such requirements do not exist, the following formula may be used:

$$\text{ARL} = \frac{\text{ADI x E x 60}}{\text{MDI x100}}$$

where ADI = maximum acceptable daily intake of pesticide (mg/kg of body weight);

E = extraction factor, which determines the transition rate of the pesticide from the plant material into the dosage form;

MDI = mean daily intake of medicinal plant product.

The 60 in the numerator represents mean adult body weight, while the denominator incorporates a consumption factor of 100 reflecting the fact that no more than 1% of the total pesticide residue consumed should be derived from medicinal plant material.

This formula is based on the acceptable daily intake (ADI) determined by FAO and WHO.

Determination of total chlorine and phosphorus
Most pesticides contain organically bound chlorine or phosphorus.

Recommended procedure

Preparation of samples
Reduce the plant material to a fine powder, and extract with a mixture of water and acetonitrile R. Most pesticides are soluble in this mixture, while most cellular constituents (e.g. cellulose, proteins, amino acids, starch, fats and related compounds) are sparingly soluble and are thus removed. A number of polar and moderately polar compounds may also be dissolved; it is therefore necessary to transfer the pesticides to light petroleum R. For pesticides containing chlorine, further purification is seldom required, but for those containing phosphorus, further purification by column chromatography may be necessary, eluting with mixtures of light petroleum R and ether R.

Preparation of the column
Use Florisil R grade 60/100 PR (or equivalent), activated at 650°C, as the support. If this material is obtained in bulk, transfer it immediately after opening to a 500-ml glass jar or bottle with a glass stopper or foil-lined, screw-top lid. Store in the dark. Before use, heat at not less than 130°C, cool in a desiccator to room temperature and heat once again to 130°C after 2 days.

Prepare a Florisil column (external diameter, 22 mm) which contains, after settling, 10 cm of activated Florisil topped with about 1 cm of anhydrous sodium sulfate R. Pre-wet the column with 40-50 ml of light petroleum R. Place a graduated flask under the column to receive the eluate.

Method
Grind the material to pass through a sieve no. 710 or 840 and mix thoroughly. Place 20-50 g of the ground sample into a blender, add 350 ml of acetonitrile R with a water content of 35% (to 350 ml of water add sufficient acetonitrile R to produce 1000 ml). Blend for 5 minutes at a high speed. Filter under vacuum

through an appropriate funnel, diameter 12 cm, fitted with filter-paper, into a 500-ml suction flask.

Transfer the filtrate to a 250-ml measuring cylinder and record the volume. Transfer the measured filtrate to a 1-litre separating funnel and carefully add 100ml of light petroleum R. Shake vigorously for 1-2 minutes, add 10 ml of sodium chloride (400 g/l) TS and 600 ml of water. Hold the separating funnel in a horizontal position and mix vigorously for 30-45 seconds. Allow to separate, discard the aqueous layer and gently wash the solvent layer with two 100-ml portions of water. Discard the washings, transfer the solvent layer to a 100-ml glass-stoppered cylinder, and record the volume. Add about 15 g of anhydrous sodium sulfate R and shake vigorously. The extract must not remain in contact with this reagent for longer than 1 hour. Transfer the extract directly to a Florisil column; if necessary, reduce the volume first to 5-10 ml. Allow it to pass through the column at a rate of not more than 5 ml per minute. Carefully rinse the cylinder with two portions, each of 5 ml, of light petroleum R, transfer them to the column, rinse with further small portions of light petroleum R if necessary, and then elute at the same rate with 200 ml of ether/light petroleum TS1. Change the receiver and elute with 200 ml of ether/light petroleum TS2. Again change the receiver and elute with 200 ml of ether/light petroleum TS3. Evaporate each eluate to a suitable volume, as required, for further testing.

- The first eluate contains chlorinated pesticides (aldrin, DDE, TDE (DDD), o,p'-and p,p'-DDT, HCH, heptachlor, heptachlor epoxide, lindane, methoxychlor), polychlorinated biphenyls (PCB), and phosphated pesticides (carbophenothion, ethion, and fenchlorphos).
- The second eluate contains chlorinated pesticides (dieldrin and endrin) and phosphated pesticides (methyl parathion and parathion).
- The third eluate contains phosphated pesticide (malathion).

Combustion of the organic matter

Combustion of the organic matter in oxygen is the preparatory step for the determination of chlorine and phosphorus. The pesticide is extracted from the sample and purified, if necessary. The extract is concentrated, evaporated to dryness, transferred to a sample holder, and burned in a suitable conical flask flushed with oxygen. The gases produced during combustion are then absorbed in a suitable solution. The absorbed chlorine is determined as chloride and the absorbed phosphorus as orthophosphate, both colorimetrically.

Apparatus

The combustion is carried out in a 1-litre conical flask made of borosilicate glass, into the stopper of which is fused one end of a piece of platinum wire about 1 mm in diameter. To the free end of the wire is attached a piece of platinum gauze measuring about 1.5 x 2 cm to provide a means of holding the sample clear of the absorbing liquid during combustion.

Sample holder for chlorine-containing residues. For a small quantity of solid material, use a sample holder made from a piece of halide-free filter-paper about 5 cm long and 3 cm wide; for a small volume of liquid, preferably use a sample

holder in the form of a cone made from cellulose acetate film. Prepare the cone as follows: wearing cloth gloves and using a suitable cardboard template cut the film in a circle of 4 cm radius.

Manually pin the two edges together to form a cone. Seal the joined edges using heat to form a seam about 5 mm wide. Immerse the seam in acetone R to about one half of its width for 10 seconds. Remove and dry it immediately in a stream of hot air. Using forceps, wash the cone by dipping in a 1-litre beaker containing warm sodium hydroxide (~240 g/l) TS for 10 seconds at a temperature of about 60°C. Rinse the cone thoroughly with water and allow to drain dry on a piece of aluminium foil. Place each cone in a clean funnel (diameter 65 mm).

Sample holder for phosphorus-containing residues. Use a piece of halide-free filter-paper about 4 cm square as the sample holder.

Combustion of chlorine-containing residues
Transfer an aliquot of the extract as prepared above onto the sample holder which is placed in a funnel using a solvent that will not dissolve the sample holder. Allow the solvent to evaporate. Wearing rubber gloves, remove the sample holder and its dry contents from the funnel, and fold it over and up to form a small packet, about 1 cm^2 in area, and secure it in the centre of the platinum gauze. Insert a narrow strip of filter-paper, about 1 x 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 30 ml of water to the combustion flask. Moisten the neck of the flask with water. Fill the flask thoroughly with oxygen by means of a tube with its end just above the liquid. Ignite the free end of the paper strip and immediately insert the stopper. Hold the stopper firmly in place. When vigorous burning has begun, tilt the flask to prevent incompletely burned material from falling into the liquid. Immediately after combustion is completed, shake the flask vigorously for 10 minutes to dissolve the combustion products. Place a small quantity of water around the rim of the flask, and carefully withdraw the stopper. Rinse the stopper, platinum wire, platinum gauze and sides of the flask with water. Transfer the liquid and liquids used for rinsing to a 50-ml volumetric flask and dilute to volume with water.

Combustion of phosphorus-containing residues
Dip the sample holder made from filter-paper into methanolic sodium hydroxide TS, then suspend it in a current of heated air. Immediately transfer about 0.2 ml of an aliquot of the extract as prepared above to the sample holder with the aid of 0.2-ml portions of chloroform R using a micropipette. Allow the solvent to evaporate from the paper, fold it to form a small packet about 1 cm^2 in area and place it in the centre of the platinum gauze. Insert a strip of filter-paper, about 1 x 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 10 ml of sulfuric acid (~37 g/l) TS to the combustion flask and continue with the combustion as described above. Transfer the solution and the liquid used for rinsing to a 25-ml volumetric flask and dilute to volume with water.

Determination of chlorides

Apparatus
The determination is made with a spectrophotometer capable of measuring absorbance at 460 nm using absorption cells with path-lengths of 2 cm and 10 cm.

Method
Place 15 ml of the solution obtained after combustion in a 50-ml conical flask together with 1 ml of ferric ammonium sulfate (0.25 mol/l) VS and 3 ml of mercuric thiocyanate TS. Swirl the contents of the flask and allow to stand for 10 minutes. Transfer a portion of the solution to a 2-cm cell and measure the absorbance at 460 run using water in the reference cell. The reading should be made promptly to minimize absorption of chloride from the air.

Prepare a standard solution of sodium chloride R containing 5 µg of chloride per ml. Transfer aliquots of this solution (0 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml) into a series of 50-ml conical flasks and dilute to 15 ml with water. Develop the colour and measure the absorbances as described above. Plot the absorbances against the chloride content of the dilutions in µg per ml and interpolate the chloride content of the solutions of the material tested.

Determination of phosphates
The phosphomolybdate method is based on the reaction of phosphate ions with ammonium molybdate to form a molybdophosphate complex, which is subsequently reduced to form a strongly blue-coloured molybdenum complex. The intensity of the blue colour is measured spectrophotometrically. This method is applicable for the determination of any phosphates that have undergone a prior separation procedure.

Naturally occurring phosphates are present in most samples, and are often not removed during the clean-up procedure. In order to obtain background values, therefore, it is necessary to proceed with the determination for all samples, even those with no phosphate-containing pesticides. These background values should be subtracted from the results obtained on testing pesticide residues. Extracts of most uncontaminated materials contain about 0.05-0.1 mg/kg of phosphorus. Therefore, no contamination with organophosphate pesticides can be assumed for results in this range.

Apparatus
The determination is made with a spectrophotometer capable of measuring absorbance at 820 nm using an absorption cell with a path-length of 1 cm.

Method
Place 7 ml of the solution obtained after combustion in a calibrated 10-ml test-tube. Add 2.2 ml of sulfuric acid (300 g/l) TS and mix the solution well. Add 0.4 ml of ammonium molybdate (40 g/l) TS and swirl the mixture. Then add 0.4 ml of aminonaphtholsulfonic acid TS and swirl again. Heat the solution to 100°C for

12 minutes (±2 minutes), cool, and transfer a portion to a 1-cm cell. Measure the absorbance at 820 nm using water in the reference cell.

Prepare standard dilutions with a known content of phosphate and measure the absorbance as described above. Plot the absorbances against the phosphate content of the dilutions in µg per ml and interpolate the phosphate content of the solutions of the material tested.

Qualitative and quantitative determination of organochlorine pesticides

Recommended procedure

Preparation of sample
Place 20 g of powdered plant material (sieve no. 180), accurately weighed, in a 500-ml beaker (tall form), mix with 98 ml of water and allow to macerate for at least 30 minutes. Add 200 ml of acetone R; the resulting volume of extraction solvent will be 295 ml. Extract for 5 minutes, while cooling and using a high-speed mixer. Filter the homogenized mixture through a porcelain filter (Büchner funnel, diameter 70mm) fitted with a filter-paper, using a slight vacuum, into a 250-ml graduated cylinder, allowing the process to last no longer than 1 minute, and then measure the volume *(V)* of the filtrate in ml.

Method
Transfer the filtrate prepared as above to a 500-ml separating funnel. Add a quantity of sodium chloride R equivalent in grams to one-tenth of the volume of the filtrate, then add 100 ml of dichloromethane R. Shake vigorously for 5 minutes, allow the phases to separate and discard the lower (aqueous) layer. Dry the acetone-dichloromethane phase, transfer it to a 500-ml conical flask, add 25 g of anhydrous sodium sulfate R and swirl occasionally. Next, filter the solution into a 500-ml flask with a ground-glass stopper using a glass funnel (diameter 100 mm) containing purified glass-wool and anhydrous sodium sulfate R. Rinse the separating funnel, the conical flask and the glass funnel twice with 10 ml of ethyl acetate R. Add 5 ml of 2,2,4-trimethylpentane R, and concentrate the crude extract to about 2 ml in a rotary vacuum evaporator in a water-bath at 30-40°C. Expel the remaining solvent in a gentle stream of air.

