

An Elsevier Indexed Journal

ISSN-2230-7346



Journal of Global Trends in Pharmaceutical Sciences

STANDARDIZATION OF HERBAL COMPOUNDS

Rajani Kontu^{*1}, Uma Maheshwar Rao Vattikuti², Sandhya Rani.M³

^{1, 2}Teegala Ram Reddy College of Pharmacy, #4-202, Meerpet, Saroornagar (M), Hyderabad, Telangana State-500097

³Center for Pharmaceutical sciences, IST, Kukatpally, JNTU-Hyderabad, Telangana-500085

*Corresponding author E-mail: <u>k.rajini0507@gmail.com</u>

ARTICLE INFO

Key Words Traditional medicine, Standardization, Quality, Evaluation



been used by the healers as "TRADITIONAL SYSTEM OF Herbs were MEDICINE" since prehistoric civilization intended for treating ailments applied for both humans as well as animals. A large part of growing countries rely on conventional practices of herbal medicines to meet up their health concerned needs. A variety of traditional systems followed in China, Japan along with India like Traditional Chinese medicine, Japanese traditional medicine and Indian traditional medicine. Just before 18th century Indian traditional system was proficient under Ayurveda, Siddha, Unani and homeopathy. According to western way of life the use of synthetic medicines was likely to acquire more side effects with less therapeutic frequency. To get rid of these problem Now-a-day's herbal drugs has an extensive adequacy by majority of the people due to their therapeutic activity for several ailments. In order to meet the quality parameters, the herbal drugs are to be standardized. The progression of standardization includes right from its cultivation, collection of herbs until out into the market. The herbal compounds were evaluated and standardized as per regulatory guidelines of WHO with established standards for evaluation or standardization of herbal compounds that mainly focus on botanical. physicochemical, pharmacological and toxicological parameters. This review article says that herbal remedies account for a major contribute for the global market which have to approve internationally predictable guiding principles of their quality, safety, and purity.

ABSTRACT

INTRODUCTION

Herbal drugs follow ethnopharmacology which was defined as scientific study of materials obtained from plants usually fragmented parts, entire plant, algae, fungi, and lichen by using traditional medicine systems like Chinese traditional medicine, Japanese traditional medicine, Indian traditional medicine etc . Discovery of new drug from medicinal plants was increasingly used against modern medicines like Allopathy⁽¹⁾. The use of traditional medicine was habitually practiced as a vital part of their culture in different developing countries. People from western countries were more likely to be believed that

the use of herbal drugs keep their lives healthier ⁽²⁾. Herbal drugs containing single chemical constituent or in multiple combinations were used as medicinal products, dietary supplements and cosmetics. These herbal formulations can be easily bought over the counter at pharmacy stores or health food shops. Herbal medicines originated firstly in the Asian countries before spreading to the West and today they were being seen as therapeutic agents for several chronic diseases. Herbal medicinal products in the form of dietary supplements were taken to promote one's health and wellness. However, it was incorrect to ingest them without a prescription as some can lead to health problems of a different sort, some may interfere with other drugs or some may not be very effective. Standardization of dietary supplements was very important for evaluating the drug quality based on the strength of their active principles. Examples of different parts of plants used as herbal medicine was discussed in the table (1)

STANDARDIZATION

Means adjusting the herbal drugs preparation to a define content of a constituent or a multiple constituents with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparation.

Standardization of Crude Drug and Formulated Drug:

Standardization was performed mainly for crude drug and formulated crude drug with their procedures was different from each other. The following are the methods to be followed for the procedure for standardization includes:

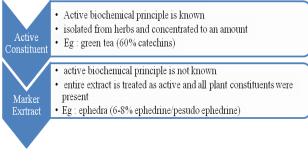
- Standardization of the crude drugs was comparison of obtained crude drug with that of the reference standard (authenticated drug). The collected test crude drug was to be accepted from herbal drug markets, and then Substitution and adulteration process should be carried out for test crude drug. Whereas for reference standard process of botanical and chemical characterization should be done, followed by specifications and limits in their documentation. For the crude drug after knowing the test adulterated compound it has to be undergoing botanical and chemical characterization unlike the reference standard.
- Standardization of formulated drug, the formulated test crude drug undergoes comparative study of classical and modern literature of information followed by location of marketed samples, study of manufacturing process and their methods and standardization done according to established Good manufacturing practices. Selection of test protocols, analysis of marketed samples and laboratory preparations were done for standardization

of formulated standard references. Then the performance of the evaluation methods was compared and related quality parameters were selected and documented. At last the final quality control standards were given by statutory body after the examination of both crude drugs and their formulation ⁽³⁾.

