EVALUATION OF HERBAL BIORESOURCES USING HISTOLOGICAL AND HISTOCHEMICAL TECHNIQUES

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India is one of the World's 12 regions having the largest bioresources. It has 45000 plant species of which 15000-20000 possess proven medicinal value (Krishna Kumar, 1996). Indian Ayurveda is a one of the noteworthy systems of traditional medicine practice that uses mainly medicinal plants for the treatments of ailments in both people and animals. Although the popularity of herbal medicine recorded a sharp decline after the introduction of allopathic chemical drugs, herbal medicines are gaining growing interest because of their cost-effective and eco-friendly attributes. Recent observations indicate that perhaps 80% of the world's population relies solely upon medicinal plants for the treatment of diseases. Furthermore, a major part of chemically synthesized drugs against infectious agents is in fact derived from natural products or from structures suggested by natural products (Kirby, 1996).

For evaluation of therapeutic efficacy of herbal drugs certain special and routine histological and histochemical techniques are of great use in development and use the understanding of general mechanisms inherited in bioresources in response to various biotic and abiotic environmental factors and the potential and useful functions of these mechanisms. This offers ample opportunities for education and research toward creating basic theories and advanced technologies applicable to the development of sustainable agriculture and innovative bioindustry.

The evaluation of a crude drug is an essential part of Pharmacognosy. The individual drug plant would undergo many procedures. One of them is Microscopic Assessment, usually for powdered drugs. Its histological appearance must match that of a known sample, and the % of adulteration with foreign substances noted. Another is by Biological Standardization. This method is usually reserved for potent drugs where a chemical assay is not possible or unreliable, for example Digitalis, (Foxglove). The assay carried out by the LD. 50 test (Lethal Dose: 50%). A number of experimental animals are used to determine the minimum dose required to kill 50% of the creatures, within a defined period of time. The various organs of these animals are collected for histological examination to study the toxic effects of such drugs. This helps in drawing conclusions for their safe/ desired use.

The various histological and histochemical studies are conducted to evaluate the therapeutic efficacy or toxicity of any herbal preparation in various conditions of animals.

Study of effect of herbal preparations on dermatological conditions of animals and its influence on wound healing employ use of various histological and histochemical techniques. Skin diseases in domestic animals are of great economic importance as they severely affect livestock production and health. Some of them are of zoonotic importance too. On histological examination efficacy of any herbal preparation can be done. There is deposition of granulation tissue, early wound contraction, and epithelisation which enhanced wound healing (Ghani *et al*., 1981; Pandey and Ghani, 1981).

Histological examination of the bursa indicated that Zeetress can help protect the follicles of the bursa against damage resulting from IBD live vaccine. While in untreated birds the majority of the follicles were atrophied due to the destruction of lymphocytes and intra- and inter-follicular oedema, the follicles of birds treated with Zeetress were partially protected or spared (Pande, 1997).

The hypothalamus initiates the responses to a stressor. It induces the anterior pituitary to increase the production of adrenocorticotropic hormone (ACTH) which in turn reaches to the adrenal gland and causes hyperactivity of its cortical cells resulting in an increased synthesis of cortisol, also a hormone. The histological and histochemical methods are of use to appreciate changes before and after use of herbal preparations to ascertain its efficacy in stress.

Tissue culture, an important new technique is being used to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. Micro propagation is one of the important contribution of Plant Tissue Culture to commercial plant propagation and has vast significance. Micro propagation is the true to type propagation of selected genotype using in vitro culture technique. This technique provides a rapid reliable system for large-scale propagation of commercially important/priority species where conventional propagation methods are inadequate to meet the growing demand.

In tissue culture cells, tissues, and organs of a plant are separated. These separated cells are grown especially in containers with a nutrient media under controlled conditions of temperature and light. The cultured plant requires a source of energy from sugar, salts, a few vitamins, amino acids, etc. that are provided in the nutrient media which can be defines, semi defined or biological. From these cultured parts, an embryo or a shoot bud may develop, which then grows into a whole new plantlet. Similarly, portions of organs or tissues can be cultured in a culture media. Generally, these give rise to an unorganized mass of cells called callus (soft tissue that forms over a cut surface).

