



CURRENT TRENDS IN STANDARDIZATION OF HERBAL FORMULATIONS IN AFRICA

Ndayazi byemero Desire^{1*}, Yassa Yoniene Piere¹, Ngolo masudi pascaline¹,
Mushagalusa Felicien² and Hameed Kadar Ali³

¹Department of Surgery, University of Goma, Democratic Republic of Congo.

³Department of Pharmacy, University of Bukavu, Democratic Republic of Congo.

³Department of Pharmacy, Cresnet University, Chennai, India.

*Corresponding author E-mail: Nuzz@hotmail.com

ARTICLE INFO

ABSTRACT

Key Words

Herbals, formulation,
Standardization and
Quality control



Herbal medicines are not a simple task since many factors influence the biological efficacy and reproducible therapeutic effect. Standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects. Pharmacological properties of an herbal formulation depend on phytochemical constituents present therein. Many cutting-edge analytical technologies have been introduced to evaluate the quality of medicinal plants and significant amount of measurement data has been produced. Development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of market/bioactive compounds and other major constituents, is a major challenge to scientists. An overview covering various techniques employed in extraction and characterization of herbal medicines as well as herbal nanomedicines standardization is reported. In addition, phytosomes increased bioavailability, bhasma as a metal nanobarrier drug delivery system, potential of metabolomics in the development of improved phytotherapeutic agents, DNA based molecular markers in adulterants, and SCAR markers for authentication and discrimination if herbs from their adulterants are reported. Nanotechnology based herbal drugs possess improved solubility and enhanced bioavailability.

INTRODUCTION

Herbal medicines (HMs) including Chinese medicine (CM) and other folk medicines are getting more and more popular nowadays globally for improving health condition of human beings as well as preventing and curing diseases. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical, standardization, and *In-vitro*, *In-vivo* parameters. The quality assessment of herbal formulations is of paramount importance in

order to justify their acceptability in the modern system of medicine [1]. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. In India, the department of Ayush, Government of India, launched a central scheme to develop standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurvedic preparations. The subject of herbal drug standardization is massively wide and deep. There is so much to know and so many

seemingly contradictory theories on the subject of herbal medicines and their relationship with human physiology and mental function. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization.

World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in natural health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards [2].

Standardization of raw materials includes the following steps: Authentication- Each and every step has to be authenticated, area of the collection, parts of the plant collection, the regional situation, as phytomorphology botanical identity, microscopic and histological analysis(characteristic features of cell walls, cell contents, starch grains, calcium oxalate crystals, hairs, fibers, vessels etc.)

Several studies of the histological parameters are list of palisade ratio, vein islet number, vein termination, stomatal number, stomatal index, trichomes, stomata, quantitative microscopy, taxonomic identity, foreign matter. Loss on drying, swelling index, foaming index, ash values and extractive values, Chromatographic and spectroscopic evaluation, Determination of heavy metals, pesticide residues, Microbial contamination, Radioactive contamination.

The parameter stability of herbal formulationsthat includes pharmacognostic parameters, physico-chemical parameters, phyto-chemical parameters, microbiological assay, chromatographic analysis.

Pharmacognostic evaluation: It includes color, odor, taste, texture, size, shape, microscopical characters, and histological parameters.

Physico-chemical parameters: It includes foreign matter, total ash, acid-insoluble ash, swelling and foaming index, assay, successive extractive values, moisture content, viscosity, PH, Disintegration time, friability, hardness,

flow capacity, flocculation, sedimentation, alcohol content.

Chemical parameters: It includes limit tests, chemical tests etc.

Chromatographic and spectroscopic analysis: It includes TLC, HPLC, HPTLC, GC, UV, IR, FT-IR, AAS, LC-MS, GC-MS, fluorimetry etc.

Microbiological parameters: It includes the full content of viable, total mould count, total coliforms count. Limiters can be used as a quantitative tool or semi-quantitative to determine and control the amount of impurities, such as reagents used in the extraction of various herbs, impurities ships directly from the manufacturing and solvents etc.

WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS

1. Quality control of crude drugs material, plant preparations and finished products.
2. Stability assessment and shelf life.
3. Safety assessment; documentation of safety based on experience or toxicological studies.
4. Assessment of efficacy by ethnomedical informations and biological activity evaluations.

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC).

Quality Control of Herbal Drugs: Quality control for efficacy and safety of herbal products is of paramount importance. Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. The term “herbal drugs” denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage [3]. Hence they are capable of variation. This variability is also caused by

differences in growth, geographical location, and time of harvesting. A practical addition to the definition is also to include other crude products derived from plants, which no longer show any organic structure, such as essential oils, fatty oils, resins, and gums. Derived or isolated compounds (e.g. strychnine from strychnous nux-vomica) or mixtures of compounds (e.g. abrin from *Abrus precatorius*).

In general, quality control is based on three important pharmacopeial definitions

- a) Identity- it should have one herb
- b) Purity – it should not have any contaminant other than herb
- c) Content or assay-the active constituents should be within the defined limits.

It is obvious that the content is the most difficult one to assess, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not [4]. Identity can be achieved by macro and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification [5, 6]. At times an incorrect botanical quality with respect to the labeling can be a problem. Purity is closely linked with safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used. In all other cases, where no active constituents or marker can be defined for the herbal drug, the

percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeia [7,8].

A special form of assay is the determination of essential oils by steam distillation. When active constituents (e.g. sennosides in *senna*) or markers (e.g. alkydamides in *Echinacea*) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy(UV/VIS), TLC, HPLC, HPTLC, GC, mass spectrometry, or a combination of GC and MS(GC/MS), can be employed [9].

Stability Assessment and Shelf Life: The past decade has seen a significant increase in the use of herbal medicines. As a result of WHO's promotion of traditional medicine, countries have been seeking the assistance of the organization in identifying safe and effective herbal medicines for use in national health care systems. Prolonged and apparently uneventful use of a substance usually offers testimony of its safety. In a few instances, however, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations has revealed previously unsuspected potential for systematic toxicity, carcinogenicity and teratogenicity. Regulatory authorities need to be quickly and reliably informed of these findings. They should also have the authority to respond promptly to such alerts, either by withdrawing or varying the licences of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription [10].

Assessment of quality: All procedures should be in accordance with good manufacturing practices.

Crude plant material: The botanical definition, including genus, species and authority, description, part of the plant, active and characteristics constituents should be specified and, if possible content limits should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label.

Plant preparations: The manufacturing procedure should be described in detail. If other substances are added during manufacture in order to adjust the plant preparation to a

certain level of active or characteristics constituents or for any other purpose, the added substances should be mentioned in the manufacturing procedures. A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances to ensure consistent quality of the preparation.

Finished product: The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms.

Stability: The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established.

Safety assessment: Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic. As a whole, herbal medicines can have a risk of adverse effects and drug-drug and drug-food interactions if not properly assessed. Assessment of the safety of herbal products, therefore, is the first priority in herbal research. These are various approaches to the evaluation of safety of herbal medicines. The toxic effects of herbal preparation may be attributed mainly to the following: Inherent toxicity of plant constituents and ingredients and Manufacturing malpractice and contamination. Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phyto-chemical and pharmacological studies. It is, however, safe to assume that, based on human experiences in various cultures, the use of toxic plant ingredients has already been largely eliminated and recent reports of toxicity could largely be due to misidentification and overdosing of certain constituents [11]. Substitution and misidentification of herbal substances, documented or regulatory approaches,

development of monitoring and surveillance systems, assessment of toxicity, risk assessment approach. The evaluation of new herbal products consists of six steps, which define the following: Characteristics of new substances, history and pattern of use, any adverse reaction, biological action, toxicity and carcinogenicity, and clinical trial data. The presence of impurities is either an intended addition, or accidental contamination via processing. The substitution of plants arises because of similar plants/ wrong identification, or the use of cheaper alternatives.