Standardized herbal extracts and their types:

The herbal extracts which undergo for standardization process includes the active constituent and the marker extract was described in the figure 1.

Figure No. 1: Types of Constituents that undergo standardization



NEED FOR STANDARDIZATION

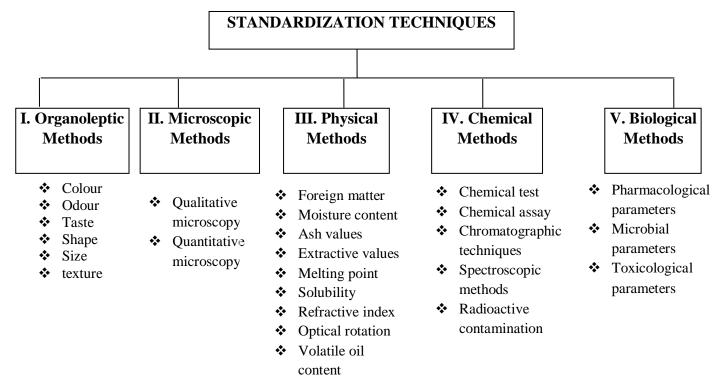
Larger number of people were moving towards use of herbal medicines in today's times, due to severe prone of side effects with that of modern medicine which became noticed every day. It the complete responsibility of was the regulatory authorities to assure the availability of pure, safe, potent and effective herbal medicines to the consumer Various established quality standards were laid down in formularies, pharmacopoeias or manufacturing operation which was to be followed.

Herbal drugs or products, whether they have their origin from different alternative systems of medicines, need to follow standardized procedures in their manufacture. Standardization of herbal crude drugs was a process of setting specific defined as standards and their limits that carry the quality, safety, efficacy and strength of the formulated herbal product⁽²⁾. So, need for standardization should be done right from cultivation of medicinal plants, collection, harvesting, storage, and application drving in to clinical laboratories. The need for standardization is required Herbal remedies obtained from these

Sl. No	Biological Name	Family	Common Name	Part Used	Chemical Constituent	Used to Treat
1	Embilica officinalis	Euphorbiaceae	Amla	Fruit	Vitamin-c	Scurvy, laxative, hyper acidity
2	Terminalia arjuna	Combretaceae	Arjun	Dried bark	Glycoside: arjunone	Mild diuretic, ischaemic heart diseases
3	Aloe barbadensis	Liliaceace	Aloe	Dried juice of the leaves	Barbaloin, glycosides	Cosmetics, laxative
4	Saraca indica	Leguminosae	Asoka	Dried stem bark	epicatechin	Astringent, uterine disorders
5	Brassica nigra	Cruciferae	Mustard	Dried ripe seeds	Glycerides of oleic,linoleic, linolinic acids	Emetic
6	Papaver somnifereum	Papaveraceae	Opium	Latex obtained from unripe capsule	morphine	Analgesic, hypnotic, narcotic
7	Pinus palustris	Pinaceae	Turpentine oil from long-leaf pine	resin	Alpha- pinene, Beta- pinene	Mild antiseptic, counter-irritant
8	Theobroma cacao	Sterculiaceae	Cacao	seeds	xanthines	Nervous system stimulant
9	Curcuma longa	Zingiberaceae	Turmeric	Dried rhizomes	Curcuminoids: curcumin	Anti- inflammatory, carminative
10	Catharanthus roseus	Apocynaceae	Vinca	Whole plant	vincristine	Anti-neoplastic, treatment for hodgkins disease

Table No: (1) LIST OF VARIOUS HERBS USED AS MEDICINES⁽³⁾





drugs account for a major share of the global market and hence it is necessary to adopt internationally recognized guidelines for their quality control. Some of the drawbacks concerned in standardization procedures were⁽³⁾.

- Identification of botanical source
- Adulteration
- Climatic conditions
- Harvesting
- Drying & storage

STANDARDIZATION OF HERBAL COMPOUNDS - METHODS AND PROCESS

As per World Health Organization (WHO), standardization defines as a process of evaluation of crude drug to authenticate its identity, determination of quality and purity, detection of nature of adulterants' by various parameters

The given figure (2) explains the contents which give an idea about the scope of work involved^(3, 6).

I. Organoleptic /Macroscopic Methods:

Also known as Macroscopic evaluation which refers to evaluation of crude drug through sensory characters ^(4, 6) discussed in the table (2).