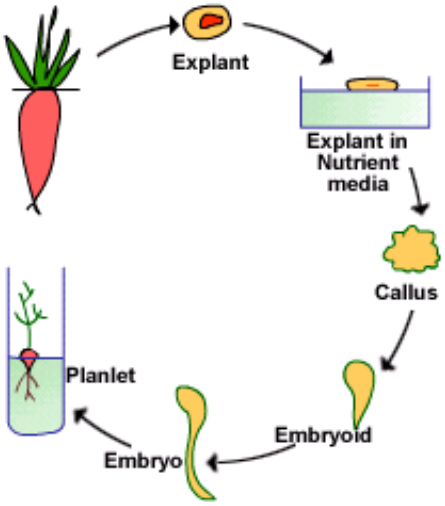

Figure: Tissue culture

Cloning: Genetically identical plants derived from an individual are called clones. Processes that produce clones can be put under the term ‘cloning’. This includes all the methods of vegetative propagation such as cutting, layering, and grafting. Propagation by tissue culture also helps in producing clones. Using the shoot tip, it is possible to obtain a large number of plantlets. This technique is used extensively in the commercial field for micro propagation of plants with medicinal values. Thus an unlimited number of plants that are genetically similar or are clones can be produced in a short span of time by tissue culture.

The most outstanding premiums offered by this aseptic propagation technology, over the conventional methods are;

1. In a relatively short time and space large number of plants can be produced, starting from single explant.

2. Unlike the conventional methods of plant propagation, micro propagation of even temperature species may be carried out throughout the year.
3. Tissue cultured plants are generally free from fungal and bacterial diseases. Virus eradication and maintenance of plants in a virus-free- state are also readily achieved in tissue culture.
4. Most of the herbal medicinal plants can be cultivated at various parts of the country.

Extracts obtained from the plants like *Catharanthus roseus, Taxus baccata, Solanum khasium, Artemisia annua, Digitalis lanata, Commifora mukal, Cassia angustifolia* etc have found a tremendous export potential (Ghatnekar and Kavian, 2000).

The demand for **vinblastine and vincristine** isolated from Periwinkle or *Catharanthus roseus* is only met due to tissue culture techniques. The **podophyllotoxin** a plant product has very good market potential which is used in the manufacture of 'etoposside'. *Podophyllum hexandrum*, a rhizome, is cultivated in Kashmir and now can be cultivated in the hilly regions of Maharashtra and Gujarat through tissue culture techniques. Another product, which has a good export potential, is **Camptothecin**, which is isolated from Nothapodytes foetida. Camptothecin is present in leaves and the bark of the plant. This plant can be cultivated throughout the Western Ghats and hilly tracks of Gujarat. **Taxol**, which is isolated from Taxus baccata (Indian Yew) is present in the bark and the spines. Although a native of North Eastern India, the plant, through the technique of tissue culture can be cultivated in many parts of Maharashtra and Gujarat. All these four products mentioned above possess antineoplastic {anti cancer} activity.

The most important mandate for cultivators of medicinal plants is use of chemical fertilizers and chemical pesticides is complete taboo. If chemical fertilizers and pesticides are used then quality as well as quantity of active ingredients is adversely affected. Nevertheless, cultivators can freely use soild and liquid biofertilizers like Growmore and Shootup, which are found to be totally safe and without any side effects whatsoever to increase he yield of active ingredients.

So various histological and histochemical techniques are at first used to evaluate the drug, its therapeutic efficacy / toxicity in animals and humans. Then the plants or part of plant with desired values can be micropropagated in large scale in all seasons at various parts of the countries in short time and space using tissue culture techniques. This helps to meet the growing demand of medicinal plants.

References:

1. Kirby, G. C. 1996. Medicinal plants and the control of protozoal diseases, with particular reference to malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 90:605-609
2. Krishna Kumar, P. R. 1996. Indian medicine industry under the Emerging patent regimes. *Ancient Science of Life.* 15(3):161
3. Ghani, A., S. K. Pandey, and G. N. Kolte. 1981. Histological & histochemcial evaluation of certain local medicaments as accelerator of wound healing. *Indian Journal of Indigenous Medicine.* (Suppl. 1).
4. Pandey, S. K. and A. Ghani. 1981. Clinical evaluation of certain local medicaments as accelerator in wound healing. *Indian Journal of Indigenous Medicine.* 2:1-10.
5. Ghatnekar, S.D. and Kavian, M.F.. 2000. Tissue culture opens up huge opportunities in growth of medicinal plants. Monday, August 28, 2000. Indian Express Newspapers (Bombay Edition).