Investigation of Medicinal Plants by Scientific Methods

by

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1. *Selection of Medicinal Plants:* It is advisable to refer the ethnomedical reports and books on medicinal plants in the flora before the selection. The plant selected has to show biological activity. Once it is collected a voucher specimen has to be prepared and this is useful in the identification of plant and for future reference.

2. *Extraction of Plant material:* Once the plant material is collected it has to be extracted in order to isolate the biologically active compounds. Extraction itself is twofold. You can do either a cold extraction or a hot extraction depending on the solubility or/and stability of the active compound(s) in the selected solvent. In cold extraction you have to dip the material in the solvent and shake it well at room temperature. But the hot extraction needs a special set-up called soxhlet apparatus where extractions are done under refulx conditions. Solvents can be either organic (hexane, $CHCl_3$, methanol, etc.) or aqueous (H_2O, dilute acid, etc.). After the extraction is completed the solvent has to be removed by evaporation under reduced pressure.

3. *Biological and Phytochemical screening of the extract:*

3.1. *Biological Screening* A large number of biological tests are available. Some examples are described below.

3.1.1. *Antitumour:* Antitumour active principles are the substances capable of inhibiting the growth of cancer cells. Cytotoxic tests following tumour inhibiting or tumour destroying assays are available for the screening of these extract. *In vitro* tests are often used for the screening.

3.1.2. *Antifertility* : Antifertility agents are the substances capable of presenting or terminating pregnancy. Both *in-vivo* and *in-vitro* techniques are available for screening of a plant extract. The test can be based on either female antifertility activity or male antifertility activity.

3.1.3. *Antimicrobial* : This area covers the activity of an extract against both fungal and bacterial growth. Antimicrobial testing is often done *in-vitro* due to its efficiency. Thin Layer Chromatographic (TLC) bioassay, spore germination test and agar plate test are used for antifungal assay. The most widely used procedure for antibacterial assay is the disc diffusion method.

3.2. *Phytochemical Screening* Phytochemical screening is carried out in order to get an idea about the important phytochemical groups present in an extract. Some of the

important phytochemical groups responsible for biological activity are alkaloids, saponius, sterols, terpenes, flavoncids, polyphenols, coumarius etc. A large number of tests are available for screening plant extracts for different phytochemical groups.

4. *Bioactivity directed fractionation of extracts* : *Fractionation of an extract can be done either by solvent partion or by chromatography. Efficiency of solvent partition method depends upon the distribution constant, volumes of two phases used and on the number of extraction.*

Chromatographic separation is the most widely used method and it is based on differences in the distribution of solutes between a mobile and a stationary phase. Chromatographic methods can be classifed as column chromatography and open-bed chromatography. In column chromatography fractionation is carried out in a column and both gases and liquids can be used as mobile phase. Thin layer chromatography and paper chromatography are examples for the open-bed chromatography.

However, in activity directed fractionation one has to monitor the biological activity of each of the fractions obtained in each stage of fractionation or separation until the active compound(s) are isolated.

5. *Purification of active compounds* : *The most widely used method of purification is recrystallization. Once purified, it is better for a researcher to record all the physical properties such as colour, melting point (m.p.), optical rotation (L_D).*

6. *Structure elucidation of active compounds* : *Two different methods known as chemical methods and physical methods are used in structure elucidation.*

6.1. *Chemical methods* : *There are certain chemical tests which give different products or answers depending on the functional groups present in the compound. eg. 2.4. DNP test for carbonyl groups, Tollen's reagent for aldehyde groups etc.*

6.2 *Physical methods* : *Due to the availability of modern techniques physical methods predominate over chemical methods in structure elucidation. These techniques are easy to handle, time saving and one can recover the sample after obtaining the required data. The most widely used techniques are Ultra-violet (UV), Infra-red (IR), Nuclear Majnehi Resonance (NMR) and Mass spectroscopy.*

6.2.1. *UV spectroscopy* : *Ultra violet radiation is the part of the electromagnetic radiation between x-rays and visible regions which is energetic enough to affect on some electronic energy levels. UV absorption peaks are observed due to absorption of certain amount of energy of the radiation by the different chromophones present in the compound. Chromophone is an isolated functional group capable of absorbing UV or visible radiation. These groups confer colour on substance and most of them have unsaturated bonds such as C = C, C = O, N = N etc.*

6.2.2. *IR Spectroscopy* : IR radiation which extends beyond visible into microwave region is capable of affecting both the vibrational and rotational energy levels of a molecule. IR spectrum gives characteristic absorption for each functional group.