Table No. 2: List of crude drugs with theirOrganoleptic characters

organoleptie enuractors			
Colour	Brown Colour	Cinnchona	
Odour	Aromatic	Umbelliferous Fruits	
Taste	Pungent	Capsicum	
Shape	Disc Shaped	Nux Vomica	
Size	Long- 10-30cm	Disitalia	
Size	Wide- 4-10 Cm	– Digitalis	
Texture	Fractured	Cascara Bark	
I CAUI C	Structure	Cascal a Dal K	

II. Microscopic Methods:

This method is used to find out the qualitative and quantitative histological characters of crude drug through transverse section (T.S) or longitudinal section (L.S) by using different staining reagents^(4, 6).

i) Qualitative microscopy: It determines xylem, phloem, stomata, trichomes etc.

Eg: Anomocytic (ranunculaceous) stomata – Foxglove, Anisocytic (cruciferous) stomata – Belladonna. Covering trichomes (uniseriate) – Digitalis, Glandular trichomes (uni cellular glandular trichomes) – Vasaka.

- **ii) Quantitative microscopy:** It includes palisade ratio, stomatal number, stomatal index, vein islet number, vein termination number.
 - a) Palisade ratio: Defined as average number of palisade cells underneath each epidermal cell.
 Eg: Cassia angustifollia 5.5-10 (upper), 4.0-7.4 (lower)
 - b) Vein islet number: Defined as number of vein-islets per square millimeter of the leaf surface between midrib to margin.

Eg: *Azadirachta indica* – 10 to 18

- c) Vein termination number: Defined as number of vein let termination per square millimeter of leaf surface midway from midrib to margin.
 Eg: Atropa belladonna 6.3-10.3
- d) Stomatal number: Defined as average number of stomata per square millimeter of leaf epidermis.
 Eg: Bacopa monniera 65-128 (upper),

90-145 (lower)

e) **Stomatal index:** Defined as percentage which the number of stomata forms to the total number of epidermal cells. calculated by given formula:

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

Eg: *Bacopa monniera* - 12.9-17.8 (upper), 12.4-16.4 (lower)

f) Lycopodium spore method: This method is to assist by counting the number of particles taken for measuring the portion of area having definite thickness and a known density with the help of a microscope. Lycopodium clavatum L are the spores composed in lycopodium which have a uniform size of 25μ m. The method can be simplified by weighing a single spore or expressed as 1mg of powdered lycopodium contains 94000 spores.

III. Physical Methods:

Physical methods includes the following parameters like

Ash Values indicates the measure of total ash with removal of all carbons produced after entire incineration of the drug compound heated at a temperature of about 450°C. This total ash usually consists of inorganic salts which also include physiological and non- physiological ash. Ash values are helpful in determining the quality and purity of an herbal compound. Different types of ash values are total ash, water soluble ash, and acid insoluble ash.

Foreign Matter in herbal drugs was seen during the cultivation& collection of medicinal plants. It refers to presence of part of a medicinal herb along with other foreign matters like animal excreta, moulds, insects sometimes it also includes mineral admixtures like stones, sand, dust which reduce the quality and purity of the medicinal to be obtained was discussed in the below table $(3)^{(7,8)}$.

Table 3: Limits for Ash value and ForeignOrganic matter

SI. No	Drug	Total ash	Acid insoluble ash	Foreign organic matter
1	Ashwagandha	≤7%	≤1.2%	≤2%
2	Ergot	≤5%	-	-
3	Rauwolfia roots	≤8%	≤2%	-

Extractives were the extracts of the exhausting crude material. To find out the evaluation of herbal crude drug, to identify the nature of active phytochemical constituents and their solubility criteria these extractive values were used .to obtain extractive values by water-soluble or ether-soluble extraction Soxhlet extractor is used. Different type of solvents was used for extraction process and their limits were mentioned in the below table (4) ^(8, 6, 1)

Types of Extraction	Herbal drug	Extractive values
Water- soluble extractive	Glycyrrhiza	Not less than 20.0% w/w
Alcohol – soluble extractive	Asafoetida	Not less than 50.0% w/w
Ether – soluble extractive	Nutmeg	Not less than 25.0% w/w

Volatile oil content was used to find out the volume of volatile oil present in the crude drug by distillation process. For the type of hydro distillation, specially designed apparatus called Clavenger's apparatus was used. The separation of volatile oil from the crude drug with the help of Clavanger's will take 4-5hours of heating due to boiling point elevation is created in it. The quality of an volatile (essential) oil containing drug is expressed in terms of percentage of oil present in the evaluating crude drug.