To purify by gel chromatography, macerate 50g of suitable beads (e.g. S-X3 bio-beads) in an elution mixture of cyclohexane R and ethyl acetate R (1:1) and pour them into a chromatographic column (length 600 mm, diameter 25 mm) adapted for use with a vacuum pump. Rinse the gel bed with the elution mixture under air-free conditions. Dissolve the extract in the flask with 5.0 ml of ethyl acetate R. Add 2 g of anhydrous sodium sulfate R, swirl gently and add 5.0 ml of cyclohexane R. Filter the completely dissolved crude extract through a rapid filter into a 10-ml test-tube with a ground-glass stopper and close the tube immediately. Then transfer 5.0 ml of the filtrate onto the gel column. Elute with the elution mixture at an average rate of 5.0 ml/minute. Plant material components leave the gel column first, followed by the active ingredients of pesticides. Fractionation must be determined for each column, using appropriate reference substances.

Discard the first fraction (about 100 ml) containing the impurities. Collect the organochlorine pesticides appearing in the next eluate (about 70ml) in a flask with a ground-glass stopper. Add l0ml of 2,2,4-trimethylpentane R and concentrate the solution to about 5 ml in a rotary vacuum evaporator and a water-bath at 30-40°C. Pipette another 5 ml of 2,2,4-trimethylpentane R into the flask and carefully evaporate the solution to about 1 ml (do not allow to become completely dry).

Calculate the amount of plant material in g in the purified extract using the following formula:

$$\frac{V}{590} \text{ x sample weight in g}$$

where V = volume of filtrate.

To purify further, transfer 1 g of previously deactivated silica gel for column chromatography (70-230 mesh) containing 1.5% of water, to a chromatographic column (length 25 cm, internal diameter 7 mm). Put 10 mm of anhydrous sodium sulfate R on top of the content of the column and cover with purified glass-wool. Before use rinse the column with 5 ml of hexane R. Allow the solvent to reach the surface of the column filling, then transfer quantitatively, by means of a pipette, the purified extract obtained by gel chromatography from the flask to the prepared silica gel column and rinse with 1 ml of hexane R. Set the flask aside for subsequent elutions.

Using a 10-ml volumetric flask as the receiver, elute any residues of polychlorinated biphenyls from the column with 10 ml of hexane R (eluate 0). Add 2 ml of an elution mixture composed of toluene R/hexane R (35:65) to the flask and swirl. Quantitatively transfer the solution to the column. Using another 10-ml volumetric flask as the receiver, elute the majority of the organochlorine pesticides from the silica gel column using 6 ml of the same elution mixture. Dilute the contents of the flasks to volume with the elution mixture (eluate 1).

Rinse the flask with 2 ml of toluene R and transfer it quantitatively to the column. Collect the eluate in a third 10-ml volumetric flask. Add 8 ml of toluene R to the flask, swirl and transfer the solution to the silica gel column; elute the remaining organochlorine pesticides using the same receiver. Dilute the contents of the flask to volume with toluene R (eluate 2).

Evaluate the test solutions by capillary gas chromatography using an electron capture detector (ECD). Confirm the findings obtained for the main column (first separation system) with a second capillary column of different polarity (second separation system).

Determination by gas chromatography
A capillary gas chromatograph with an ECD is used for the measurement. Helium R is used as the carrier gas and a mixture of argon R and methane R (95:5) as an auxiliary gas for the detection.

First separation system
Use a vitreous silica column, 30 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 5% phenyl, 95% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.5 minutes;
- increase the temperature at a rate of 30°C per minute to 160°C and maintain this temperature for 2 minutes;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use a "split/split-free" injector to inject the sample solution and maintain the injection port at a temperature of 240°C. Inject a volume of 1 µl at a rate of 30 seconds ("split-free"). The detector temperature should be 300°C.

Second separation system
Use a vitreous silica column, 15 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 7% cyanopropyl, 7% phenyl, 86% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.2 minutes;
- increase the temperature at a rate of 30°C per minute to 180°C and maintain this temperature for 1 minute;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use an on-column injector to inject a volume of 1 µl of the sample solution. The detector temperature should be 300°C.

Use the "external standard" method for the qualitative and quantitative evaluation of the organochlorine pesticides in the test solutions with reference solutions of the following pesticides: α-, β-, γ- and δ-hexachlorocyclohexane (HCH); hexachlorobenzene; quintozene; aldrin; dieldrin; endrin; α- and β-endosulfan; endosulfan sulfate; heptachlor, heptachlorepoxide; camphechlor; TDE, DDE and DDT (both *o,p'*- and *p,p'*-isomers); methoxychlor.

Measure the peak height of the pesticides obtained in the chromatograms and calculate the concentration of the residues in mg/kg using the following formula:

$$\frac{h_t \text{ x10}}{w} x \frac{w_r}{h_r}$$

where h_t = peak height obtained for the test solution in mm,
w = quantity of sample in the purified extract (g),
w_r = quantity of pesticide in ng in the reference solution injected,
h_r = peak height obtained for the reference solution in mm.

Analysis of esters of organophosphorus compounds

The extraction and the clean-up procedures can be performed as described above, but the detection requires a phosphorus flame ionization detector (P-FID).

Determination of specific pesticide residues in plant material

General recommendations
For the total determination, mix thoroughly 1 kg of plant material.

In order to obtain reliable chromatographic results, do one or more of the following:

- repeat the separation using another column;
- use a different separation system;
- use a different detector system;
- apply a coupling technique;
- prepare a derivative;
- perform chromatography with a mixture of the sample and a reference substance;
- change the sample preparation;
- use a fractionated elution during the column-chromatography clean-up procedure of the plant extract and test every fraction by chromatography; and
- compare the distribution coefficient of the material with that of a reference substance.

Prior to the quantitative determination of the material to be tested, check whether there is a linear relationship between the values obtained for the reference substance and its concentration over the range 0.1-2 times the standard concentration. Otherwise, prepare another concentration range or evaluate the results using a reference curve. Use any suitable mechanical or manual technique for the chromatographic determination.

Store the reference solutions protected from light to prevent decomposition. Use glass vessels closed with glass stoppers and keep them in a container saturated with the solvent employed to avoid any increase in concentration due to evaporation. Check the loss by evaporation by interim weighing of the vessels.

Use concentrated reference solutions within 6 months and diluted reference solutions within 4 weeks.

Rate of recovery
The rate of recovery (R) is the percentage of the reference material, originally added to the plant material, that is determined using the method described below.

Determination of desmetryn, prometryn, and simazine residues

Preparation of the plant material extract
Place 10.0 g of powdered plant material in a 500-ml conical flask and add 125.0 ml of chloroform R. Shake the mixture for 60 minutes and filter under reduced pressure through a filter-paper (medium grade) into a round-bottomed flask. Wash the residue with 3 successive volumes each of 25.0 ml of chloroform R.

Method
Concentrate the combined filtrates to a volume of 3-5 ml using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the extract to a chromatographic column as prepared below, rinsing the round-bottomed flask twice with 5.0 ml of chloroform R.

Preparation of chromatographic column
Use a glass tube (internal diameter 20-22 mm) with a restricted orifice and protected with a sintered-glass plate (e.g. P10 or P16, glass filter G4; or P40, glass filter G3). Fill the column with chloroform R, then pour purified aluminium oxide R into it to form a 100-mm thick layer. The support material should remain covered with chloroform R. After transferring the extract and the rinsing liquids to the column, elute with 150.0 ml of chloroform R, at a rate of 1-2 drops per second, collecting the eluate in a round-bottomed flask. The first purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To the residue add 10.0 ml of light petroleum R and transfer the mixture to a chromatographic column containing a layer of purified aluminium oxide R, 50 mm thick, in light petroleum R. Elute the mixture with 90.0 ml of light petroleum R, using this to rinse the round-bottomed flask, at a rate of 1-2 drops per second. Discard the eluate. Dissolve any remaining residue which has not dissolved in light petroleum R in 10.0 ml of a mixture composed of 60 volumes of chloroform R and 40 volumes of light petroleum R and transfer the solution to the column. Rinse the round-bottomed flask twice more with 10.0 ml of the solvent mixture. Transfer the liquid used for rinsing to the column. Elute with 120.0 ml of the same solvent mixture, at a rate of 1-2 drops per second and collect the eluate in a round-bottomed flask. The second purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To prepare a purified extract for the determination by gas chromatography, dissolve the residue in sufficient acetone R to produce a volume of 10.0 ml. If an especially purified extract is required, proceed as described below.

To the residue add 10.0 ml of light petroleum R and 10.0 ml of dimethyl sulfoxide R. Shake the mixture and transfer it to a separating funnel. Extract the dimethyl sulfoxide layer twice with 10.0 ml of light petroleum R. Discard the petroleum ether extract. Then add 100 ml of water to the dimethyl sulfoxide layer and extract 3 times, each with 20.0 ml of chloroform R. Extract the combined chloroform extracts twice with 20.0 ml of water and evaporate them to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the residue along with a mixture of 10.0 ml of light petroleum R and 10.0 ml of hydrochloric acid (1 mol/l) VS to a separating funnel and extract the mixture first with 10.0 ml and then with 5.0 ml of hydrochloric acid (1 mol/l) VS. Discard the petroleum ether layer and adjust the pH of the combined aqueous solutions to a value between 7 and 8 using sodium hydroxide (1 mol/l) VS. Extract the solution 3 times, each with 20.0 ml of chloroform R. Dry the combined chloroform extracts with anhydrous sodium sulfate R and filter into a round-bottomed flask, rinsing the funnel 3 times with 10.0-ml portions of chloroform R. Evaporate the filtrate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Dissolve the residue in sufficient acetone R to produce 10.0 ml of especially purified extract to be used for the determination by gas chromatography.

Use the extracts as indicated below for the following plant materials:

No.	Material	No.	Material
1	Flores Calendulae	10	Fructus Foeniculi
2	Flores Chamomillae	11	Herba Millefolii
3	Folia Melissae	12	Herba Plantaginis anceolatae
4	Folia Menthae piperitae	13	Radix Althaeae
5	Folia Salviae	14	Radix Angelicae
6	Folia Thymi	15	Radix Levistici
7	Fructus Carvi	16	Radix Petroselini
8	Fructus Coriandri	17	Radix Valerianae
9	Fructus Cynobasti		

For materials no. 1 and 2, use an especially purified extract (see page 58); for materials no. 3-17, use a purified extract (see page 58).

Determination of the rate of recovery

Prepare five individual samples using each of the following procedures:

1. To prepare solution S_2, first dissolve separately 0.040 g of each of the reference substances, desmetryn R, prometryn R and simazine R, in sufficient acetone R to produce 100.0 ml. Then place 5.0 ml of each solution into a 100-ml volumetric flask and dilute the mixture to volume with acetone R (S_2). Place 10.0 g of powdered plant material into a 500-ml conical flask and add 1.0 ml of solution S_2. Shake this mixture

mechanically for 60 minutes; if necessary, repeat the operation manually and then proceed as described under "Preparation of the plant material extract". Use either the purified or especially purified extract for the determination by gas chromatography, as specified in the test procedure for the plant material concerned.

2. Treat 10.0 g of powdered plant material as described under "Preparation of the plant material extract". Use either the purified extract or the especially purified extract for the determination by gas chromatography, as specified in the test procedure for each individual plant material.