6.2.3. *NMR Spectroscopy* : Compounds also capable of absorbing radiowaves which causes the transition between two orientations of nucleus in the presence of magentic field, are known as nuclear magnetic resonance (NMR). Most widely used NMR technique is Proton NMR. Here the important point is that the chemical environment of H changes absorbing radio wave also changes for the resonance. So HNMR spectrum shows number and environment of protons present in the molecule. Similarly ^{13}C NMR gives number and environment of carbon atoms in the compound.

6.2.4. *Mass Spectroscopy* : In this technique, the molecule is bombarded with energised electrons and molecular ion is formed. The molecular ion is then accelarated under an electric field and let it to travel in a circular path. Depending on the mass of the fragment ions formed, different ions fall in different places and are detected. These ions appear as peaks in the spectrum with molecular weights. High resolution mass spectrum gives molecular formula of the compound.

In summary, UV spectrum gives the information about the choromphone, IR gives an idea about functional groups, ^{1}H and ^{13}C NMR gives different types and number of H and C atoms in the molecule and MS analysis gives the moleculer formula. So combining these information and using the knowledge of chemistry we can get chemical structure of a given compound.

APPENDIX I

SOME COMMONLY USED MEDICINAL PLANTS OF SRI LANKA

Botanical name (Family)	Sinhala/Tamil/English name	Important uses
1. *Abrus precatorius* (Leguminosae)	S - Olinda T - Kandam E - Indian Liquorice	Sore throat, conjunctivitis, ulcers
2. *Acalypha indica* (Euphorbiaceae)	S - Kuppamehiya T - Kuppamoni E - Three seeded mercury	Anthelmintic, bronchitis, asthma, ringworm
3. *Acorus calamus* (Araceae)	S - Wadakaha T - Vashambu E - Cinnamon Sedge	Cough, fever, anthelmintic
4. *Aerra lanata* (Amaranthaceae)	S - Polpala T - Siripulai E -	Diuretic, coughs, headaches
5. *Allium sativum* (Liliaceae)	S - Sudu lunu T - Vellaipoodu E - Garlic	Anthelmintic, diuretic, asthma, high blood pressure, gastric stimulant, bronchitis
6. *Aloe vera* (Liliaceae)	S - Komarika T - Veligam E - Aloe	Asthma, burns, dyspepsia
7. *Asparagus racemosus* (Liliaceae)	S - Hathawariya T - Sathavari E - -	Jaundice, diuretic, gonorrhoea
8. *Azadirachta indica* (Meliaceae)	S - Kohomba T - Malugam E - Margosa tree	Carminative, samllpox, Anthelmintic, Chronic ulcers, sores, Rheumatism
9. *Bacopa monnieri* (Scrophuloriaceae)	S - Lunuwila T - Brami E - -	Epilepsy, diuretic, Snake bites
10. *Briophyllum pinatrum* (Crassulaceae)	S - Akka-pana T - Malai Kalli E - -	Urinary disorders Paultice