Eg: Fennel -4.0% v/w^(7,8).

Moisture content set down for the process of drying at the time of harvesting and cleaning the crude drugs due to the growth of microbes. The significance of drying helps in preservation, fixing to enzymatic or hydrolytic reactions that alter the chemical composition and decrease the weight of the drug. Lower the moisture content greater will be the stability, purity of the drug. It is determined by loss of drying, Azeotropic distillation method by Deane Stark Apparatus and Karl Fischer method⁽⁸⁾.

Bitterness value was used to determine the measure of bitter taste of a therapeutically active crude drug. As per WHO bitterness property of the medicinal crude plant material can be found out by comparing concentration of threshold bitterness of crude extract with that of the diluted solutions of quinine hydrochloride(1 gm of quinine hydrochloride in 2000ml of water)⁽⁹⁾.

Swelling Index or Swelling Factor refers to quantify the volume of mucilage up on dilution produced by 1gram of plant material. Swelling factor was mainly observed in the families of Plantago ovate, P.psylium etc. Its determination was known by adding water or swelling agent to the tested crude drug. Sometimes pectin and hemi-cellulose can also be predicted. By using glass-stopper measuring cylinder, the tested crude drug was shaken repeatedly for 1hour then the volume in milli liters of swelling factor can be determined^(9,8).

Foaming Index refers to herbal drugs containing Saponins when treated with aqueous decoction up on shaking can cause unrelenting foam so the ability of foaming of the herbal extracts can be determined by this parameter. Foaming index can be calculated by the formula:

Foaming index =
$$\frac{1000}{4}$$

Where A = volume in ml of diluted decoction

Solubility helps in determining the nature of solvent used to soluble the crude drug for the process of evaluation. For example alkaloidal free salts soluble in water, bases in organic solvents, fixed oils & fats in ether, chloroform.

Refractive index defines as When a ray of sodium light pass from one medium to the another medium of different densities bends at its original path, thus the ratio of velocity of light in vacuum to its velocity in the substance is said to be refractive index.

Eg: Mustard oil – 1.4758 to 1.4798.

Optical rotation Crude drugs either in solid state or liquid state which are optically active posses a property of rotating the plane of polarized light. This is measured by Polarimeter using sodium lamp as a light at temperature of 25°C.

Eg: Peppermint oil: -18° to -33° .

IV. Chemical Methods:

This method was evaluated for the chemical nature of the active constituent with the help of chemical tests, chemical assays, spectroscopic methods and chromatographic techniques.

i) Chemical test helps in Detection of specific groups of the active constituent in the crude extract. It is the easier and the fastest

method to be studied for the analyses of the particular active compound. The chemical tests can be performed by both qualitative and quantitative methods.

Qualitative methods: The organic constituents are the pharmaceutically important groups of the medicinal plants that include carbohydrates, alkaloids, tannins, glycosides, flavanoids etc. To detect the organic active moiety in crude drug special chemical tests are performed depending upon the colour reactions when treated with certain chemical reagents and some of the examples were given in the table 5

Table 5: Organic Constituents and their qualitative tests for Herbal Drugs

	qualitative tests for merbar brugs			
SI. No	Organic Constituents Containing Drugs	Specific Chemical Tests	Results	
1	Alkaloids -	Dragandroffs	Yellow/Orange	
	Cinchona, Vasaka	test	precipitate	
2	Glycosides -	Molisch test	Violet ring	
	Digitalis, Senna		formed at	
			junction of two	
			layers	
3	Steroids-	Lieberman	Green colour	
	Dioscorea	buchard test		
4	Tannins -	Ferric	Dark blue colour	
	Myrobalan	chloride test		
5	Flavanoids -	Shinoda test	Pink colour	
	Citrus			

• **Quantitative Chemical tests:** Applied for non cellular products such as fixed oils, Volatile oils, Waxes (Table 6).