Calculate the rate of recovery (R) in % using the following formula:

$$\frac{2\,(a - b)}{c}$$

where a = average quantity in mg/kg of the 5 residues obtained using procedure 1,
b = average quantity in mg/kg of the 5 residues obtained using procedure 2,
c = quantity of reference substances in mg contained in solution S_2 during procedure 1.

The rate should be within the range 70-120%. It is specific for each drug.

Determination by gas chromatography
Perform the determination as described in Volume 1 of *The international pharmacopoeia* (5).

Apparatus
The equipment consists of:

- a glass column 1.2 m long, internal diameter 2 mm;
- a suitable stationary liquid phase;
- a suitable diatomaceous support.

Use nitrogen R as the carrier gas with a flow rate of 30.0 ml/min. The sample injection block should be maintained at 230°C, the column at 190°C and the detector, which should be nitrogen-selective, at 300°C. In addition:

- volume of sample solution to be injected: 2.0 µl;
- separation characteristics: $h \leq 1.2 \times 10^{-3}$ for desmetryn R; $R_S \geq 1.2$ for prometryn R and simazine R;
- relative standard deviation (precision of chromatographic system): $s_r \leq 0.05$ for desmetryn R, prometryn R and simazine R.

Method
Chromatogram T. To determine the separation characteristics, inject solution S_2 (for the preparation of solution S_2 see "Determination of the rate of recovery" above). Chromatograms A_1-A_5. To determine the relative standard deviation, inject solution S_2 and repeat the determination 5 times.

Chromatogram S_2. Inject 1.0 ml of solution S_2 for the determination of the rate of recovery. Dilute 1.0 ml of solution S_2 to 10.0 ml with acetone R and inject it for the chromatographic determination. On the chromatogram the peaks occur in the following sequence: prometryn, simazine, desmetryn.

Chromatogram P_2. Inject the purified extract or the especially purified extract. Determine using an external standard: $a = 0.0005$To convert the values obtained to percentage by weight, multiply the concentration in mg/kg by 10^4.

The total maximum permissible amount of residues due to desmetryn, prometryn and simazine is 2 mg per kg of plant material.

17. Determination of arsenic and heavy metals

Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides.

Limit test for arsenic

The amount of arsenic in the medicinal plant material is estimated by matching the depth of colour with that of a standard stain.

Recommended procedure

Preparation of the sample by acid digestion
Place 35-70g of coarsely ground material, accurately weighed, in a Kjeldahl flask, capacity 800-1000 ml. Add 10-25 ml of water and 25-50 ml of nitric acid (~1000 g/l) TS and then carefully add 20 ml of sulfuric acid (~1760g/l) TS. Heat cautiously so that no excessive foaming takes place. Gradually add nitric acid (~10008/l) TS, drop by drop, until all the organic matter is destroyed. This is achieved when no further darkening of the solution is observed with continued heating, and a clear solution with copious vapours of sulfur trioxide is obtained. Cool, and add 75 ml of water and 25 ml of ammonium oxalate (25 g/l) TS. Heat again until sulfur trioxide vapours develop. Cool, transfer with the help of water to a 250-ml volumetric flask, and dilute to volume with water.

Apparatus
A suitable type of apparatus is constructed as follows. A wide-mouthed bottle of about 120-ml capacity is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of about 200mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). The lower end of the tube is drawn out to an internal diameter of about 1 mm, and there is a hole not less than 2 mm in diameter blown in the side of the tube, near the constricted part. The tube is positioned so that when the bottle contains 70ml of liquid the constricted end is above the surface of the liquid and the hole in the side is below the bottom of the bung. The upper end of the tube has a flat, ground surface at right-angles to the axis of the tube, with slightly rounded-off edges.

One of two rubber bungs (about 25 mm x 25 mm), each with a central hole of exactly 6.5 mm diameter, is fitted at the upper end of the tube. The other bung is fitted with a piece of glass tube about 3 mm long and with an internal diameter of exactly 6.5 mm and with a similar ground surface. One end of each of the tubes is flush with the larger end of the bungs, so that when these ends are held tightly together with a rubber band or a spring clip, the openings of the two tubes meet to form a true tube. Alternatively, the two bungs may be replaced by any suitable construction satisfying the conditions described in the test.

Method
Moisten some cotton-wool with lead acetate (80g/l) TS, allow to dry, and lightly pack into the tube which fits into the wide-mouthed bottle to not less than 25 mm from the top. Between the flat surfaces of the tubes, place a piece of mercuric bromide paper AsR that is large enough to cover their openings (15 mm x 15 mm). The mercuric bromide paper AsR can be fitted by any other means provided that:

- the whole of the evolved gas passes through the paper;
- the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and
- the paper is protected from sunlight during the test.

Place an aliquot (25-50ml) of the solution being tested, prepared as described above, in the wide-mouthed bottle, add 1 g of potassium iodide AsR and l0g of granulated zinc AsR, and place the prepared glass tube assembly quickly in position. Allow the reaction to proceed for 40 minutes. Compare any yellow stain that is produced on the mercuric bromide paper AsR with a standard stain produced in a similar manner with a known quantity of dilute arsenic AsTS. Examine the test and standard stains without delay in daylight; the stains fade with time.

The most suitable temperature for carrying out the test is generally about 40°C but, as the rate of evolution of the gas varies somewhat with different batches of granulated zinc AsR, the temperature may have to be adjusted to obtain an even evolution of gas. The reaction may be accelerated by placing the apparatus on a warm surface, care being taken to ensure that the mercuric bromide paper AsR remains dry throughout.

Between successive tests, the tube must be washed with hydrochloric acid (~250g/l) AsTS, rinsed with water and dried.

Preparation of standard stain
Add 10 ml of stannated hydrochloric acid (~250g/l) AsTS and 1 ml of dilute arsenic AsTS to 50 ml of water. The resulting solution, when treated as described in the general test, yields a stain on mercuric bromide paper AsR referred to as the standard stain (10 μg of As).

Limit test for cadmium and lead
The method of determination is left to the analyst. Nevertheless, the determination must be consistent and sensitive enough to allow comparison with a reference material.

Recommended procedure

Apparatus
The equipment consists of a digestion vessel, consisting of a vitreous silica crucible (DIN 12904), "tall form", height 62mm, diameter 50mm, capacity 75ml, with a vitreous silica cover.

Materials used are:

- *digestion mixture*: 2 parts by weight of nitric acid (~1000g/l) TS and 1 part by weight of perchloric acid (~1170g/l) TS.
- *reference materials*: olive leaves (*Olea europaea*)[1] and hay powder[2].

Clean scrupulously with nitric acid (~1000g/l) TS the digestion vessel and all other equipment to be used for the determination, rinse thoroughly several times with water and dry at 120°C.

Preparation of the sample
For the wet digestion method in an open system, place 200-250mg of air-dried plant material, accurately weighed, finely cut and homogeneously mixed, into a cleaned silica crucible. Add 1.0ml of the digestion mixture, cover the crucible without exerting pressure and place it in an oven with a controlled temperature and time regulator (computer-controlled, if available).

Heat slowly to 100°C and maintain at this temperature for up to 3 hours; then heat to 120°C and maintain at this temperature for 2 hours. Raise the temperature very slowly to 240°C, avoiding losses due to possible violent reactions especially in the temperature range of 160-200°C, and maintain at this temperature for 4 hours. Dissolve the remaining dry inorganic residue in 2.5 ml of nitric acid (~1000g/l) TS and use for the determination of heavy metals.

Every sample should be tested in parallel with a blank.

Method
The contents of lead and cadmium may be determined by inverse voltametry or by atomic absorption spectrophotometry.

The following maximum amounts in dried plant materials, which are based on the ADI values, are proposed:
- lead, 10 mg/kg;
- cadmium, 0.3 mg/kg.

[1] BCR reference material CRM No. 62 Community Bureau of Reference, obtainable from BCR, Directorate-General X11, Commission of the European Communities, 200 rue de la Loi, B-1049 Brussels, Belgium.
[2] Obtainable from IAEA/V-10, International Atomic Energy Agency, Analytical Quality Control Services, Laboratory Geibersdorf, P.O. Box 1'00, A-Vienna, Austria.

18. Determination of microorganisms

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spore-forming bacteria frequently predominate. Current practices of harvesting, handling and production may cause additional contamination and microbial growth. The determination of Escherichia coli and moulds may indicate the quality of production and harvesting practices.

Methods for decontamination are restricted. For example, the use of ethylene oxide has been forbidden within countries of the European Union. Treatment with ionizing irradiation is also forbidden or requires a special registration procedure in some countries.

In addition, the presence of aflatoxins in plant material can be hazardous to health if absorbed even in very small amounts. They should therefore be determined after using a suitable clean-up procedure.

Test for specific microorganisms

The conditions of the test for microbial contamination are designed to minimize accidental contamination of the material being examined; the precautions taken must not adversely affect any microorganisms that could be revealed.

Recommended procedure

Pretreatment of the material being examined

Depending on the nature of the crude medicinal plant material, grind, dissolve, dilute, suspend or emulsify the material being examined using a suitable method and eliminate any antimicrobial properties by dilution, neutralization or filtration.

Water-soluble materials

Dissolve or dilute 10 g or 10 ml of plant material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test, adjust the volume to 100ml with the same medium. (Some materials may require the use of a larger volume.) If necessary, adjust the pH of the suspension to about 7.

Non-fatty materials insoluble in water

Suspend 10g or 10ml of material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test; dilute to 100ml with the same medium. (Some materials may require the use of a larger volume.) If necessary, divide the material being examined and homogenize the suspension mechanically. A suitable surfactant, such as a solution of polysorbate

80 R containing 1 mg per ml may be added. If necessary, adjust the pH of the suspension to about 7.

Fatty materials
Homogenize 10g or 10ml of material, unless otherwise specified in the test procedure for the material concerned, with 5g of polysorbate 20R or polysorbate 80R. If necessary, heat to not more than 40°C. (Occasionally, it may be necessary to heat to a temperature of up to 45°C, for the shortest possible time.) Mix carefully while maintaining the temperature in a water-bath or oven. Add 85 ml of lactose broth or another suitable medium proven to have no antimicrobial activity in the conditions of the test, heated to not more than 40°C if necessary. Maintain this temperature for the shortest time necessary until an emulsion is formed and, in any case, for not more than 30 minutes. If necessary, adjust the pH of the emulsion to about 7.

Enterobacteriaceae and certain other Gram-negative bacteria

Detection of bacteria
Homogenize the pretreated material appropriately and incubate at 30-37°C for a length of time sufficient for revivification of the bacteria but not sufficient for multiplication of the organisms (usually 2-5 hours). Shake the container, transfer 1g or 1ml of the homogenized material to 100ml of Enterobacteriaceae enrichment broth-Mossel and incubate at 35-37°C for 18-48 hours. Prepare a subculture on a plate with violet-red bile agar with glucose and lactose. Incubate at 35-37°C for 18-48 hours. The material passes the test if no growth of colonies of Gram-negative bacteria is detected on the plate.

Quantitative evaluation
Inoculate a suitable amount of Enterobacteriaceae enrichment broth-Mossel with quantities of homogenized material prepared as described under "Detection of bacteria" above, appropriately diluted as necessary, containing 1.0g, 0.1g and 10µg, or 1.0ml, 0.1 ml and 10µl, of the material being examined. Incubate at 35-37°C for 24-48 hours. Prepare a subculture of each of the cultures on a plate with violet-red bile agar with glucose and lactose in order to obtain selective isolation. Incubate at 35-37°C for 18-24 hours. The growth of well-developed colonies, generally red or reddish in colour, of Gram-negative bacteria constitutes a positive result. Note the smallest quantity of material that gives a positive result. Determine the probable number of bacteria using Table 5.