Assessment of toxicity: Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important [12]. Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique Standardization techniques to adequately model toxicity.

Assessment of efficacy: Herbal medicines are inherently different from conventional pharmacological treatments, but presently there is no way to assess their efficacy other than by currently used conventional clinical trial methodologies, in which efficacy is conventionally assessed by clinical, laboratory, or diagnostic outcomes: Clinical outcomes include parameters such as improved morbidity, reduced pain or discomfort, improved appetite and weight gain, reduction of blood pressure, reduction of tumor size or extent, and improved quality of life. Laboratory /other diagnostic outcomes include parameters such as reduction of blood glucose, improvement of hemoglobin status, reduction of opacity as measured by radiological or imaging techniques, and improvement in electrocardiogram (ECG) findings. Implementation of a standardized approach for the herbal practitioners and collection of the prospective data necessarily creates an interventional design which, if planned properly, may closely resemble single-blind randomized trials. Even if it differs from double-blind randomized trials in the degree of rigor, the design may be the optimum, both biologically and economically, for rapid evaluation of herbal products. Standardization, however, may sometimes be incompatible with the existing legislative framework and caution

is needed regarding the ethical implications of such studies.

CONVENTIONAL METHODS FOR STANDARDISATION OF HERBAL FORMULATION

Standardization of herbal formulation requires implementation of Good Manufacturing Practices (GMP) [13, 14, 15]. In addition, study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, shelf-life, toxicity evaluation, chemical profiling of the herbal formulations is considered essential [16]. Other factors such as pesticide residue, aflatoxine content, heavy metals contamination, Good Agricultural Practices (GAP) in herbal drug standardization are equally important [17]. The standardization of various marketed herbal and poly herbal formulation [kasisa bhasma, a traditional formulation, containing the mixture of *Emblica officianalis* and lime juice used in the treatment of anaemia, hepatotoxicity [18], Ashwagandhalehyam, a traditional formulation, used in the treatment of inflammation, and epileptic seizures have been reported Ashwagandharistam, a traditional formulation, having good anti-epileptic activity have been reported [Madhumehari churna (Baidynath) containing the mixture of eight herbal antidiabetic drugs [21]. Panacasa churna known to be effective in gastrointestinal disorder [22], Dashamularishta, a traditional formulation, used in the normalization of physiological processes after child birth [23], Gokshuradi churna, Megni, Jawarish-e Darchini [24,25] have been reported. But still there are many poly herbal formulations which require standardization as there are frequently used based only on their ethanobotanical use [26]. Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the poly herbal formulations [27]. Methiorep premix (a combination of herbs viz. *Cicer arietinum*, *Phaseolus mungo*, *Mucuna pruriens*, *Triticum sativum*, *Allium cepa* & richer source of protein with highly bioavailable methionine) has been recommended as a safe product to replace synthetic methionine in poultry ration and for supplementation in basal diet for regular usage [28]. TLC and HPTLC fingerprint were used for deciding the identity, purity and strength of the poly herbal formulation and also for fixing

standards for this Ayurvedic formulation [29].

MODERN TECHNIQUES OF EXTRACTION METHODS

Supercritical fluid extraction (SFE)

Supercritical fluid extraction is the most preferable process for the extraction of the active constituents from the medicinal and aromatic plants [30]. SFE has emerged as a highly promising technology for the production of herbal medicines and nutraceuticals with high potency of active ingredients [31]. SFE techniques have been found useful in isolating the desired phytoconstituents from plant extracts [32]. The use of supercritical fluids for the extraction of a range of materials including plant products of medicinal, flavouring and cosmetic interest has during the last decade, become of increasing economic and research interest. In 1822, Cagniard de la Tour reported that above a certain temperature and pressure, single substances do not condense or evaporate but exist as a fluid. Under these conditions the gas and liquid phase both possess the same density and no division exist between the two phases. This is the critical state. In phytochemistry these properties can be exploited to maximize the extraction of plant constituents. Super critical fluid extraction (SFE) involves use of gases, usually CO₂, and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. There are many other gases and liquids that are highly efficient as extraction solvents when put under pressure.

