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WHO Guideline for Standardization and Evaluation of Herbal Medicine

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ABSTRACT:
Herbal drug has been employed in numerous conditions since ancient times by saints and munis. Herbal drug has numerous active ingredients for numerous conditions but the proper knowledge must be necessary for the medication of herbal expression else active ingredients will be damaged. Considering this fact present composition emphasis the herbal expression medication. Knowing these data we can use herbal expression for utmost complicated conditions and save humans from the effect of synthetic medicine side effect.

Keywords: Herbal medicines, Active Constituents, Test, Crude, Ethanol.

I. INTRODUCTION:

1.Herbal Standardization and Evaluation

In recent times, there has been great demand for Factory deduced products in developed countries. These Products are decreasingly being sought out as medicinal Products, nutraceuticals and cosmetics(1). There are Around 6000 herbal manufacturers in India. Further than 4000 units are produced Ayurveda drugs. Due to Lack of architectures, professed force dependable styles, and strict Nonsupervisory laws, utmost of these Manufacturers produce their product on the veritably Conditional Base(2).

In order to have a good collaboration between the quality of raw accoutrements , in process Accoutrements and the final products, it has come essential to develop dependable, specific and Sensitive quality control styles using a combination of classical and ultramodern necessary system of Analysis. Standardization is an essential dimension for icing the quality control of the herbal medicines(3). " 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 factory to its clinical operation. It also means conforming the herbal Medicine medication to a defined content of a element or a group of substances with known remedial Exertion independently by adding excipients or by mixing herbal medicines or herbal medicine Medications(4). " Evaluation " of a medicine means evidence of its identity and determination of its Quality and chastity and discovery of its nature of contamination(5).

Standardization of herbal medicines isn't an easy task as multitudinous factors impact the memoir Efficacity and reproducible remedial effect. In order to gain quality acquainted herbal products, care Should be taken right from the proper identification of shops, season and area of collection and their birth and sanctification process and attributing the combination in the case of polyherbal medicines(3).

The Standardization of crude drug materials includes the following steps-
Authentication: - Each and every step has to be authenticated.

a) Stage of collection.
b) Parts of the collected plant.
c) Regional status.
d) Botanical identity like phytomorphology,

Microscopical and histological analysis (characteristic of cell walls, cell contents, starch grains, calcium oxalate crystals, Trichomes, fibers, vessels etc.) (6).
Various histological parameter studies are:-

a) Leaf constant: - Palisade ratio, Vein islet number, Vein termination, Stomatal number and Stomatal index.
b) Trichomes.
c) Stomata.

International Journal of Pharmaceutical Research and Applications
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d) Quantitative microscopy.
e) Taxonomical identity.
f) Foreign matter.
g) Organoleptic evaluation.
h) Ash values and extractive values.
i) Moisture content determination.
j) Chromatographic and spectroscopic evaluation.
k) Heavy metal determination.
l) Pesticide residue.
m) Microbial contamination.
n) Radioactive contamination.

The herbal expression, in general, can be formalized schematically as to formulate the cure using raw Accoutrements collected from different points and a relative chemical efficacity of different batches of expression are to be observed. The medications with better clinical efficacity are to be named. After all the routine physical, chemical and pharmacological parameters are to be checked for all the batches to Elect the final finished product and to validate the whole manufacturing process(6).

The stability parameters for the herbal phrasings which include physical, chemical and microbiological Parameters are as follows:

Physical parameters include color, odor, appearance, clarity, density, humidity content, pH, decomposition time, frangibility, hardness, flowability, flocculation, sedimentation, settling rate and ash values.

Chemical parameters include limit tests, chemical tests, chemical assays etc. Chromatographic analysis of herbals can be done using TLC, HPLC, HPTLC, GC, UV, GC- MS and fluorimetryetc.