Escherichia coli
Transfer a quantity of the homogenized material in lactose broth, prepared and incubated as described above, and containing 1 g or 1 ml of the material being examined, to 100 ml of MacConkey broth and incubate at 43-45°C for 18-24 hours.

Table 5
Determination of Enterobacteriaceae and certain other Gram-negative bacteria

Result for each quantity or volume			Probable number of bacteria per g of material
1.0 g or 1.0 ml	0.1g or 0.1 ml	0.01 g or 0.01 ml	
+	+	+	More than 10^2
+	+	–	Less than 10^2 but more than 10
+	–	–	Less than 10 but more than 1
–	–	–	Less than 1

Prepare a subculture on a plate with MacConkey agar and incubate at 43-45°C for 18-24 hours. Growth of red, generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of E. coli. This may be confirmed by the formation of indole at 43.5-44.5°C or by other biochemical reactions. The material passes the test if no such colonies are detected or if the confirmatory biochemical reactions are negative.

Salmonella spp.
Incubate the solution, suspension or emulsion of the pretreated material prepared as described above at 35-37°C for 5-24 hours, as appropriate for enrichment.

Primary test
Transfer 10 ml of the enrichment culture to 100 ml of tetrathionate bile brilliant green broth and incubate at 42-43°C for 18-24 hours. Prepare subcultures on at least two of the following three agar media: deoxycholate citrate agar; xylose, lysine, deoxycholate agar; and brilliant green agar. Incubate at 35-37°C for 24-48 hours. Carry out the secondary test if any colonies are produced that conform to the description given in Table 6.

Table 6
Description of Salmonella colonies appearing on different culture media

Medium	Description of colony
Deoxycholate citrate agar	Well developed, colourless
Xylose, lysine, deoxycholate agar	Well developed, red, with or without black centres deoxycholate agar
Brilliant green agar	Small, transparent and colourless, or opaque, pink or white (frequently surrounded by a pink to red zone)

Secondary test
Prepare a subculture of any colonies showing the characteristics described in Table 6 on the surface of triple sugar iron agar using the deep inoculation technique. This can be achieved by first inoculating the inclined surface of the culture medium followed by a stab culture with the same inoculating needle and incubating at 35-37°C for 18-24 hours. The test is positive for the presence of Salmonella spp. if a change of colour from red to yellow is observed in the deep culture (but not in the surface culture), usually with the formation of gas with or without production of hydrogen sulfide in the agar. Confirmation is obtained by appropriate biochemical and serological tests.

The material being examined passes the test if cultures of the type described do not appear in the primary test, or if the confirmatory biochemical and serological tests in the secondary test are negative.

Pseudomonas aeruginosa
Pretreat the material being examined as described on pages 64-65 but using buffered sodium chloride-peptone solution, pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test, in place of lactose broth. Inoculate 100 ml of soybean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1g or 1 ml of the material being examined. Mix and incubate at 35-37°C for 24-48 hours. Prepare a subculture on a plate of cetrimide agar and incubate at 35-37 °C for 24-48 hours. If no growth of microorganisms is detected, the material passes the test. If growth of colonies of Gram-negative rods occurs, usually with a greenish fluorescence, apply an oxidase test and test the growth in soybean-casein digest medium at 42°C. The following method may be used. Place 2 or 3 drops of a freshly prepared 0.01 g/ml solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride R on filter-paper and apply a smear of the suspected colony; the test is positive if a purple colour is produced within 5-10 seconds. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Staphylococcus aureus
Prepare an enrichment culture as described for *Pseudomonas aeruginosa*. Prepare a subculture on a suitable medium such as Baird-Parker agar. Incubate at 35-37°C for 24-48 hours. The material passes the test if no growth of microorganisms is detected. Black colonies of Gram-positive cocci often surrounded by clear zones may indicate the presence of *Staphylococcus aureus*. For catalase-positive cocci, confirmation may be obtained, for example, by coagulase and deoxyribonuclease tests. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Validation of the tests for specific microorganisms
If necessary, grow separately the test strains listed in Table 7 on the culture media indicated at 30-35°C for 18-24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 so that the test suspensions contain about 10^3; microorganisms per ml. Mix equal volumes of

each suspension and use 0.4 ml (approximately 10^2 microorganisms of each strain) as an inoculum in tests for *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus*, in the presence and absence of the material being examined, if necessary. The test method should give a positive result for the respective strain of microorganism.

Table 7
Test strains and culture media for use in validating the tests for specific microorganisms

Microorganism	Strain number[a]	Medium
Escherichia coli	e.g. NCIMB 8545 (ATCC 8739, CIP 53.126)	lactose broth
Pseudomonas aeruginosa	e.g. NCIMB 8626 (ATCC 9027, CIP 82.118)	soybean-casein digest medium
Salmonella typhimurium	No strain number is recommended. Species not pathogenic for humans, such as Salmonella abony (NCTC 6017, CIP 80.39), may be used	lactose broth
Staphylococcus aureus	e.g. NCIMB 8625 (ATCC 6538 P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)	soybean-casein digest medium

[a]See section 20, page 78.

Total viable aerobic count

The total viable aerobic count of the material being examined is determined, as specified in the test procedure, for the plant material concerned using one of the following methods: membrane-filtration, plate count or serial dilution.

Pretreatment of the material being examined
Pretreat the material as described in the "Test for specific microorganisms", page 64, but in place of lactose broth use buffered sodium chloride-peptone solution pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test.

Membrane filtration
Use membrane filters with a nominal pore size of not greater than 0.45 μm, the effectiveness of which in retaining bacteria has been established. For example, cellulose nitrate filters are used for aqueous, oily and weakly alcoholic solutions, and cellulose acetate filters for strongly alcoholic solutions. The technique described uses filter discs of about 50mm in diameter. For filters of a different diameter, adjust the volumes of the dilutions and washings accordingly. Sterilize the filtration apparatus and the membrane by appropriate means. They are

designed to permit the solution being examined to be introduced and filtered under aseptic conditions, and the membrane to be transferred to the culture medium.

Transfer 10ml or a solution containing 1g of the material to each of two membrane filters and filter immediately. If necessary, dilute the pretreated material to obtain an expected colony count of 10-100. Wash each membrane, filtering three or more successive quantities of approximately 100ml of a suitable liquid such as buffered sodium chloride-peptone solution, pH 7.0. For fatty materials, a suitable surfactant may be added, such as polysorbate 20R or polysorbate 80R. Transfer one of the membrane filters, intended primarily for the enumeration of bacteria, to the surface of a plate with casein-soybean digest agar and the other, intended primarily for the enumeration of fungi, to the surface of a plate with Sabouraud glucose agar with antibiotics. Incubate the plates for 5 days, unless a more reliable count can be obtained otherwise, at 30-35°C for the detection of bacteria and at 20-25°C for the detection of fungi. Count the number of colonies formed. Calculate the number of microorganisms per g or per ml of the material tested, if necessary counting bacteria and fungi separately.

Plate count
For bacteria. Use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the pretreated material as described above to obtain an expected colony count of not more than 300. Prepare at least two dishes using the same dilution and incubate them at 30-35°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the plate with the largest number of colonies, up to a maximum of 300.

For fungi. Use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied Sabouraud glucose agar with antibiotics at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the pretreated material as described above to obtain an expected colony count of not more than 100. Prepare at least two dishes using the same dilution and incubate them at 20-25°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the dish with not more than 100 colonies.

Serial dilution
Prepare a series of 12 tubes each containing 9-10ml of soybean-casein digest medium. To each of the first three tubes add 1 ml of the 1:10 dilution of dissolved, homogenized material prepared as described on pages 64-65. To the next three tubes add 1 ml of a 1:100 dilution of the material and to the next three tubes add 1 ml of a 1:1000 dilution of the material. To the last three tubes add 1 ml of the diluent. Incubate the tubes at 30-35°C for at least 5 days. No microbial growth

should appear in the last three tubes. If the reading of the results is difficult or uncertain owing to the nature of the material being examined, prepare a subculture in a liquid or a solid medium, and evaluate the results after a further period of incubation. Determine the most probable number of microorganisms per g or ml of the material using Table 8.

If, for the first column, the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per ml is less than 100.

Effectiveness of the culture medium and validity of the counting method
The following strains are normally used (see also section 20):

Staphylococcus aureus	NCIMB 8625 (ATCC 6538-P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)
Bacillus subtilis	NCIMB 8054 (ATCC 6633, CIP 52.62)
Escherichia coli	NCIMB 8545 (ATCC 8739, CIP 53.126)
Candida albicans	ATCC 2091 (CIP 1180.79) or ATCC 10 231 (NCPF 3179, CIP 48.72)

Allow the test strains to grow separately in tubes containing soybean-casein digest medium at 30-35°C for 18-24 hours, except for Candida albicans which needs a temperature of 20-25°C for 48 hours.

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to obtain test suspensions containing about 100 viable microorganisms per ml. Use the suspension of each microorganism separately as a control of the counting methods, in the presence and absence of the material being examined, if necessary.

Table 8
Determination of total viable aerobic count

Number of tubes 100mg or 0.1 ml per tube	with microbial 10mg or 0.01 ml per tube	growth[a] 1mg or 0.001 ml per tube	Most probable number of microorganisms per g or ml
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

[a] Amounts in mg or ml are quantities of original plant material.

To validate the method, a count for the test organism should be obtained differing by not more than a factor of 10 from the calculated value for the inoculum. To test the sterility of the medium and the diluent, as well as aseptic performance, carry out the total viable aerobic count using sterile buffered sodium chloride-peptone solution pH 7.0 as the test preparation. There should be no growth of microorganisms.

Microbial contamination limits in medicinal plant materials

Different limits are set according to the use of the material and the material itself.

- For contamination of "crude" plant material intended for further processing (including additional decontamination by a physical or chemical process) the limits, adapted from the provisional guidelines established by an international consultative group (12), are given for untreated plant material harvested under acceptable hygienic conditions:
  - *Escherichia coli*, maximum 10^4 per gram;
  - mould propagules, maximum 10^5 per gram.

- For plant materials that have been pretreated (e.g. with boiling water as used for herbal teas and infusions) or that are used as topical dosage forms:
  - aerobic bacteria, maximum 10^7 per gram;

- yeasts and moulds, maximum 10^4 per gram;
- Escherichia coli, maximum 10^2 per gram;
- other enterobacteria, maximum 10^4 per gram;
- salmonellae, none.

- For other plant materials for internal use:
  - aerobic bacteria, maximum 10^5 per gram;
  - yeasts and moulds, maximum 10^3 per gram;
  - Escherichia coli, maximum 10 per gram;
  - other enterobacteria, maximum 10^3, per gram;
  - salmonellae, none.

Test for aflatoxins

This test is designed to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2, which are highly dangerous contaminants in any material of plant origin.

Recommended procedure

Preparation of samples
Grind or reduce not less than 100g of crude medicinal plant material to a moderately fine powder (sieve no. 355/180). The larger the sample size, i.e. 500g-1kg or more, the greater the possibility of detecting pockets of contamination.

Weigh 50g of the powdered material, transfer to a conical glass-stoppered flask, and add 170 ml of methanol R and 30 ml of water. Using a mechanical device, shake vigorously for not less than 30 minutes. Filter through a medium-porosity filter-paper. If a special clean-up procedure is required (see below), collect 100ml of filtrate (A) from the start of flow; otherwise discard the first 50ml and collect 40ml of filtrate (B).