SI. No	Quantitative Chemical Tests ⁽¹⁰⁾	Definition	Calculated by	Examples
1	Acid Value	Defined as number of milligrams of Potassium hydroxide(KOH) required to neutralize the free acid in 1gm of substance	acid value= $\frac{a \times 0.00561 \times 1000}{w}$	Yellow bees wax - 5to 8mg KOH/g
2	Saponification Value	Defined as no.of KOH required to neutralize the free fatty acid after hydrolysis of 1gm of oil/fat	saponification value = $\frac{(b-a) \times 0.02805 \times 1000}{w}$	Shark liver oil – 150 to 200mg KOH/g
3	Iodine Value	Expressed in grams, quantity of iodine which is absorbed by 100g substance	iodine value= $\frac{(b-a) \times 0.01269 \times 1000}{w}$	Olive oil- 79 to 88
4	Ester Value	Defined as no.of milligrams of KOH required for neutralizing the acids resulting for complete hydrolysis of lgm of substance	ester value= $\frac{(B_{Hel} - V_{Hel}) \times 28.05}{w}$	Carnauba wax – 74 to 78

Table 6: Definition of Quantitative Chemical tests and their examples

ii) Chemical assays:

For evaluating the specific group of constituent present in a crude drug it can be assayed by titrimetric and gravimetric methods, in some cases it can also be assayed by colorimetric technique. For example alkaloidal content can be assayed by titrimetric method present in a alkaloidal drugs i.e., Quinine from cinchona can be assayed by titrimetric method like acid-base titration. Pure form of drug can be obtained by these chemical assays.

iii) Spectroscopic methods:

The spectroscopical analysis of a drug to be evaluated can helps in determining the capacity of absorbed vibrations at specific wavelengths and structural determination. So the spectral data can be given by various techniques employed in pharmaceutical analysis includes Ultra-violet, Infra-red, Nuclear Magnetic Resonance (NMR), Mass spectroscopy etc.

iv) Chromatographic Techniques:

It represents a group of methods for separating molecular mixtures that depend on differential affinities of solute between two immiscible phases i.e. Mobile phase and a Stationary phase. The mobile phase will be in liquids or gaseous state where as stationary phase will be coated as thin layer on an inert supporting material present in porous or finely divided liquid state. The mobile phase runs over the stationary phase for separation of mixtures into individual components. Various instrumental chromatographic techniques includes Thin Chromatography (TLC), Laver High Performance Thin Layer Chromatography (HPTLC), Gas Liquid Chromatography Performance Liquid (GLC), High Chromatography (HPLC), Gel Permeation Chromatography (Gel filtration).

v) Radioactive Contamination is the radio nuclides present in the environment leads to the nuclear accidents. This contamination is due to radio-isotopes deposited in soil and in surroundings.for example the sample called Peperomia pellucida contains ²³⁸ U, ²³⁴ U, ²³² Th, ²²⁶ Ra, ²¹⁰ Pb radio-nuclides. As per WHO there are no certain limits given to measure radioactive contamination⁽¹⁴⁾.

V. Biological Methods:

i) Pharmacological Methods:

To demonstrate pharmacological effects, observations done on animal models. Protocols for the assessment of biological efficacy and their activities have discussed below:

Anti – Ulcer Activity: Various factors responsible for increase in acidity due to diet, alcohol & stress. Herbal drugs showing antiulcerogenic activity are Liquorice, Atropine and Hyoscine, Carica papaya. For example aqueous extract of Carica papaya was administered at a dose of 50 and 100mg/kg orally in rats against ethanol induced gastric ulcers. This extract protects the gastric mucosa against ethanol effects and helps in reduction of volume of gastric juice and gastric acidity. Sometimes, this activity can be assessed by ulcer index where the number and size of ulcerative lesions are considered.

Anti – Diabetic Activity: Diabetic mellitus is a disorder caused by increase blood glucose of intake food. For hypoglycemic activity number of plant extracts like Cinnamomum, Allium sativum, Cassiaauriculata, Aloe barbadensis, Glycerrhiza glabra were been used from traditional medicines. If there is an increase hepatic metabolism and increase insulin release, aqueous homogenate of garlic (10ml/kg/day) is administered orally to sucrose fed rabbits (10gm/kg/day in water for 2 months) due to Sallyl cystein sulfoxide (SACS), the precursor of allicin and garlic oil of Allium sativum helps in control lipid peroxidation better than Glibenclamide insulin. and After administration, it leads to increase hepatic glycogen, free amino acid content, decrease fasting blood glucose have been seen in invitro conditions (11).