Microbiological parameters include total feasible content, total earth count, total enterobacterial and their count. Limiters can be employed as a quantitative or semi-quantitative tool to ascertain and control a number of contaminations like the reagents used during abstraction of colorful sauces, contaminations coming directly from the manufacturing vessels and from the detergents.

2. Guidelines for Herbal Drug Standardization

2.1 WHO Guidelines

The subject of herbal medicine standardization is largely wide and deep. The guidelines set by who can be epitomized as follows-

- Reference to the identity of the medicine. Botanical evaluation- sensitive characters, foreign matter, microscopical, histological, histochemical etc.
- Refers to the physicochemical character of the medicine. Physical and chemical identity, chromatographic fingerprints, ash values, extractive values, humidity content, unpredictable oil painting and alkaloidal assays, quantitative estimation protocols etc.
- A reference to the pharmacological parameters, natural exertion biographies, bitterness values, hemolytic indicator, astringency, swelling factor, raging indicator etc.
- Toxin details fungicide remainders, heavy essence, microbial impurity like total feasible count, pathogens like. Coli, salmonella. Aeruginosa. Aureus, enterobacteria etc.
- Microbial contamination.
- Radioactive contamination.

2.2 Modern herbal Ayurvedic monographs

In the ultramodern herbal ayurvedic studies the standardization parameters are bandied in a comprehensive way. According to the ultramodern ayurvedic causerie, the quality control protocols include the following the antonyms, publication related to the factory, ingredients present, logical styles. Evaluation description of the medicine, phytomorphological, atomic, organoleptic evaluation and foreign matter,etc.

3. WHO Guidelines Monograph Title

Botanical: - sensory evaluation, foreign matter, microscopy measurement.
Physicochemical TLC: - ash, extractable matter, water content and volatile matter, volatile oils.
Pharmacological: - bitterness value, Haemolytic activity, astringency, sterling index, foaming index.
Toxicological: - pesticide residue, arsenic, metals.
Microbial contamination: - total viable count, pathogens, aflatoxins, radioactive contamination.

4. Standardization of Herbal Drug/Products

Commercial production of herbal medicines and their trade are the fastest growing sector of industry today, due to increasing demand of medicinal plants; the supply line is adversely affected leading to the adulteration and substitution for genuine drugs.

I. **Fluorescence quenching**: When a plant extract is spotted on a fluorescent silica gel layer and exposed to UV light, it appears as a spot on a fluorescent background, thus causing quenching and

International Journal of Pharmaceutical Research and Applications
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is directly proportional to the concentration of the extract. The silica gel GF plate was used as an adsorbent for fluorescence quenching. Solvents took hexane toluene, ether, ethyl acetate, butanol, methanol and water (8).

II. **Use of fingerprinting and marker compounds for identification and standardization of botanical drugs:** Chemical and chromatographic techniques may be used to aid in identification of an herbal material or extract. Chromatographic technique such as HPLC, TLC, GC and capillary electrophoresis and spectroscopic methods such as IR, NMR and UV may also be used for fingerprinting. DNA fingerprinting has been widely used in many species, e.g. DNA fingerprinting of panax species and their adulterants (9). Marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and obtain stability profiles (10).

III. **Densitometry thin layer chromatographic determination of aescin in an herbal medicinal product containing Asculum and visit dry extract:** A TLC method is developed to analyze the total saponin content, also referred to as the aescin content, in an herbal medicinal product containing two dry extracts in capsules. After a purification step using c (18) solid phase extraction, the samples are analyzed on a silica gel HPTLC plate with the upper layer of a mixture of acetic acid/water/butanol (10/40/50v/v/v) as the mobile phase. Spots are visualized by spraying with anisaldehyde reagent and heating the plate for 5-10 min. (100-105oc) and measured at a wavelength of 535nm (11).