Examples involving the extraction of phytochemicals with supercritical carbon dioxide follow:

- 1) Alkaloids: decaffeination of green coffee Isolation of vindoline from *Cathranthus roseus*.
- 2) Pigments: extraction of annatto sees
- 3) Diterpene: extraction of *Taxus brevifolia* and *T. cuspidata*
- 4) Acylphloroglucinols: oxygenated Hyperforin derivative of *Hypericum* [33].

Coupled SFE-SFC: System in which a sample is extracted with a supercritical fluid which then places the extracted material in the

inlet part of a supercritical fluid chromatographic system. The extract is then chromatographed directly using supercritical fluid.

Coupled SFE-GC and SFE-LC: System in which a sample is extracted using a supercritical fluid which is then depressurized to deposit the extracted material in the inlet part or a column of gas or liquid chromatographic system respectively. SFE is characterized by robustness of sample preparation, reliability, less time consuming, high yield and also has potential for coupling with number of chromatographic methods [34].

Solid phase extraction (SPE): SPE technique is applied for isolation of analyte from a liquid matrix and purified herbal extracts. This technique has many advantages such as: high recovery of the analyte, concentrate of analyte, highly purified extracts, ability to simultaneously extract analytes of high polarity range, ease of automation, compatibility with instrumental analysis and reduction in organic solvent in comparison with more traditional sample preparation techniques [35]. The solid phase extraction was introduced for determining thirteen organochlorine pesticide residues including alpha-benzene hexachloride (BHC), beta BHC, gamma BHC, delta BHC, p,p'-dichlorodiphenyl dichloro ethylene (pp'DDE), p,p'-dichloro-di-phenyl dichloroethane (pp'DDD), o,p'-dichloro-diphenyl trichloroethane (op'-DDT), pp'-DDT heptachlor (HEPT), aldrin, heptachlor epoxide (HCE), dieldrin and andrin in *scutellaria baicalensis*, *Salvia miltiorrhiza*, *Belamcanda chinensis*, *Paeoniae lactiflora*, *Angelica dahurica*, *Arisaema erubescens*, *Fructus arctii*, *Anemarrhenaa sphenolodes* and *Platycodon grandiflorum*.

The organochlorine pesticides were extracted from herbs with mixed solvents of acetone and n-hexane by ultrasonic and cleaned up by Florosil solid phase extraction column [36]. Solid phase extraction was used to prepare the test solution for the analysis of aristolochic acid I and II in herbal medicines [37].

Spouted bed extraction: In certain instances, as in the production of annatto powder from the seeds of *Bixa orellana*, the physical removal of the pigment layer of the seed-coat

can yield a less impaired product than that produced by solvent extraction. Such methods can involve the use of ball mill or a spouted bed unit. A development of the latter, the conical spouted bed extractor, has been investigated for annatto production. Basically it consists of a cylinder tapered at both ends and containing the seeds at the lower end through which a jet of hot air is forced. Seeds and pigment-loaded fine particles are propelled into the space above from whence the seeds fall back to be recirculated and the annatto powder moves to a cyclone from which it is collected [38].