In order to eliminate interfering plant pigments use a special clean-up procedure: transfer 100 ml of filtrate A to a 250-ml beaker and add 20 ml of zinc acetate/aluminium chloride TS and 80 ml of water. Stir, allow to stand for 5 minutes, add 5 g of a filter aid, such as diatomaceous earth, mix and filter through a medium-porosity filter-paper. Discard the first 50ml and collect 80ml of filtrate (C).

Transfer either filtrate B or C to a separating funnel. Add 40ml of sodium chloride (100 g/l) TS and 25 ml of light petroleum R, and shake for 1 minute. Allow the layers to separate and transfer the lower layer to a second separating funnel. Extract twice with 25 ml of dichloromethane R and shake for 1 minute. Allow the layers to separate and combine each of the lower layers in a 125-ml conical flask. Add several boiling chips and evaporate almost to dryness on a water-bath. Cool the residue, cover the flask and keep it for the determination by thin-layer chromatography or for a further clean-up procedure by column chromatography.

If necessary, remove further interfering compounds using a column 300 mm long with an internal diameter of 10 mm, a stopper and either a medium-pore sintered disc or a glass-wool plug. Prepare a slurry by mixing 2 g of silica gel R with 10 ml of a mixture of 3 volumes of ether R and 1 volume of light petroleum R, pour into the column and wash with 5 ml of the same solvent mixture. Allow the adsorbent to settle and add to the top of the column a layer of 1.5 g of anhydrous sodium sulfate R. Dissolve the residue from above in 3 ml of dichloromethane R and transfer it to the column. Rinse the flask twice with 1-ml portions of dichloromethane R and add them to the column, eluting at a rate not faster than 1 ml/min. Then add successively to the column 3 ml of light petroleum R, 3 ml of ether R and 3 ml of dichloromethane R, and elute at a rate not faster than 3 ml/min. Discard the eluates. Add to the column 6 ml of a mixture of 9 volumes of dichloromethane R and 1 volume of acetone R and elute at a rate not faster than 1 ml/min, preferably without using vacuum. Collect this eluate in a small vial, add a few boiling chips and evaporate just to dryness on a water-bath.

Method

To either of the residues obtained above, add 0.2 ml of a mixture of 98 volumes of chloroform R and 2 volumes of acetonitrile R, close the vial and shake vigorously until the residues are dissolved, preferably using a vortex mixer.

Carry out the test as described in section 6, "Thin-layer chromatography", using silica gel G as the coating substance and a mixture of 85 volumes of chloroform R, 10 volumes of acetone R and 5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 2.5 µl, 5 µl, 7.5 µl and 10 µl of aflatoxin mixture TS, then apply three volumes, each of 10 µl, of the sample residues. Further superimpose on one of these spots 5 µl of aflatoxin mixture TS. Place the plate in an unsaturated chamber and develop. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in a dark room under ultraviolet light (365 nm).

Four clearly separated blue fluorescent spots are obtained from the aflatoxin mixture. Observe any spot obtained from the solutions of the residues that coincides in hue and position with those of the aflatoxin mixture. Any spot obtained from the solutions of the residues with the superimposed aflatoxin mixture should be more intense than the corresponding spot for the test solution, and should show no sign of separation or tailing, which would be a sign of dissimilar compounds.

Interpretation of results

No spots corresponding to aflatoxin should be obtained from any of the sample residues. If any such spot is obtained, compare its position with the spots obtained from the aflatoxin mixture to identify the type of aflatoxin present. An approximate estimation of the concentration of aflatoxin in the sample may be obtained by comparing the intensity of the spots with those of the aflatoxin mixtures.

19. Radioactive contamination

A certain amount of exposure to ionizing radiation cannot be avoided since there are many sources, including radionuclides occurring naturally in the ground and the atmosphere. These sources are described extensively in the booklet Facts about low-level radiation (13).

Dangerous contamination may be the consequence of a nuclear accident. The World Health Organization, in close collaboration with several other international organizations, has developed guidelines for use in the event of widespread contamination by radionuclides resulting from a major nuclear accident (14). This publication emphasizes that the health risks from food accidentally contaminated by radionuclides depend not only on the specific radionuclide and the level of contamination but also on the quantity of food consumed.

The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long-lived and short-lived fission products, actinides, and activation products. The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).

The amount of exposure to radiation depends on the intake of radionuclides and other variables such as age, metabolic kinetics, and weight of the individual (also known as the dose conversion factor).

Even at maximum observed levels of radioactive contamination with the more dangerous radionuclides, significant risk is associated only with consumption of quantities of over 20 kg of plant material per year so that a risk to health is most unlikely to be encountered given the amount of medicinal plant materials that would need to be ingested. Additionally, the level of contamination might be reduced during the manufacturing process. Therefore, no limits for radioactive contamination are proposed.

Method of measurement
Since radionuclides from accidental discharges vary with the type of facility involved, a generalized method of measurement is so far not available. However, should such contamination be of concern, suspect samples can be analysed by a competent laboratory. Details of laboratory techniques are available from the International Atomic Energy Agency (IAEA).[3]

[3] International Atomic Energy Agency (IAEA), Analytical Quality Control Services, Laboratory Seibersdorf, PO Box 100, Vienna, Austria.

20. Culture media and strains of microorganisms

Culture media

The following media are satisfactory, but other media may be used if they have similar nutritive and selective properties for the microorganisms to be tested.

Baird-Parker agar
Procedure. Dissolve 10.0 g of pancreatic digest of casein R, 5.0 g of beef extract R, 1.0g of water-soluble yeast extract R, 5.0 g of lithium chloride R, 20.0 g of agar R, 12.0 g of glycine R and 10.0 g of sodium pyruvate R in sufficient water to produce 950 ml. Heat to boiling for 1 minute, shaking frequently and adjust the pH to 6.6-7.0 using sodium hydroxide (0.5 mol/l) VS. Sterilize in an autoclave at 121°C for 15 minutes, cool to 45-50°C and add 10 ml of a sterile 0.01 g/ml solution of potassium tellurite R and 50 ml of egg-yolk emulsion.

Brilliant green agar
Procedure. Dissolve 10.0 g of dried peptone R (meat and casein), 3.0 g of water-soluble yeast extract R, 5.0 g of sodium chloride R, 10.0 g of lactose R, 10.0 g of sucrose R, 20.0 g of agar R, 0.08 g of phenol red R and 12.5 mg of brilliant green R in sufficient water to produce 1000 ml. Heat to boiling for 1 minute. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 6.7-7.1. Immediately before use, sterilize in an autoclave at 121°C for 15 minutes, cool to 50°C and pour into Petri dishes.

Buffered sodium chloride-peptone solution pH 7.0
Procedure. Dissolve 3.56 g of potassium dihydrogen phosphate R, 7.23 g of disodium hydrogen phosphate R, 4.30 g of sodium chloride R and 1.0 g of dried peptone R (meat and casein) in sufficient water to produce 1000ml. Polysorbate 20 R or polysorbate 80 R may be added, 0.001-0.01 g per ml. Sterilize in an autoclave at 121°C for 15 minutes.

Casein-soybean digest agar
Procedure. Dissolve 15.0g of pancreatic digest of casein R, 3.0g of papaic digest of soybean meal R, 5.0 g of sodium chloride R and 15.0 g of agar R in sufficient water to produce 1000 ml. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 7.1-7.5. Sterilize in an autoclave at 121°C for 15 minutes.

Cetrimide agar
Procedure. Dissolve 20.0 g of pancreatic digest of gelatin R, 1.4g of magnesium chloride R, 10.0 g of potassium sulfate R, 0.3 g of cetrimide R, 13.6 g of agar R and 10.0 ml of glycerol R insufficient water to produce 1000 ml. Heat to boiling for 1 minute with shaking. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 7.0-7.4. Sterilize in an autoclave at 121°C for 15 minutes.

Deoxycholate citrate agar
Procedure. Dissolve 10.0 g of beef extract R, 10.0 g of dried peptone R (meat), 10.0 g of lactose R, 20.0 g of sodium citrate R, 1.0 g of iron(III) citrate R, 5.0 g of sodium deoxycholate, 13.5 g of agar R and 20 mg of neutral red R in sufficient

Introduction

- The demand for plant based medicines,
  - health medicines,
  - health products,
  - pharmaceuticals,
  - food supplements,
  - cosmetics, etc.,
- is increasing in both developing and developed countries,

- Due to the growing recognition that
  - The natural products are non-toxic,
  - Have less side effects,
  - Easily available at affordable prices and sometimes
  - The only source of health care available to the poor.
- Global estimation indicate that
  - over 80 % of the world population can't afford the products of the Western pharmaceutical industry and
  - they have to rely upon the use of traditional plant-based medicines.

- To reduce the financial burden on the developing countries, which spend 40-50 % of their total budget on health.
- WHO currently encourages, recommends and promotes the inclusion of herbal drugs in national health care programmes because such drugs are easily available at a price within the reach of a common man and safer than the modern synthetic drugs.

- India has been identified as one of the top 12 mega-diversity rich flora of medicinal and aromatic plants occurring in diverse eco-systems.
- India has a long history of more than 5000 years as a supplier of Medicinal and fragrant materials to the Western Civilizations in Greece, Rome and Egypt.

- The Indian medicinal plant-based industry faces many problems and is affected by number of factors which include absence of well defined policies/guidelines and strategies for promotion of cultivation and post harvest technology, standardization and marketing.
- The most alarming problem of the plant-based industry is the decreasing supply of plant materials from natural resources.

- A national policy on medicinal plants with a view to preserve endangered species and promoting cultivation of plants which are being extensively used by industry, will help in solving the major problems of the industry.

- Special attention is required on medicinal plants on which significant research leads have been obtained.
- **E.g. *Acorus calamus* (Tranquilizer)**
- ***Albizia leback* (immunomodulator)**
- ***Andrographis paniculata* (Antihepatotoxic)**
- **Boswellia serrata (antiarthritic & antihepatotoxic)**
- ***Commiphora mukul (antihypercholesteolaemic)***
- ***Coleus forskohlii (cardiotonic)***
- ***Centella asiatica (brain tonic)***
- ***Phyllanthus amarus (antihepatotoxic)***
- ***Sida rhombifolia (anabolic)***
- ***Valeriana wallichii (Tranquillizer)***
- ***Withania somnifera (Adaptogen)***

- Indian industry is based on small family based units which are unable to compete at global level and need structural changes in production (size) and productivity (unit) through corporate entry in farming and utilization of technological innovation.
- There is need to have separate marketing and development board for medicinal and aromatic plants and phytopharmaceuticals.
- Such board could interact with the growers and users industry to bring stability in their production, demand, price, quality and also help in international trade.

- Global Herbal Medicine Market, by category, is segmented into herbal pharmaceuticals, herbal dietary supplements, herbal functional foods, and herbal beauty products.
- The herbal pharmaceutical segment is growing owing to the increasing research and development on various herbs to find their medicinal properties, growing demand for natural medicines having fewer side-effects, and rising prevalence of chronic diseases such as diabetes, arthritis, cancer, sleep disorders, and digestive problems.

