INTRODUCTION

1.1 Introduction to fungal infections

Fungal infections are common as a primary disease or as a secondary infection developed following the antibiotic therapy. Individuals suffering from malignancy, diabetes mellitus and those under corticosteroid therapy are more prone to fungal infections[1].

Many of the fungi, that are now known to cause infection, usually live in association with humans as commensals without harming the host. For the last 3 decades there has been an increase in the incidence of at least secondary systemic serious fungal infections. This has resulted mainly because of the widespread use of highly potent antibiotics which kill even the nonpathogenic bacteria that normally compete with these fungi and keep their growth arrested; and secondly because of a decrease in host defense either because of AIDS or by the use of immunosuppressant drugs or by the use of cancer chemotherapeutic agents.

Fungal infections (also called mycoses) are less frequent than bacterial or viral infections but have become common in hospital settling and immunocompromised patients. Fungi are also more complex than bacteria or viruses. They have different ribosomes and possess a rigid outer cell wall which consists of a heavy amount of polysaccharides. Inner to this cell wall lies the cell membrane which contains ergosterol (instead of cholesterol)[2].

1.1.1 Classification of fungal infections[3,4]

Fungi may be classified as either yeasts or moulds (Table 1). Yeast like pathogenic fungi include *Blastomyces, Candida, Coccidioides, Histoplasma, Cryptococcus* and fungi that cause *chromoblastomycosis* (e.g. *Phialophora spp.*). Mould group of pathogenic fungi include *Aspergillus spp., dermatophytes* and *mucorales* fungi. Among fungi, *Candida species* and *Pneumocystis carini* are not pathogenic unless the normal defense

mechanisms are compromised. Hence the infections caused by them are called as opportunistic infections. Clinically, the fungal infections are classified as local (if it is in a restricted area of the body) or invasive (when it spread into nearby tissues) or disseminated (when the infection has spread from the primary site to different organs throughout the body). But the fungal infections are more conveniently classified in three main groups of fungal infection: the superficial, subcutaneous, and systemic infection.

Superficial infections (affecting skin, nails, scalp or mucus membrane)

The superficial infections are worldwide in their distribution. The superficial infections can be classified into the dermatomycoses and candidiasis. *Dermatomycoses* are infections of skin, hair and nails most commonly caused by *Trichophyton*, *Microsporum* and *Epidermophyton spp*. In superficial candidiasis, the yeast-like organism infects the mucous membranes of the mouth, vagina or skin.

Subcutaneos infections (affecting deep region of skin)

These infections are largely confined to the subcutaneous tissue and dermis, but may extend to the epidermis as well as deeper, for example, to bone. Organisms are usually implanted from sources in the external environment such as plants or soil. These infections are sometimes known as the mycoses of implantation because of this route of entry.

Systemic infections (affecting deeper tissues and organs)

Systemic fungal disease is systemic candidiasis- an infection with a yeast-like organism. Other more serious conditions are cryptococcal meningitis or endocarditis, pulmonary aspergillosis, and rhinocerebral mucormycosis. The commonest systemic fungal infections are blastomycosis, histoplasmosis, and paracoccidiomycosis; these are often primary infections, i.e. they are not secondary to reduced immunological function or altered commensally microorganisms.

Table 1: Classification of fungal infections

Sr. No.	Classes	Examples
1.	Superficial mycoses	Dermatophytosis Superficial candidosis Disease due to Malassezia Others (eg, Scopulariopsis, Scytalidium infections)
2.	Subcutaneous mycoses	Sporotrichosis Mycetoma Chromoblastomycosis Phaeohyphomycotic cyst Subcutaneous zygomycosis
3.	Systemic mycoses	Opportunistic Systemic candidosis, Aspergillosis, Zygomycosis, Fusariosis Cryptococcosis Histoplasmosis African histoplasmosis Blastomycosis Paracoccidioidomycosis Infection due to Penicillium marneffei

1.1.2 Antifungal drugs[1]

Drugs used in fungal infections are classified as follows:

(a) Those acting locally

Antibiotics : Nystatin, Pimaricin, Hamycin, Candidicin and Tricomycin

Chemical agents : Benzoic acid, Salicylic acid, Chlorphenasin, Tolnaflate, Dithranol, Chrysarobin, Ichthamol, Selenium sulfide, Mesulfan, Holoprogin, Buclosamide, Cictopiroxolamine, Quinidochlor.