Counter-current extraction: This is a liquid-liquid extraction process and the principle involved is similar to partition chromatography. Briefly, a lower, stationary phase is contained in a series of tubes and an upper, moving immiscible liquid is transferred from tube to tube along the series, the immiscible liquids being shaken and allowed to separate between each transference. The mixture to be fractionated is placed in the first tube containing the immiscible liquids and the apparatus is agitated and the layers are allowed to separate. The components of the mixture will be distributed between the two layers according to their partition coefficients. The upper phase is moved along to the second tube containing lower phase and more moving phase is brought into contact with the lower phase of tube 1. Shaking and transference again takes place and continues along a sufficient number of tubes to give a fractionation of the mixture. The distribution of each substance over a given number of tubes can be ascertained by the terms of the binomial expansion. If buffer solutions are used as the stationary phase, the exploits differences in ionization constants of the various solutes. In some instances the effectiveness of the separation can be improved by using a series of buffer solutions of graded pH value as the lower phase. Solutions of acids and alkalis also find some application as lower phase. A development of the extractor is the steady state distribution machine. This allows true counter-current extraction and can be programmed so that both solvents move in opposite directions. The mixture to be fractionated is fed continuously into the centre of the train of cells and, according to the solvents, the solutes may move in either direction [39].

Microwave –assisted extraction (MAE):

MAE technology includes the extraction of high-value compounds from natural sources including phytonutrients, nutraceutical and functional food ingredients and pharmaceutical actives from biomass [40]. MAE find a utility in production of cost effective herbal extracts and helpful in extraction of carotenoids from single cells, taxanes from taxus biomass, essential fatty acids from microalgae and oilseeds, phytosterols from medicinal plants, polyphenols from green tea, and essential oils from various sources. Compared to conventional solvent extraction methods, advantages of this technology include: a) improved product,-purity of crude extracts, -stability of marker compounds and use of minimal toxic solvents. b) Reduced processing costs, increased recovery and purity of marker compounds, very fast extraction rates, reduced energy and solvent usage [41,42].

Modern techniques in herbal drug identification and characterization

HPLC

The preparative and analytical HPLC are widely applicable in pharmaceutical industry for isolating and purification of herbal compounds. They are of basically two types in preparative HPLC: those are low pressure HPLC (typically under 5 bars) and high pressure HPLC (pressure greater than 20 bar) [43,44]. The most important parameters to be considered are of resolution sensitivity and fast analysis time in analytical HPLC however both the degree of solute purity and the amount of compound that can be produced per unit time that is recovery in preparative HPLC [45]. The main aim is to isolate the herbal compounds, where as in analytical work the aim is to get the information about sample. The preparative HPLC is the closest to analytical HPLC than the traditional PLC, because it is having higher column efficiencies and faster solvent velocities permit more difficult separation to be conducted more quickly [46]. This is most important in the pharmaceutical industries because newer formulations have to be introduced in the market as early as possible. The combination of HPLC and LC/MS is presently powerful technique for the quality control of Chinese medicine i.e. liquorice [47]. Kankasava is a fermented polyherbal formulation prepared with Kanaka and other ingredients [48]. It is used in chronic Bronchitis, asthmatic cough

and breathlessness. Kankasava is analysed by RP HPLC. It is a simple, precise, accurate RP-HPLC method was developed for the quantitative estimation of atropine in kankasava polyherbal branded formulations. The separation was achieved with a column RP C-18 (250mm×4.6mm×5 micron) using mobile phase mixture of methanol & 10 mmol dihydrogen phosphate buffer in a ratio of 50:50 v/v at a flow rate of 1 ml/min, and analysis was screened with UV detector at 254 nm. The retention time for standard atropine sulphate was found to be 4.0667 minutes. Linearity was found to be $r^2 = 0.998$.