CLASSIFICATION OF MEDICINAL PLANT BASED INDUSTRY

Six categories:

- i. Plant drugs for Indian system of Medicine (traditional system) covering- Ayurveda, Unani and Siddha.
- ii. OTC, non-prescription products consisting of plant parts, extracts and galenicals.
- iii. Essential oils industry
- iv. Phytopharmaceuticals
- v. Natural health products
- Health foods, Nutraceuticals, Recombinant proteins
- vi. Cosmeceutical industry

Based on indigenous systems

- Several traditional healthcare systems of medicine are being practiced in India subcontinents.
- The most commonly used one are Ayurvedic, Siddha, Unani and Tibbi system of medicine.
- All alternative systems introduced at different stages co-exist with its indigenous systems of medicine in the multiethnic states of India.
- There are many small manufacturing units using medicinal plants and thousands of Vaidyas preparing their own drugs from various plants.
- Herbal industry in India uses about 8000 medicinal plants;

- Frequently used plants in number of traditional herbal formulations.
- T. chebula, T. belirica, E. officinalis (219)
- G. glabra (141)
- Piper longum (135)
- Adhatoda vasica (110)
- Withania somnifera (109)
- Cyperus rotundus (102)
- Tinospora cordifolia (88)
- Berberis aristata (65)
- Holarrhena antidysentrica (59)
- Boerhavia diffusa (52)

SOME IMPORTANT MEDICINAL PLANTS USED BY INDIAN HERBAL INDUSTRY

Plant name	Consumption (tonnes)	Plant name	Consumption (tonnes)
Aloe leaf	200	Acorus rhizome	150
Kalmegh	250	Shatavari	500
Bacopa	700	Berberis	500
Cinnamon	200-300	Guggul	500
Kesar	5	Nagar motha	150
Bhringraj	500	Cardamom	60
Embelia	200	Glycyrrhiza	5000
Anantmool	200	Kurchi	150
Vasaka	500	Mucuna	200
Jaiphal	500	Jatamansi	200
Amla	10000	Kutki	200
Pipparimool	200	p. Nigrum	150

Plant name	Consumption (tonnes)	Plant name	Consumption (tonnes)
Chitrak	500	Vidarikand	200
Ashoka	1200	Senna	1000
Nux-vomicA	1000	Chirata	300
Clove	150	Cumin	300
Baheda	500	Harde	500
Galo	1000	Tagar-valerian	150
Ashwagandha	500	Ginger	500

- Several decade ago-medicine prepared by the practicing physician themselves
- Recent times-this practice replaced by the establishment of organized indigenous drug industry
- 25000 licensed pharmacies of Ind. Medicinal system
- 700000 registered practitioner
- 1000 single drugs, 3000 compound formulations
- Siddha-600-800 medicinal plants
- Unani 700-800
- Tibetian- 500-600
- Still exact quantification is not possible as no reliable data exists.

DEVELOPMENT OF HERBAL MEDICINE INDUSTRY

- Herbal medicines are the finished, labeled medicinal products that contain active ingredients from aerial or underground parts of plant or other plant materials, or combination thereof, whether in the crude state or as plant preparations.
- Herbal medicines may contain excipients in addition to active ingredients.
- Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants are not considered to be herbal medicines.

Non-prescribing (OTC)

- The direct utilization of plant material is a feature of traditional medicines not only in the developing countries but also in Europe and USA.
- Europe has a long history of research and processing of botanical extracts and has strict regulations, established quality control procedures and details of clinical data to support the products.
- Overall, the European market is as well regulated as the drug industry and many of the compounds sold in USA as dietary supplements are marketed as drugs in other countries.

- The current trend is to procure standardized extracts of plants as raw material, for which, they are trying to establish their own R&D unit as per the guideline issued by WHO.
- The objectives of these guidelines, therefore, is to define basic criteria for evaluation of quality, safety and efficacy of herbal medicines.
- Herbal and related extracts will see the strongest growth based on expanding scientific evidence of health benefits and rising popularity of alternative medicines.

- Govt. institutions involved in the standardization of raw material as well as formulations…
- Pharmacopoeial committees for Ayurveda, Siddha, Unani and Homeopathy systems.
- The Pharmacopoeial Laboratory for Indian Medicines (PLIM) and the Homeopathy Pharmacopoeial Laboratory (HPL) at Ghaziabadare providing technical backup to these committees.
- 178 monographs are ready
- Two volumes of Ayu. Formulary -635 formulations
- Siddha pharmacopoeia committee- seven volumes containing standards of 910 drugs.

- The Unani Pharmacopoeial Committee has published one National Formulary of 441 formulations.
- 45 monographs of single unani drugs
- The homeopathic Pharmacopoeial Committee has brought out 7 volumes containing standards of 910 drugs.

Essential Oil Industry

- The essential oil industry was traditionally a cottage industry in India.
- During the last 65 years, a number of industrial companies have been established for a large scale production of essential oils, oleo-resins and perfumes.
- The essential oils from plants being produced in India are more than 500 tonnes accounts for 90% of the world production.
- E.g. Ajowain oil, Celery oil, Citronella oil, Cedarwood oil, Devana oil, Eucalyptus oil, Lemon grass oil, Mentha oil, Geranium oil, Lavender oil, Palmrosa oil, Rose oil, Orange oil, Jasmine oil, Vetiver oil, Coriander oil, Sandalwood oil etc..

- In India the production of turpentine oil and the resin from pines is well-established industry; having annual production about 35000-40000 tonnes annually.
- Another big established oil industry is of mentha oil, eucalyptus oil and lemon oil.
- Annual world production of limonene is about 75000 tonnes and Brazil is biggest producer in world market.
- It is the by product of citrus industry though turpentine oil and eucalyptus oil also yield limonene but the best economically cheap raw material is the discarded orange and lemon peel which is being used by Brazilian Phytochemical Industry.

phytopharmaceuticals

- Before independence, plant-based phyto pharmaceutical industry in India was confined only to Quinine from Cinchona in the three state-owned factories.
- The very first industry was established by British Government at Mungpoo in Darjeeling.
- During the past 55 years, bulk production of plant-based drug has become major part of Indian Pharmaceutical Industry.

- Major pharmaceuticals produced in India are:
- Morphine, Codeine, Papaverine, Thebain, Emetine, Reserpine, Quinine, Quinidine, Digoxin, Caffeine, Hyoscyamine, Berberine, Colchicine, Rutin, Vinblastine, Vincristine, Brucine, Strychnine, Ergot Alkaloids, Senna glycosides, Diosgenin, Podophyllotoxin resin and citral.
- Indian Institute of Chemical Technology (IICT), Hyderabad has developed methods for etoposide and teniposide production; and CIPLA is now producing it on commercial bases.

- National chemical Laboratory, Pune developed the method for Vincristine (VCR) and Vinblastine (VLB) production and CIPLA has further improved the production technique and they are the third largest manufacturer of VCR and VLB in the world.

Natural Health Product Industry

- In these days the interest in herbal medicine is on rise, not only in the phytodrugs but also in natural health products, which include, health foods, nutraceuticals and personal care products.
- Health food: are the food products supplemented with herbal ingredients.
- Vitamines, minerals and nutrients or ingredients isolated from plants.
- They have physiological benefits and reduce the risk of chronic diseases.

- **NUTRACEUTICALS**
- It is a latest term for health food, first innovated by Stephen Deffice, founder of the Foundation for Innovation in Medicine of New Jersey, USA.
- The word nutraceutical is an amalgamation of the term “Nutrition” and “Pharmaceutical” or it can be more correctly defined as parts of a food that have a medical or health benefit including the prevention and treatment of disease.
- The three main constituents, which make-up nutraceuticals are herbal and related extracts, vitamins, minerals and nutrients.

- Antioxidant and herbal teas also form an important part of the nutraceutical market.
- The leading antioxidant phytochemicals in demand are Vit. A, C and E; Carotenoids and flavonoids.
- Indian nutraceuticals market CAGR of 21 % and reach $ 10 billion by 2022 from $ 4 billion in 2017
- Globally, too, the nutraceutical market is expected to reach $ 241 billion in 2019 from $ 172 billion in 2014.
- CAGR 7.3% (2015-2021)
- Japan is the 3rd largest producer of nutraceuticals in the world and largest in the Asia pacific region. About half of the all patents for nutraceuticals have been developed in Japan.

- Nutraceuticals are the most progressing sector for health food and pharmaceutical industry based on plants.
- Many functional food/nutraceutical companies are part of larger food or pharmaceutical industries.
- A number of large food and pharmaceutical companies, such as Abbott laboratories, GSK, Ledrle, Dabur, Himalaya, Zandu, Allen lab, and Aimil pharmaceuticals are also manufacturing nutraceuticals.

Herbal cosmetics and personal care products

- Rs. 80370 crore (2018) CAGR 22% -2025
- Cosmetic and personal care products containing natural products have rapidly growing trend in the market.
- Beginning in the early 1990's, cosmetic manufacturers began to use the term cosmeceuticals to describe the OTC skin care products.
- Claiming therapeutics benefits, the cosmeceutical products contain phytoconstituents in the extracts form or in the purified form such as alpha-hydroxy acids, vitamins, antioxidants and emollient oils rich in Vit A and E, botanical extracts, plant acids/enzymes and essential oils.

- Botanical extracts:
- Canola (Brassica napus)
- Chamomile (Matricaria chamomilla) dry extracts
- Marigold (Calendula officinalis) dry extracts
- Echinacea (echinacea spc..)
- Pumpkin seed (Cucurbita pepo) lipophilic extracts
- Ivy (Rhus toxicodendron) soft extracts
- Peruvian bark (Cinchona succirubra) fluid extracts
- Ginkgo (ginkgo biloba)
- Centella asiatica leaf extractsetc..

PHARMACOGNOSY SECTION

- MICROSCOPE BINOCULAR
- DISSECTING MICROSCOPE
- MICROTOME
- PHYSICAL BALANCE
- ALLUMINIUM SLIDE TRAYS
- STAGE MICROMETER
- CAMERA LUCIDA (PRISM & MIRROR TYPES)
- CHEMICALS, GLASS WARES ETC.

Industries:

- 1.Dabur India Ltd
- 2.Hamdard Laboratories
- 3.Baidyanath Pharmaceuticals
- 4.Himalaya Herbals
- 5.Surya Herbals Limited
- 6.Charak Pharmaceuticals
- 7.Zandu Realty Limited
- 8.Zealous Herbals
- 9.Vicco Laboratories
- 10.Divya Pharmacy
- 11. Patanjali

Institutions:

- Council of Scientific and Industrial Research (CSIR)
- 38 national laboratories, 39 outreach Center, 3 innovation complexes and 5 units
- 4600 active scientists, 8000 scientific and technical personnel
- National botanical Research Institute
- Central Institute of Medicinal and Aromatic Plants (CIMAP)
- Central Drug Research Institute
- National Medicinal Plant Board
- Indian Council for Medical Research
- Indian Agriculture Research Institute

[SCHEDULE T]

[See Rule 157]

GOOD MANUFACTURING PRACTICES FOR AYURVEDIC, SIDDHA AND UNANI MEDICINES

The Good Manufacturing Practices (GMP) are prescribed as follows in Part I and Part II to ensure that:

(i) Raw materials used in the manufacture of drugs are authentic, of prescribed quality and are free from contamination.
(ii) The manufacturing process is as has been prescribed to maintain the standards.
(iii) Adequate quality control measures are adopted.
(iv) The manufactured drug which is released for sale is of acceptable quality.
(v) To achieve the objectives listed above, each licensee shall evolve methodology and procedures for following the prescribed process of manufacture of drugs which should be documented as a manual and kept for reference and inspection. However, under IMCC Act 1970 registered Vaidyas, Siddhas and Hakeems who prepare medicines on their own to dispense to their patients and not selling such drugs in the market are exempted from the purview of G.M.P.

PART-I
GOOD MANUFACTRING PRACTICES

Factory Premises:

The manufacturing plant should have adequate space for:-

(i) Receiving and storing raw material
(ii) Manufacturing process areas
(iii) Quality control section
(iv) Finished goods store
(v) Office
(vi) Rejected goods/drugs store

1.2 General Requirements:

1.1(A) **Location and surroundings-** The factory building for manufacture of Ayurveda, Siddha and Unani medicines shall be so situated and shall have such construction as to avoid contamination from open sewerage, drain, public lavatory or any factory which produces disagreeable or obnoxious odour or fumes or excessive soot, dust or smoke.