(b) Those acting systemically

Griseofulvin, Amphotericin-B, Flucytosine.

(c) Those acting systemically as well as topically

Ketoconazole, Miconazole, Itranconazole, Fluconazole.

1.2 Introduction to herbals

Herbal medicines are being used by about 80% of the world population in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability & lesser side effects. Further, modern medicines are costly, have produce side effects & require personal control.

Today, Herbal medicines have tremendous market growth in worldwide. For the European and USA markets alone, the requirement will reach about US $ 7 billion and US $ 5 billion per annum, respectively, in recent years[5]. In India, the value of herbal medicine related trade is about US $ 20 million with an annual export of US $ 1.1 billion. Annual production of herbal drugs is reached about US $ 60 billion in china. The main importers of china's herbal exports (about 66% shares) are Korea, Japan, Singapore and Hong Kong and have thus attracted the interest of larger pharmaceutical companies[6]. Now with advent of new Indian patent (amendment act) 2005, under which the herbal products can be patented, it is needed to establish bench-marks for Indian system of medicine to validate them scientifically for their worldwide acceptance and for the country's benefit before these are patented in the western countries.

1.2.1 Terms relating to herbal medicines:

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products[7].

Herbs include crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered[7].

Herbal materials

Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by

various local procedures, such as steaming, roasting, or stirbaking with honey, alcoholic beverages or other materials[7].

Herbal preparations

Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification or concentration or by other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials[7].

Finished herbal products

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term **mixed herbal product** can also be used. Finished herbal products and mixed herbal products may contain excipients in addition to the active ingredients. In some countries, herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal materials and mineral materials). Generally, however, finished products or mixed products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal[7].

1.2.2 Standardization & quality control of herbal drugs

In recent years, there has been fantastic demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics. There are around 6000 herbal manufacturers in India. More than 4000 units are producing Ayurveda medicines. Due to lack of infrastructures, skilled

manpower reliable methods and stringent regulatory laws most of these manufacturers produce their product on very tentative basis[7].

In order to have a excellent coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs. "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations. "Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration. Standardization of herbal drugs is not an simple task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in case of polyherbal drugs[7].

Standardization of herbal medicine is used to describe all measures which are performed during the production of herbal medicine and quality control leading to a reproducible quality[6]. Standardization gives an idea about the entire field of study from birth of a plant to its clinical application. Standardization is used to ensure the consistent efficacy that manufacturers should use to ensure batch to batch consistency or their products[8]. A

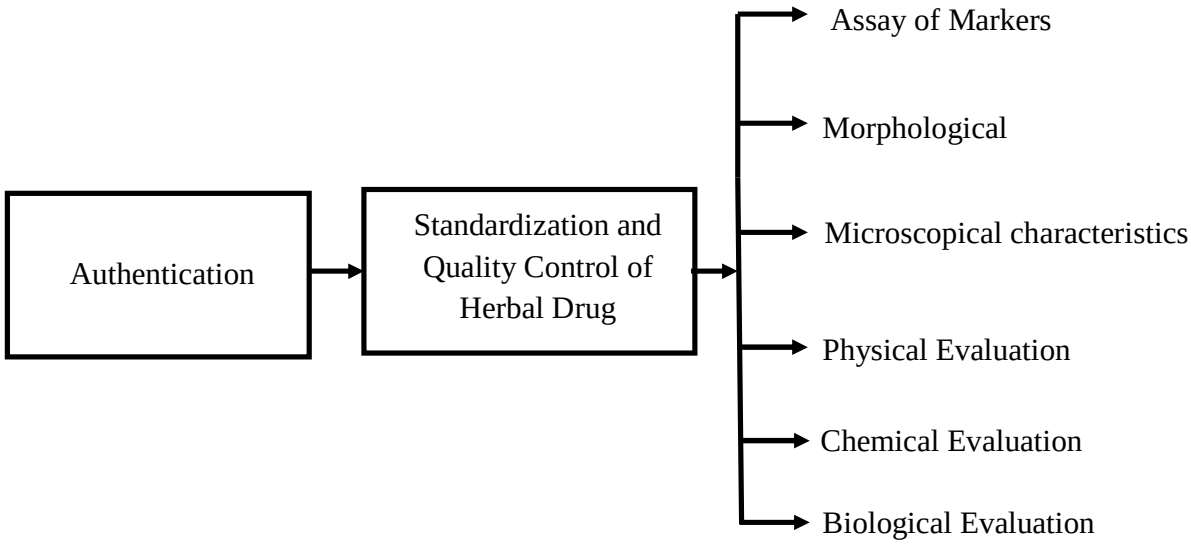
validated and standard methodology in herbal medicine is a must and requires correlation of traditional knowledge with recent scientific knowledge[6].