11.	*Calotropis gigantea* (Asclepiadaceae)	S - Wara T - Arukkam E - Giant milk weed	Tumour inhibiting, Cough, cold, asthma, loss of appetite
12.	*Cannabis sativa* (Canabidaceae)	S - Ganja T - Ganja E - cannabis	Dysentery, tetanus, headache, appetizer
13.	*Carica papaya* (Caricaceae)	S - Gas-labu T - Pappali E - Papaya	Dyspepsia, intestinal irritation, ulcers
14.	*Cassia auriculata* (Leguminosae)	S - Ranawara T - Ponnayarasum E - Tannar's cassia	Diabetes, laxative, eye diseases
15.	*Centella asiatica* (Umbelliferae)	S - Gotukola T - Vallara E - Water Navel	Indigestion, dysentry, Eczema
16.	*Chenopodium ambrosioides* (Chenopodiaceae)	S - Chenopodium T - Sinnapodium E - Worm seed	Anthelmintic, Vermifuge
17.	*Coleus Malabricus* (Labiatae)	S - Eriwaria T - E -	Stomach ailments
18.	*Coscinium fenestratum* (Mernsperaceae)	S - Weni-wal T - Imalum E - Calumba Wood	Antiseptic, tetanus
19.	*Crotelaria vermucosa* (leguminosae)	S - Nil-andanahiriya T - Kilukluppai E - Blue rattle-pod	Scabies, impetigo
20.	*Croton tiglium* (Euphorbiaceae)	S - Jayapala T - Naganam E - Purging Croton	Purgative, cancer, rheumatism
21.	*Curcuma longa* (Zirigiberaceae)	S - Kaha T - Manjal E - Turmeric	Bruises, dysentry, anthelmintic
22.	*Cyperus rotundus* (Cyperaceae)	S - Kalandum T - Kora E - Nut sedge	Antiseptic, dysentry, diuretic.
23.	*Datura metal* (Solanaceae)	S - Kalu-attana T - Ayigam E - Purple Datura	Narcotic, asthma
24.	*Erratamia divaricata* (Apocynaceae)	S - Watu-sudda T - Nandiyar-vattam E - Moon flower	Sore eyes, Inflammation of the cornea

25. *Gloriosa superba* *(Liliaceae)*	*S - Niyandagala* *T - Anaram* *E - Glory lilly*	*Bruises, sprains, leprosy*
26. *Hemidesmus indicus* *(Asclepiadaceae)*	*S - Iramusu* *T - Nannari* *E - Indian Sarsaparilla*	*Diuretic, fever*
27. *Ixora coccinea* *(Rubiaceae)*	*S - Rat mal* *T - Vedchi* *E - Red ixora*	*Dysentery, Sedative, Bronchitis*
28. *Mesva ferrea* *(Guttiferae)*	*S - Na* *T - Irul* *E - Iron Wood*	*Astringent, stomachic dysentery, vomiting, coughs*
29. *Michelia champaca* *(Magnoliaceae)*	*S - Sapu* *T - Amariyam* *E - Golden champa*	*Carminative, stimulant, anstispasmodic, diuretic stomachic*
30. *Mimsoa pudica* *(Leguminosae)*	*S - Nidi kumba* *T - Thottasuringi* *E - Humble plant*	*Kidney diseases, bronchitis*
31. *Mormodica charantia* *(Cucurbitaceeae)*	*S - Karawila* *T - Pakal* *E - Bitter gourd*	*Diabetes, dysentery, vermifuge*
32. *Moringa oleifera* *(Moringaceae)*	*S - Murunga* *T - Achuram* *E - Drumstick*	*Aphsodiscae, diurectic, digestive, authelminitic*
33. *Murraya kuenigii* *(Rutaceae)*	*S - Karapincha* *T - Karuvembu* *E - Curry leaf*	*Diarrhoea, dysentery*
34. *Nelumbium speciosum* *(Nymphaeaceae)*	*S - Nelum* *T - Ambal* *E - Lotus*	*Diuretic, cardiac tonic, diarrhoea*
35. *Olimum sanctum* *(Labiatae)*	*S - Maduvutala* *T - Thulasi* *E - Basie*	*Gonorrhoea, malaria, catarrh*
36. *Paretta indica* *(Ruhiaceae)*	*S - Pavatta* *T - Pavattai* *E - Indian pellet shrub*	*Haemorroids Reumatism, visceral obstruction*