Anti- Hypertensive activity: Elevated blood pressure in the coronary arteries causes hypertension, which leads to heart failure, coronary artery diseases, arterial fibrillation etc. Reserpine was one of the first drugs used on large scale to treat systemic hypertension. It acts irreversibly by blocking the uptake of neither biogenic amines like nor- epinephrine, dopamine& serotonin levels in storage vesicles of adrenergic neurons, thus leaving catecholamine's to be destroyed by intra neuronal monoamine oxidase. Thus reserpine helps in depletion of catecholamine's by its sympatholytic & anti hypertensive activity. Reserpine lowers blood pressure by decrease cardiac output, peripheral vascular resistance, hear rate & rennin secretion.Daily oral dose of reserpine 0.25mg and 0.05mg when given along with diuretic, when whole root is used adult dose of 50 to 200mg/day is administered once daily $^{(12)}$.

ii) Micro Biological Parameters:

Heavy metals comprise of Arsenic, Lead, and Cadmium etc were abundantly found in nature. When medicinal plants contaminated with these heavy metals, cause environmental pollutions and severe toxicity. As per Ayurvedic pharmacopeia of India (API) it was given some limits mentioned table (7)⁽¹³⁾.

Table	7:	Limits	for	Heavy	metals
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Tuble / Linnes for fieuty metuls					
Sl. No	Heavy Metal Contents	Permissible Limits			
1	Lead	10ppm			
2	Arsenic	3ppm			
3	Cadmium	0.3ppm			
4	Mercury	1ppm			

Microbial Contamination refers to the presence of several micro organisms like bacteria, fungi, yeast, and moulds etc. Due to the growth of the viable micro organisms it may contaminate the crude drug and affect the quality, purity and safety of the formulated herbal drugs. The following are permissible limits given by API ⁽¹²⁾ was described in the table (8).

Haemolytic property, medicinal plants belonging to families like Caryophyllaceae, Primulaceae, Araliaceae, and Dioscoreaceae contains Saponins. It has a ability of causing haemolysis in the blood, saponins changes the erythrocytic cell membrane by diffusion of haemoglobin in the surrounding atmosphere of the blood. Haemolytic property can be determined by comparison of reference solution (about 10milli gram of saponins in pH 7.4 phosphate buffer, diluted upto 100ml of volumetric flask) which has a haemolytic property 1000 units per gram Pesticide Residue refers to the presence of any foreign matter along with the microbial growth during collection, storage & marketing. It can be detached by spraying pesticides that may causes toxic effects. To retain the problem with pesticides, pest control procedures and limits have been given by WHO and API $^{(13)}$ table (9).

Table. 9 Permissible limits for the substances
used as pesticides

Sl. No	Substance	Limits		
1.	Chlorpyrifos-methyl	0.1mg/kg		
2.	Fenitrothion	0.51mg/kg		
3.	Parathion	0.51mg/kg		

Aflatoxins are the toxic compounds found in fungal species like Aspergillus and Pencillium. These aflatoxin producing fungi in herbal compounds are very dangerous for handling and also for administration. The myotoxic compounds of Aspergillus species have the capability to produce different aflatoxins like B_1 , B_2 , G_1 and G_2 . As per Ayurvedic pharmacopeia ⁽¹²⁾ the following limits were discussed in table (10)

Table 10: Permissible limits for Aflatoxins

Aflatoxin	Permissible Limit
B_1	< 2 ppb
$B_1 \! + \! B_2 \! + \! G_1 \! + \! G_2$	< 5 ppb

Table 8: Type of micro organisms and their permissible limits for herbal extracts and powders
and plant materials.

Sl. No	Type of Micro organisms	Parameters Permissible Limits For Herbal Extracts & Powders	Permissible Limits For Plant Materials Which Will Be Treated Before Use
1.	Staphylococcus Aureus/G	Absent	-
2.	Salmonella Sp./G	Absent	Absent
3.	Pseudomonas Aeruginosa/G	Absent	-10 ⁵
4.	Escherichia Coli	Absent	10
5.	Total Yeast & Mould	10 ³ /G	10 ⁵ /G

CONCLUSION

In the Current scenario were health related ailments are treated with allopathic system of medicines, since it is giving the faster relief but the side effects are more prone to produce complication also. At present the approach of the mordent medicine is mostly changing towards the alternative medicine (traditional medicines) such as Ayurveda, Siddha, Unani and homeopathy, were these medicines are used to cure the health related issues in long terms and with fewer side effects. Hence there is a huge require for the herbal medicines and to meet the demand enhanced technology needed for the isolation of active chemical constituents from the medicinal plants to get effective quality of the product. So standard protocols should be adopted for the standardization and validation of these herbal drugs right from cultivation, pre and post harvesting, time of collection and storage given by WHO, Ayurvedic pharmacopeia of India.

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