In the past the collection, identification, preparation of ayurvedic medicines were done by the Acharyas themselves. So drugs made by them were more efficacious, authentic and genuine. In the present age, the collection is made by the suppliers. There are so many drugs, which lost their effectiveness with the passage of time, yet they are used by the manufactures because of its inclusion in yoga. Sometimes a adulterant is asked in place of the told drugs, which is not used properly. These big pharmacies never see the effectiveness always in hurry to prepare the drug to make and fulfill their supply asked by the consumer. They never hesitate to make the adulteration by adding the low quality drugs. This causes the lowering of the genuine character of the drug and makes it less efficacious[9].

Herbal medicine standardization of medicinal plant parts should be authentic and free from harmful materials like pesticides, arsenic and heavy metals, aflatoxin contaminations, radioactive contaminations and microbial contaminations etc. The extract should then be checked for indicated biological activity in an experimental animal(s). The bioactive extract should be standardized on the basis of active principal or major compounds along with fingerprints. Another important criterion is stabilization of the bioactive extract with a minimum shelf life of over a year.

Standard and consistent quality of herbal products is only possible if the quality of the raw material is herbs and is controlled in a detailed manner. WHO has taken necessary steps to control the quality of herbal drugs and to ensure the higher safety margins of the herbal drugs[6,10]. An authenticated protocol for quality control of raw material for the finished product is shown in figure 1.

Figure: 1 An authenticated protocol for quality control of raw material for the finished product



1.2.2.1 Important quality control parameters for making a consistent quality of herbal medicine[11,12,13]:

Physical parameters:

Foreign Matter : Foreign organic matter,
Microbial contamination,
Radio active contamination,
Aflatoxin contamination,
Moisture content.

Ash Values : Total ash, Acid insoluble ash,
Water soluble ash, Sulphated ash.

Extractive Values : Water soluble extractive,
Ethanol soluble extractive,
Petroleum ether soluble extractive,
Volatile matter

Physical constants : Melting Point, Freezing Point, R_f Value, Refractive Index, Optical Rotation.

Chromatographic Methods : T.L.C., H.P.L.C. H.P.T.L.C., G.C.

Spectroscopic Methods : U.V.- Visible Spectroscopy, I.R. Spectroscopy, N.M.R. Spectroscopy, Mass Spectroscopy, X-Ray Crystallographic Study.

Chemical Evaluation:

Aqueous Titrametric Method

Non aqueous Titrametric Method

Gravimetric Analysis

Colorimetric Method

Simple Chemical Test Analysis & Phytochemical Screening

Biological Evaluation:

Bioassay

Microbial Assay

Morphological Characteristics:

Sensory Study : Colour, Odour, Taste, Texture, Shape & Size, Surface characteristics etc...

Systemic Study : Stem, Fruits, Leaves, Flowers etc…

Microscopical Characteristics:

Transverse Sections

Longitudinal Sections : Radial L.S., Tangential L.S.

Surface Constants : Stomatal Index, Stomatal Number,

Palisade ratio, Vein Islet Number,

Vein Termination Number.

At present, modern analytical tools are available for finding the content of crude drugs. The International Conference on Hormonization (ICH) guidelines provides internationally acceptable validated methods and procedures for determining active constituents present in the plant drugs. An authentified and standard protocol for production of herbal drug is shown in figure 2.

Figure 2: An authenticated and standard protocol for production of herbal drug