IV. **Determination of stigmasterol, beta-sitosterol and stigmasterol in oral dosage forms using HPLC with evaporative light scattering detection:** A validated and repeatable HPLC method with online evaporative light scattering was developed for the analysis of two sterols, stigmasterol, beta-sitosterol and a stanol found to be common in many herbal formulations and health care supplements. This method was used to assay commercially available products formulated as oral dosage forms purported to contain African potato and associated sterols and stanol (12).

V. **Elemental analysis of herbal preparations for traditional medicines by neutron activation analysis with the ko standardization method:** Medicinal herb preparations prescribed for specific treatment purposes were purchased from markets and were analyzed by instrumental neutron activation analysis with ko standardization. 500-700mg of each sample was palletized under a pressure of six tones and irradiated together with monitors for alpha and neutron flux ratio determination for about 6h in a thermal flux of 2.29 x 10 (12) n/cm2/s (13).

VI. **Liquid chromatography UV-determination and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry characterization of sitosterol and stigmasterol in soya bean oil**: a narrow bore HPLC-UV method was developed for the analysis of two of the most abundant naturally occurring phytosterols in vegetable oils: sitosterol and stigmasterol. The method enabled detection of the compounds at a concentration of 0.42µ/ml and quantization at a concentration of 0.52 and 0.54µ/ml for sitosterol and stigmasterol, respectively (14).

VII. **Simultaneous determination of cinnamaldehyde, eugenol and paeonol in traditional Chinese medicinal preparations by capillary GC-fid**: a capillary GC method was established for simultaneous determination of cinnamaldehyde (Cnmd), eugenol (el) and paeonol (pl) in two traditional Chinese herbal medicinal preparations, wei tong ding tablet (wtdt) and guifu dihuang pill (gdhp). The assays were based on a programmed temperature GC in a 30m x 0.53mm capillary columns with nitrogen as the carrier and fid detector. Good linearity was obtained over ranges of 0.45-0.452mg/l cnmd, 0.31-0.625mg/l el and 0.30-610mg/l pl, respectively (15).

International Journal of Pharmaceutical Research and Applications
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VIII. **HPTLC fingerprinting of marketed formulation containing shankhpushpi**: these are the important ayurveda formulations used for perinatalcare of mother and child health. Standardization of churnas was carried out by organoleptic study, phytochemical analysis; qualitative organic and inorganic analysis, thin layer chromatography, UV-visible spectrophotometer and HPLC fingerprint studies. Qualitative organic analysis of both the churnas revealed the presence of alkaloids, steroids, phenols, tannins, glycosides, resins, saponin and flavonoids (16).

5. Evaluation of Herbal Drug/Products

1) **Biological parameter (bioassay):** It is well Established that the biological potency of the herbal Constituents is due to not one but a mixture of Bioactive plant constituents and the relative Properties of a single bioactive compound can vary From batch to batch while the biological activity Remains within the desirable limits (1). Some of the Examples are:

a) **Evaluation of adaptogenic activity profiles of Herbal preparation:** Adaptogens help the body to Come up with stress and enhance general health And performance. AVM is an herbal formulation. Composition- Emblica officinalis, Withania Somnifera, Asparagus racemosus, Ocimum Sanctum, Tribulus terrestris and Piper longum. AVM shows significant antistress, Immunomodulatory and anabolic activities indifferent animal models thereby proving a promising adaptogen (17).

b) **Evaluationidant activity of herbal products:** A new test method for measuring the antioxidant power of herbal products, based on solid phase spectrophotometry using tetrabenzo-b, f, j, n, l, 5, 9, 13- tetraazacy-clohexadecin- Cu (II) complex immobilized on silica gel is proposed. The method represents an alternative to the mostly used scavenging capacity assays. The method was approved in the analysis of the most popular herbal beverages and drugs Echinacea determined spectrophotometrically (18).