1.1(B) **Buildings-** The building used for factory shall be such as to permit production of drugs under hygienic conditions and should be free from cobwebs and insects/rodents. It should have adequate provision of light and ventilation. The floor and the walls should not be damp or moist. The premises used for manufacturing, processing, packaging and labeling will be in conformity with the provisions of the Factory Act. It shall be located so as to be:

(i) Compatible with other manufacturing operations that may be carried out in the same or adjacent premises.
(ii) Adequately provided with working space to allow orderly and logical placement of equipment and materials to avoid the risk of mix-up between different drugs or components thereof and control the possibility of cross-contamination by other drugs or substances and avoid the risk of omission of any manufacturing or control step.
(iii) Designed, constructed and maintained to prevent entry of insects and rodents. Interior surface (walls, floors and ceilings) shall be smooth and free from cracks and permit easy cleaning and disinfection. The walls of the room

in which the manufacturing operations are carried out shall be impervious to and be capable of being kept clean. The flooring shall be smooth and even and shall be such as not to permit retention or accumulation of dust or waste products.

(iv) Provided with proper drainage system in the processing area. The sanitary fittings and electrical fixtures in the manufacturing area shall be proper and safe.

(v) Furnace/Bhatti section could be covered with tin roof and proper ventilation, but sufficient care should be taken to prevent flies and dust.

(vi) There should be fire safety measures and proper exits should be there.

(vii) Drying space- There should be separate space for drying of raw material, in process medicine or medicines which require drying before packing. This space will be protected from flies/insects/dusts, etc., by proper flooring, wiremash windows, glass pances or other material.

1.1(C) **Water Supply-** The water used in manufacture shall be pure and of potable quality. Adequate provision of water for washing the premises shall be made.

1.1(D) **Disposal of Waste-** From the manufacturing sections and laboratories the waste water and the residues which might be prejudicial to the workers or public health shall be disposed off after suitable treatment as per guidelines of pollution control authorities to render them harmless.

1.1(E) **Containers' Cleaning-** In factories where operations involving the use of containers such as glass bottles, vials and jars are conducted, there shall be adequate arrangement separated from the manufacturing operations for washing, cleaning and drying of such containers.

1.1(F) **Stores-** Storage should have proper ventilation and shall be free from dampness. It should provide independent adequate space for storage of different types of material, such as raw material, packaging material and finished products.

1.1(F)(A) **Raw Materials-** All raw materials procured for manufacturing will be stored in the raw materials store. The manufacture based on the experience and the characteristics of theparticular raw material used in Ayurveda, Siddha and Unani system shall decide the use of appropriate containers which would protect the quality of the raw material as well as prevent it from damage due to dampness, microbiological contamination or rodent and insect infestation, etc. If certain raw materials require such controlled environmental conditions, the raw materials stores may be sub-divided with proper enclosures to provide such conditions by suitable cabinization. While designing such containers, cabins or areas in the raw materials store, care may be taken to handle the following different categories of raw materials:-

(1) Raw material of metallic origin.
(2) Raw material of mineral origin.
(3) Raw material from animal source.
(4) Fresh Herbs.
(5) Dry Herbs or plant parts.
(6) Excipients, etc.
(7) Volatile oils/perfumes & flavours.
(8) Plant concentrates/extracts and exudates/resins.

Each container used for raw material storage shall be properly identified with the label which indicates name of the raw material, source of supply and will also clearly state the status of raw material such as 'UNDER TEST' or 'APPROVED' or 'REJECTED'. The labels shall further indicate the identity of the particular supply in the form of Batch No. or Lot. No. and the date of receipt of consignment. All the raw materials shall be sampled and got tested either by the in-house Ayurvedic, Siddha and Unani experts (Quality control technical person) or by the

laboratories approved by Government and shall be used only on approval after verifying. The rejected raw material should be removed from other raw materials store and should be kept in a separate room. Procedure of 'First in first out' should be adopted for raw materials wherever necessary. Records of the receipt, testing and approval or rejection and use of raw material shall be maintained.

1.1(F)(B) **Packaging Materials-** All packaging materials such as bottles, jars, capsules, etc. shall be stored properly. All containers and closures shall be adequately cleaned and dried before packing the products.

1.1(F)(C) **Finished Goods Stores-** The finished goods transferred from the production area after proper packaging shall be stored in the finished goods stores within an area marked "Quarantine". After the quality control laboratory and the experts have checked the correctness of finished goods with reference to its packing/labelling as well as the finished product quality as prescribed,, then it will be moved to 'Approved Finished Goods Stock" area. Only approved finished goods shall be dispatched as per marketing requirements. Distribution records shall be maintained as required.
If any Ayurvedic, Siddha and Unani drug needs special storage conditions, finished goods store shall provide necessary environmental requirements.

1.1(G) **Working Space-** The manufacturing area shall provide adequate space (manufacture and quality control) for orderly placement of equipment and material used in any of the operations for which these are employed so as to facilitate easy and safe working and to minimize or to eliminate any risk of mix-up between different drugs, raw materials and to prevent the possibility of cross-contamination of one drug by another drug that is manufactured, stored or handled in the same premises.

1.1(H) **Health, Clothing, Sanitation and Hygiene of Workers-** All workers employed in the Factory shall be free from contagious diseases. The clothing of the workers shall consist of proper uniform suitable to the nature of work and the climate and shall be clean. The uniform shall also include cloth or synthetic covering for hands, feet and head wherever required. Adequate facilities for personal cleanliness such as clean towels, soap and scrubbing brushes shall be provided. Separate provision shall be made for lavatories to be used by men and women, and such lavatories shall be located at places separated from the processing rooms. Workers will also be provided facilities for changing their clothes and to keep their personal belongings.

1.1(I) **Medical Services-** The manufacturer shall also provide:-

(a) Adequate facilities for first aid;
(b) Medical examination of workers at the time of employment and periodical check up thereafter by a physician once a year, with particular attention being devoted to freedom from infections. Records thereof shall be maintained.

1.1(J) **Machinery and Equipments-** For carrying out manufacturing depending on the size of operation and the nature of product manufactured, suitable equipment either manually operated or operated semi-automatically (electrical or team based) or fully automatic machinery shall be made available. These may include machines for use in the process of manufacture such as crushing, grinding, powdering, boiling, mashing, burning, roasting,filtering, drying, filling, labeling and packing, etc. To ensure ease in movement of workers and orderliness in operations a suitably adequate space will be ensured between two machines or rows of machines. These machinery and equipments and machinery recommended is indicated in Part II-A. Proper standard operational procedures (SOPs) for cleaning maintaining and performance of every machine should be laid down.

1.1(K) **Batch Manufacturing Records-** The licensee shall maintain batch manufacturing record of each batch of Ayurvedic, Siddha and Unani drugs manufactured irrespective of the type of product manufactured (classical preparation or patent and proprietary medicines). Manufacturing records are required to provide and account of the list of raw materials and their quantities obtained from the store, tests conducted during the various stages of manufacture like taste, colour, physical characteristics and chemical tests as may be necessary or indicated in the approved books of Ayurveda, Siddha and Unani mentioned in the First Schedule of the Drugs and Cosmetics Act, 1940 (23 of 1940). These tests my include any in-house or pharmacopoeial test adopted by the manufacturer in the raw material or in the process material and in the finished product. These records shall be duly signed by Production and Quality Control Personnel respectively. Details of transfer of manufactured drug to the finished products store including dates and quantity of drugs transferred along with record of testing of the finished product, if any, and packaging, records shall be maintained. Only after the manufactured drugs have been verified and accepted quality shall be allowed to be cleared for sale.

It should be essential to maintain the record of date, manpower, machine and equipments used and to keep in process record of various shodhana, bhavana, buring in fire and specific grindings in terms of internal use.

1.1(L) **Distribution Records-** Records of sale and distribution of each batch of Ayurveda, Siddha and Unani Drugs shall be maintained in order to facilitate prompt and complete recall of the batch, if necessary.

The duration of record keeping should be the date of expiry of the batch, Certain categories of Ayurvedic, Siddha and Unani medicines like Bhasma, Rasa, Kupi-pakva, Parpati, Sindura, Karpu/Uppu/Puram, Kushta, Asava-arista, etc. do not have expiry date, in contrast their efficacy increases with the passage of time. Hence, records need to be maintained up to 5 years of the exhausting of stock.

1.1(M) **Record of Market Complaints-** Manufacturers shall maintain a register to record all reports of market complaints received regarding the products sold in the market. The manufacturer shall enter all data received on such market complaints, investigations carried out by the manufacturers regarding the complaint as well as any corrective action initiated to prevent recurrence of such market complaints shall also be recorded. Once in a period of six months the manufacturer shall submit the record such complaints to the Licensing Authority. The Register shall also be available for inspection during any inspection of the premises.

Reports of any adverse reaction resulting from the use of Ayurvedic, Siddha and Unani drugs shall also be maintained in a separate register by each manufacturer. The manufacturer shall investigate any of the adverse reaction to find if the same is due to any defect in the product, and whether such reactions are already reported in the literature or it is a new observation.

1.1(N) **Quality Control-** Every licensee is required to provide facility for quality control section in his own premises or through Government-approved testing laboratory. The test shall be as per the Ayurveda, Siddha and Unani pharmacopoeial standard. Where the tests are not available, the test should be performed according to the manufacturer's specification or other information available. The quality control section shall verify all the raw materials, monitor in process, quality checks and control the quality of finished product being released to finished goods store/warehouse. Preferably for such quality control there will be a separate expert. The quality control section shall have the following facilities:

(1) There should be 150 sq feet area for quality control section.

(2) For identification of raw drugs, reference books and reference samples

should be maintained.

(3) Manufacturing record should be maintained for the various processes.

(4) To verify the finished products, controlled samples of finished products of each batch will be kept till the expiry date of product.

(5) To supervise and monitor adequacy of conditions under which raw materials, semi-finished products and finished products are stored.

(6) Keep record in establishing shelf life and storage requirements for the drugs.

(7) Manufacturers who are manufacturing patent proprietary Ayurveda, Siddha and Unani medicines shall provide their own specification and control references in respect of such formulated drugs.

(8) The record of specific method and procedure of preparation, that is, "Bhavana", "Mardana" and "Puta" and the record of every process carried out by the manufacturer shall be maintained.

(9) The standards for identity, purity and strength as given in respective pharmacopoeias of Ayurveda, Siddha and Unani systems of medicines published by Government of India Shall be complied with.

(10) All raw materials will be monitored for fungal, bacterial contamination with a view to minimize such contamination.

(11) Quality control section will have a minimum of-

(i) One person with Ayurveda/Unani/Siddha qualification recognized under Schedule II of Indian Medicine Central Council Act, 1970. Two other persons, one each with Bachelor qualification in Botany/Chemistry/Pharmacy could be on part-time or on contractual basis.

(ii) The manufacturing unit shall have a quality control section as explained under Section 35(ii). Alternatively, these quality control provisions will be met by getting testing, etc., from a recognized laboratory for Ayurveda, Siddha and Unani drugs; under Rule 160-A of the Drugs and Cosmetics Act. The manufacturing company will maintain all the record of various tests got done from outside recognized laboratory.

(iii) List of equipment recommended is indicated in Part II-C.