37. *Piper betle* *(Piperaceae)*	*S - Bulath* *T - Ilaikkodi* *E - Betel*	*Antiseptic, febrifuge, small pox.*
38. *Piper longum* *(Piperceae)*	*S - Thippili* *T - Argadi* *E - Long pepper*	*Fever, cough, bronchitis*
39. *Plumbago zeylanica* *(Plumbaginaceae)*	*S - Ela-kitul* *T - Akkini* *E - Ceylon Leadwort*	*Dyspepsia, diarrhoea*
40. *Punica grantum* *(Pumicaceae)*	*S - Delum* *T - Kalumal* *E - Pomegranate*	*Astringent, diarrhoea, dysentry, bronchitis*
41. *Raurolfia serpentina* *(Apoynaceae)*	*S - Ekaweriya* *T - Sorannamilbori* *E - Snake root*	*Arthelmintic, blood pressure, insanity*
42. *Ruta graveolens* *(Rutaceae)*	*S - Arudha* *T - Arudha* *E - Garden rue*	*Bronchitis, pneumonia*
43. *Sesamum indicum* *(pedaliaceae)*	*S - Thala* *T - Ellu* *E - Sesame*	*Vermifuge, catarrh*
44. *Sida acuta* *(Malvaceae)*	*S - Gas-berila* *T - Chittamatti* *E - ---*	*Haemorrhoich, fever*
45. *Solanum jaguini* *(Solanaceae)*	*S - Katuwelbatu* *T - Sutturam* *E - ---*	*Coughs, asthma*
46. *Tinospora cordifòlia* *(Menispermaceae)*	*S - Rasakinda* *T - Chintil* *E - ---.*	*Stomachache, fever*
47. *Veronica cineria* *(Compositae)*	*S - Monara kudumbiya* *T - Seedeviar senkulunir* *E - Fleabane*	*Anti-rheumatic conjunctivities Wormifuge*
48. *Vetiveria zizanioides* *(Graminae)*	*S - Savandara* *T - Vettiver* *E - Khus-Khus*	*Typhoid fever, anaemia, oedema*

49. *Vitex trifolia* (Verbenaceae)	S - Nika T - Nochi E - ---	Sprains, rhematism
50. *Zingiber officinale* (Zingiberaceae)	S - Inguru T - Allan E - Ginger	Dyspepsia, colds, fever

APPENDIX - II

Glossary of Medical Terms

ABSCESS	*A collection of pus or matter in any internal or external part of the body.*
ANTHELMINTIC	*A drug that is capable of killing intestinal worms. vermifuge.*
ANTACID	*A drug which nutralises the acidity of the gastric juice.*
ANAEMIA	*A disease in which the blood is deficient in quantity or in red blood cells.*
ASTRINGENT	*A drug which induces contraction of tissues, or arrests secretion or bleeding.*
ANTICEPTIC	*A drug which destroys disease germs.*
COLIC	*Pain due to spasmodic contraction of the abdomen.*
CANCER	*Any malignant growth.*
CONJUCTIVITIS	*Inflamation of the conjunctiva or the mucous membrane covering the eye ball.*
CARMINATIVE	*A drug which relieves flatulence or the feeling of over fulness of the stomach.*
DIURETIC	*A drug which increases the amount of urine.*
DROPSY	*A disease marked by an excessive collection of a watery fluid in tissues.*
LAXATIVE	*Relieves pain. Takes off the stress and strain.*
ECZEMA	*A skin disease.*
EMETIC	*A drug which causes vomitting.*
FEBRIFUGE	*A drug used for reducing fever.*

GALLACTAGOGE ..... *An agent that promotes the secretion and flow of milk.*

LEPROSY ..... *A chronic wasting disease caused by a germ.*

NARCOTIC ..... *A drug which induces deep sleep.*

OPTHALMIA ..... *Inflamation of the eye.*

STYPTIC ..... *An agent which checks bleeding.*