c) **Evaluation of microbial contamination reduction on plants through a technological process of decoction and spray dry:** The technological process of raw material has many stages, generally, adverse to microbial growth, but its complete elimination depends on the initial and work condition utilized. The aim of this work was to verify the microbial contamination, such as extracting solution (SE) and spray dried extract (PSA) with the purpose of evaluating the decrease of contamination after the decoction and the spray dry. The microbiological analysis of the products was performed by total plate count and MPN coliform (19).

d) **Evaluation oxide scavenging activity of selected medicinal plants used in inflammatory diseases:** Four traditional medicinal plants, namely Ventilago madraspatana Gaertn., Rubbia cordifolia Linn., Lantana camera Linn. And Morinda citrifolia Linn. Were selected for a study on the inhibition of nitric oxide (NO), a key mediator in the phenomenon of inflammation, signifying the presence of effective anti-inflammatory constituents therein. Plant samples were extracted with different solvents for evaluation of their inhibitory activity on NO produced in vitro from sodium nitroprusside, and in LPS-activated murine peritoneal macrophages, ex-vivo (20).

e) **The lipid peroxidation inhibitory activity:** The reaction mixture contained mice liver homogenate(0.2ml, 10% w/v) in 0.15 KCl, KCl (0.1ml, 150µm), Tris buffer (0.4ml, PH 7.5) and various concentrations of test extracts. In vitro lipid peroxidation was initiated by addition of FeSO4.7H2O (0.1ml, 10µm). The reaction mixture was incubated at 37oC for 1 h. After the incubation period, the reaction was terminated by addition of thiobarbituric acid (TBA-2ml, 0.8%) and by heating the contents for 15 min. for development of colored complex. The tubes were then centrifuged at 4000 rpm for 10 min. and cooled. The % inhibition of lipid peroxidation was determined by comparing the results of test compound with those of controls not treated with extracts by monitoring the color intensity at 532nm. Gallic acid was used as a positive control (21).

2) **Evaluation of marketed polyherbal antidiabetic formulations using biomarker charantin**: charantin is one of the phytoconstituents present in momordica charantia. It is well known to possess antihyperglycemia, anticholesterol, immunosuppressive, antiulcerogenic, antispermatogenic and androgenic activities. HPTLC method is fast, precise, sensitive and reproducible with good recoveries for

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standardization of polyherbal formulations. The recovery values of charantin were found to be about 98.89% (2).