1.2 **Requirement for Sterile Product:**

(A) **Manufacturing Areas–** For the manufacture of sterile Ayurvedic, Unani and Siddha drugs, separate enclosed areas specifically designed for the purpose shall be provided. These areas shall be provided with air locks for entry and shall be essentially dust free and ventilated with an air supply. For all areas where aseptic manufacture has to be carried out, air supply shall be filtered through bacteria retaining filters (HEPA Filters) and shall be at a pressure higher than in the adjacent areas. The filters shall be checked for performance on installation and periodically thereafter the record of checks shall be maintained. All the surfaces in sterile manufacturing areas shall be designed to facilitate cleaning and disinfection. For sterile manufacturing routine microbial counts of all Ayurvedic, Siddha and Unani drug manufacturing areas shall be carried out during operations. Results of such count shall be checked against established in-house standards and record maintained.

Access to manufacturing areas shall be restricted to minimum number of authorized personnel. Special procedure to be followed for entering and leaving the manufacturing areas shall be written down and displayed.

For the manufacturing of Ayurvedic, Siddha and Unani drug that can be sterilized in their final containers, the design of the areas shall preclude the possibility of the products intended for sterilization being mixed with or taken to be products already sterilized. In case of terminally sterilized products, the design of the areas shall preclude the possibility of mix-up between non-sterile products.

(B) **Precautions against contamination and mix:**

(a) Carrying out manufacturing operations in a separate block of adequately isolated building or operating in an isolated enclosure within the building,
(b) Using appropriate pressure differential in the process area.
(c) Providing a suitable exhaust system.
(d) Designing laminar flow sterile air system for sterile products.
(e) The germicidal efficiency of UV lamps shall be checked and recorded indicating the burning hours or checked using intensity.
(f) Individual containers of liquids and ophthalmic solutions shall be examined against black-white background fitted with diffused light after filling to ensure freedom from contamination with foreign suspended matter.
(g) Expert technical staff approved by the Licensing Authority shall check and compare actual yield against theoretical yield before final distribution of the batch.

All process controls as required under master formula including room temperature, relative humidity, volume filled, leakage and clarity shall be checked and recorded.

PART-II

A. LIST OF RECOMMENDED MACHINERY, EQUIPMENT AND MINIMUM MANUFACTURING PREMISES REQUIRED FOR THE MANUFACTURE OF VARIOUS CATEGORIES OF AYURVEDIC, SIDDHA SYSTEM OF MEDICINES

One machine indicated for one category of medicine could be used for the manufacturing of other category of medicine also. Similarly some of the manufacturing areas like powdering, furnace, packing of liquids and Avaleha, Paks, could also be shared for these items.

Sl.No. (1)	Category of Medicine (2)	Minimum manufacturing space required (3)	Machinery/equipment recommended (4)
		1200 Square feet covered area with separate cabins or partitions for each activity. If Unani medicines are manufactured in same premises an additional area of 400 sq. feet will be required.	
1.	Anjana/Pisti	100 sq. feet.	Karel/mechanized/motorized, karel. End runner/Ball-Mill Sieves/Shifter.
2.	Churna / Nasya/ Manjan/Lepa/ Kwath Churn	200 sq feet	Grinder/disintegrator/Pulveriser/ Powder mixer/sieves/shifter.
3.	Pills/Vati /Gutika Matirai and tablets	100 sq. feet	Ball Mill, Mass mixer/powder mixer, Granulator, drier, tablet compressing machine, pill/vati cutting machine, stainless steel trays/container for storage and sugar coating, polishing pan in case of sugar-coated tablets,mechanised chattoo (for mixing guggulu) where required.

Sl.No. (1)	Category of Medicine (2)	Minimum manufacturing space required (3)	Machinery/equipment recommended (4)
4.	Kupi pakava/Ksara/ Parpati/LavanaBhasma Satva/Sindura Karpu/ Uppu / Param	150 sq. feet	Bhatti, Karahi/Stainless steel Vessels/Patila Flask, Multani Matti/Plaster of Paris, Copper Rod, Earthern container, Gaj Put Bhatti, Mufflefurnace(Electrically operated) End/EdgeRunner, Exhaust Fan, Wooden/S.S.Spatula.
5.	Kajal	100 sq. feet	Earthern lamps for collection of Kajal, Triple Roller Mill, End Runner, Sieves, S.S.Patila, Filling/ packing and manufacturing room should be provided with exhaust fan and ultra violet lamps.
6.	Capsules	100 sq. feet	Air Conditioner, De-humidifier, hygrometer, thermometer, Capsule filling machine and chemical balance.
7.	Ointment/Marham Pasai	100sq. feet	Tube filling machine, Crimping Machine/Ointment Mixer, End Runner/ Mill (Where required) S.S. Storage Container S.S.Patila.
8.	Pak/Avaleh/Khand/ Modak/Lakayam	100 sq. feet	Bhatti section fitted with exhaust fan and should be fly proof, Iron Kadahi/S.S. Patila and S.S. Storage container.
9.	Panak, Syrup / Pravahi Kwath Manapaku	150 sq, feet	Tincture press, exhaust fan fitted and fly proof, Bhatti section, Bottle washing machine, filter press / Gravity filter, liquid filling machine, P.P. Capping Machine.
10.	Asava / Arishta	200 sq. ft	Same as mentioned above. Fermentation tanks, containers and distillation plant where necessary, Filter Press.
11.	Sura	100 sq. ft	Same as mentioned above plus Distillation plant and Transfer pump.
12.	Ark Tinir	100 sq. ft	Maceration tank, Distillation plant, Liquid filling tank with tap / Gravity filter/Filter press, Visual inspection box.
13.	Tail/Ghrit Ney	100 sq. ft	Bhatti, Kadahi/S.S. Patila S.S.Storage Containers, Filtration equipment, filling tank with tap/Liquid filling machine.
14.	Aschyotan / Netra Malham Panir/Karn Bindu/Nasa-bindu	100 sq. ft	Hot air oven electrically heated with thermostatic control, kettle gas or electrically heated with suitable mixing arrangements, collation mill, or ointment mill, tube filling

Sl.No. (1)	Category of Medicine (2)	Minimum manufacturing space required (3)	Machinery/equipment recommended (4)
			equipment, mixing and storage tanks of stainless steel or of other suitable material sintered glass funnel, seitz filter or filter candle, liquid filling equipment, autoclave.
15.	Each manufacturing unit will have a separate area for Bhatti, furnace boilers, puta, etc. This will have proper ventilation, removal of smoke, prevention of flies, insets, dust etc. The furnace section could have tin roof.	200 sq. ft	

B. LIST OF MACHINERY, EQUIPMENT AND MINIMUM MANUFACTURING PREMISES REQUIRED FOR THE MANUFACTURE OF VARIOUS CATEGORIES OF UNANI SYSTEM OF MEDICINES

One machine indicated for one category of medicine could be used for the manufacturing of other category of medicine also. Similarly some of the manufacturing areas like powdering, furnace, packing of liquids could also be shared for these items.

Sl.No. (1)	Category of Medicine (2)	Minimum manufacturing space required (3)	Machinery/equipment recommended (4)
		1200 square feet covered area with separate cabins, partitions for each activity. If Ayurveda / Siddha Medicines are also manufactured in same premises an additional area of 400 square feet will be required.	
1.	Itrifal Tirya/majoon/ Laooq/Jawarish Khamiras	100 sq. feet	Grinder/ Pulveriser, Sieves, powder mixer (if required), S.S. Patilas, Bhatti and other accessories, plant mixer for Khamiras.
2.	Arq.	100 sq. feet	Distillation Plant (garembic) S.S. storage tank, Boiling Vessel, Gravity filter, Bottle filling machine, Bottle washing machine, Bottle drier.
3.	Habb (Pills) and tablets.	100 sq. feet	Ball Mill, Mass Mixer/Powder mixer, Granulator drier, tablet compressing machine, pill/vati cutting machine, stainless steal trays/ container for storage and sugar coating, polishing

Sl.No. (1)	Category of Medicine (2)	Minimum manufacturing space required (3)	Machinery/equipment recommended (4)
			pan in case of sugar-coated tablets, mechanized chattoo, (for mixing guggul) where required.
4.	Sufoof (Powder)	200 sq. feet	Grinder / pulveriser, Sieves, Trays, Scoops, Powder mixer (where required).
5.	Raughan (oils) (Crushing and boiling)	100 sq. feet	Oil Expeller, S.S. Patilas Oil filter bottle, Filling machine, Bottle drier, Bhatti.
6.	Shiyaf, Surma, Kajal	100 sq. feet	End runner, mixing S.S. Vessel.
7.	Marham, Zimad (Ointment)	100 sq. feet	Kharal, Bhatti, End runner, Grinder, Pulveriser, Triple Roller Mill (if required).
8.	Qurs (Tab.)	100 sq. feet	Grinder/Pulveriser, Sieves, Powder mixer (where needed), Granulator, Drier, Tablet Compressing Machine, Die punches Trays, O.T. Apparatus, Balance with weights, Scoops, Sugar Coating Pan, polishing pan, Heater.
9.	Kushta	100 sq. feet	Bhatti, Kharal, Sil Batta, Earthen pots.
10.	Murabba	100 sq. feet	Aluminium Vessels 50-100 kgs. Capacity, Gendna, Bhatti.
11.	Capsule	100 sq. feet	Pulveriser, Powder mixer (where needed), capsule filling machine, Air conditioner, De-humidifier, Balance with weights, storage containers, glass.
12.	Sharbat and Joshanda	100 sq. feet	Tinctum Press, exhaust fan fitted, Bhatti section, Bottle washing machine, Filter Press Gravity filter, Liquid filling tank with tap/liquid filling machine, hot air oven electrically heated with thermostatic control, kettle.
13.	Qutoor-e- Chashm and Marham (Eye drops, eye ointment)	100 sq. feet	Hot air oven electrically heated with thermostatic control, kettle.

C. LIST OF EQUIPMENT RECOMMENDED FOR IN-HOUSE QUALITY CONTROL SECTION

(Alternatively, unit can get testing done from the Government approved laboratory).

(A)	CHEMISTRY SECTION	(B)	PHARMACOGNOSY SECTION
1.	Alcohol Determination Apparatus (complete set)	1.	Microscope Binoculor.

(A)	CHEMISTRY SECTION	(B)	PHARMACOGNOSY SECTION
2.	Volatile Oil Determination Apparatus.	2.	Dissecting Microscope.
3.	Boiling Point Determination Apparatus.	3.	Microtome.
4.	Melting Point Determination Apparatus.	4.	Physical Balance.
5.	Refractometer.	5.	Aluminium Slide Trays.
6.	Polarimeter.	6.	Stage Micrometer.
7.	Viscometer.	7.	Camera Lucida (Prism and Mirror Type).
8.	Tablet Disintegration Apparatus.	8.	Chemicals, Glassware etc.
9.	Moisture Meter.		
10.	Muffle Furnace.		
11.	Electronic Balance.		
12.	Magnetic Stirrer.		
13.	Hot Air Oven.		
14.	Refrigerator.		
15.	Glass/Steel Distillation Apparatus.		
16.	LPG Gas Cylinders with Burners.		
17.	Water Bath (Temperature controlled.)		
18.	Heating Mantles/ Hot Plates.		
19.	TLC Apparatus with all accessories (Manual)		
20.	Paper Chromatography apparatus with accessories.		
21.	Sieve size 10 to120 with Sieve shaker.		
22.	Centrifuge Machine.		
23.	Dehumidifier.		
24.	pH Meter.		
25.	Limit Test Apparatus.		

Note: - The above requirements of machinery, equipments, space are made subject to the modification at the discretion of the Licensing Authority; if he is of the opinion that having regard to the nature and extent of the manufacturing operations it is necessary to relax or alter them in the circumstances in a particular case.