3) **In vivo and in vitro evaluation of hair growth potential of shoe flower**: the leaves and flowers of hibiscus rosa-sinensis are used as promoters of hair growth and as an aid in healing of ulcers. Petroleum ether extract of leaves and flowers of the plant was evaluated for the potential growth in vivo and in vitro methods. In vivo, 1% extract of leaves and flowers in liquid was applied topically over the shaved skin of albino rats and monitored and assessed for 30 days. The length of hair and different cyclic phases of hair follicles, like anagen and telogen phases were determined at different time periods. In vitro, the hair follicles from albino rat neonates were isolated and cultured in dmem supplemented with 0.01mg/ml petroleum ether extract of leaves and flowers. It is concluded that the leaf extract, when compared to flower extract, exhibits more potency on hair growth (22).
4) **Clinical evaluation to assess the safety and efficacy of code herbal medicine "Dysmo-off" versus allopathic medicine "Diclofenac Sodium" for the treatment of primary Dysmenorrhoea:** the clinical study on primary dysmenorrhoea to comparatively examine the coded herbal drug formulation "dysmo-off" with authentic allopathic medicine "diclofenac sodium". A random controlled clinical trial was conducted. These evaluations were based on a verbal rating scale so as to ascertain the rate of analgesic effects on dysmenorrhoeic pain. The patients were randomly allocated with the ratio of 1:2 for controlling treatment with (nsaids) (n=40) received diclofenac sodium tablets twice daily for 4 days (50mg one day prior to and three days after the menstruation), and test treatment with dysmo-off (n=80) received powdered dysmo-off twice daily for 4 days (5g one day prior to and three days after the menstruation). Treatment lasted for 4 consecutive menstrual cycles. Haemoglobin, esr and ultrasound were measured at baseline during the study. All subjects were clinically studied (23).
5) **Thermographic evaluation**: in the present study, the authors used thermography to evaluate the effects of herbal formulations based on "sho" scientifically. In the cases that were suitable for keishi-bukuryo-gan, the so-called keishi-bukuryo-gan sho, a significant skin temperature rise was observed in the upper half of the body after the intake of keishi-bukuryo-gan. In a case that was suitable for hochuekkito, a marked elevation of skin temperature spread through the upper trunk. It suggested that thermography is useful for an objective evaluation of sho in kampo medicines, and for identification of the action site of the herbal formulation (24).
6) **Biochemical evaluation**: most of the herbal drugs are a mixture of a number of ingredients. Their cumulative effect increases the efficacy of the drug in curing the diseases. Muthu marunthu is an herbal formulation comprising of eight various plant ingredients and has been claimed to possess anticancer effect. It was observed that the growth rate in rats was normal and there was no change in blood parameters such as glucose, urea, proteins, cholesterol and also in the activities of pathophysiological enzymes such as lactate dehydrogenase (ldh), glutamate oxaloacetate transaminase (got), glutamate pyruvate transaminase (gpt), alkaline and acid phosphatase after muthu marunthu administration. The tumor weight was found to be reduced in methylcholanthrene induced fibrosarcoma rats after muthu marunthu treatment (25).
7) **Evaluation of kutaj**: ghanavati for alkaloidal principles- kutaj-ghanavati is a reputed ayurvedic preparation used in dysentery and diarrhea. It contains a water extract of kurchi bark and fine powder of aconite roots. It was evaluated quantitatively and qualitatively employing TLC and titrimetric method. In the TLC study no interference of kurchi and aconite alkaloids with one another in their respective solvent systems. The formulation was found to contain all alkaloids of kurchi and aconite (26).
8) **Organoleptic evaluation**: organoleptic evaluation of food products plays an important role in judging the censoring acceptability or rejection of food items in the market. Effect of various treatments (blanching, pricking, and lye treatment), sugar concentration (50%, 60%, 70%) and storage on the color scores; flavor scores; texture scores of intermediate moisture apricots. The overall acceptability of the products was significantly higher in 70% sugar syrup but these scores decreased as the storage period advanced (27).

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II. CONCLUSION

The subject of herbal medicine standardization is largely wide and deep. There's so important to know and so important putatively antithetical propositions on the subject of herbal drugs and its relationship with mortal physiology and internal function. For the purpose of exploration work on standardization of herbal phrasings, a profound knowledge of the important sauces set up in India and extensively used in ayurvedic expression is of utmost significance(6).

Indeed when the chemical composition of a factory excerpt is known, the pharmacologically active half may not be. Environment, climate and growth conditions impact the composition, as does the specific part of the factory and its maturity. Studies detailing standardization of active constituents would ameliorate the business. Indeed if an herbal product is formalized to, for illustration, 4 of a element, the remaining 96 of constituents isn't formalized and may affect the product's solubility, bioavailability, stability, efficacity and toxin. Just as controlled trialsare necessary to establish safety and efficacity, manufacturing norms are needed to insure product quality(28).

Currently newer and advanced styles are available for the standardization of herbal medicines like luminescence quenching, the combination of chromatographic and spectrophotometric styles, natural assays, use of biomarkers in characteristic etc. Bioassay can play an important part in the standardization of herbal medicines and can also come an important quality control system as well as for proper stability testing of the product(4).

India can crop as the major country and play the supereminent part in the product of formalized, therapeutically effective ayurvedic expression. India needs to explore the medicinally important shops. This can be achieved only if the herbal products are estimated and anatomized using sophisticated ultramodern ways of standardization similar as UV-visible, TLC, HPLC, HPTLC, GC-MS, Spectrofluorimetric and other styles(6).

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