High performance thin layer chromatography (HPTLC)

TLC is the common fingerprint technique for herbal analysis. The herbal compounds can easily identified by TLC [49]. In this technique, the authentication of various species, evaluation of stability and consistency of their preparations from different manufacturers [50]. HPTLC is the common fingerprint mainly used to analyze the compounds which is having low or moderate polarities. HPTLC technique is widely used in the pharmaceutical industry for process development, identification and detection of adulterants, substituent in the herbal products and also helps in the identification of pesticide content, mycotoxins and in quality control of herb and health products [51]. HPTLC technique was reported for simultaneous estimation of gallic acid, Rutin, Quercetin in terminalia chebula [52]. The aqueous extract of *Terminalia chebula*, precoated silica gel GF 254 as stationary phase and the mobile phase for tannins toluene: acetone: glacial acetic acid (3:1:2) and mobile phase for rutin and quercetin, ethyl acetate: dichloromethane: formic acid: glacial acetic acid: water (10:2.5:1:1:0.1). Detection and quantification were reported densitometrically at $\lambda = 254$ for gallic acid and 366nm for rutin and quercetin. The R_f values of gallic acid, rutin and quercetin are 0.30, 0.13, 0.93. The simultaneous estimation of withaferin A and beta- sinosterol- d- glucoside in four Ashwagandha formulations [53]. Syzygium jambolanum was quantitatively estimated in terms of stability, repeatability, accuracy and phytoconstituents such as glycoside, tannins, ellagic acid and gallic acid by HPTLC [54]. It was employed for detection, monitoring and quantification of bacoside A & B in Bacopa

monnieria and its formulations. Simultaneous estimation of Diosgenin and Levodopa was done by HPTLC method. Quantification was carried out at 194 nm for Diosgenin and 280 nm for levodopa using absorbance reflectance mode. The R_f value of levodopa and Diosgenin was found to be 0.27±0.2 and 0.61±0.2. The content uniformity test was carried out as per the USP specification. The proposed HPTLC method provides a faster and cost effective quantitative control for the analysis of levodopa and diosgenin [55].

Gas chromatography - mass spectroscopy (GC-MS)

Gas chromatographic equipment can easily interfaced with rapid scan mass spectrometer of various types. The flow rate of the capillary column is generally low but enough that the column. Output can easily fed directly into ionization chamber of MS. In this the simplest mass detector in GC is the Ion Trap Detector [46]. The ions trap detector is remarkable compact and less expensive than quadrupole instruments. The identification and quantification of chemical constituents present in the polyherbal oil formulation was analyzed by GC-MS method [56]. An effective fast and accurate capillary gas chromatography method was employed for determining the organochlorine pesticide residues. The SPE extract was separated by capillary column by using electrochemical detector. The split ratio is of 1:2.2 using the carrier gas N₂ with the flow rate of 1.4 ml/min. The Injector temperature is of 220^oc and the detector temperature is of 330^oc. Thus the good linearities were obtained for organochlorine pesticides. It has been used for identification of large number of components present in natural and biological systems [57].

Liquid chromatography- Mass spectroscopy (LC-MS)

LC-MS is the one of the most prominent method of choice in many stages of drug development [58]. The chemical standardization of an aqueous extract of the mixture of the herbs provided chemical compounds serving as reference markers using LC-MS [59]. It is useful to analyze the aminoglycosides showed that these drugs are highly soluble in water, showed low plasma protein binding and more than 90 percent excreted through the kidney[60]. The pharmacokinetic studies of Chinese medicinal herbs using LC-MS. Interference peaks in

biological samples are easily observed when using HPLC coupled to ultraviolet, fluorescence and electrochemical detectors. With the introduction of highly sensitive and selective LC-MS-based bioanalytical methods, sample preparation can usually be simplified to speed up the throughput of data. ME must be investigated to ensure that accuracy, precision, selectivity and robustness in the HPLC-MS- based procedures, so that the sensitivity will not be compromised. On approach to the identification of ME is the method of post- column infusion. In brief, the analyte of interest is infused constantly into the ion source of a mass spectroscopy to form a steady signal. The blank matrix after sample preparation is then injected into the HPLC/MS system, which it helps to determine the region in chromatogram by the components of the matrix. Another approach to quantitatively assess ME is the post –extraction fortification method.

Supercritical fluid chromatography: The super critical fluid and microbore liquid chromatography offer potential applications for the drug analysis. In this the mobile phase is a gas (CO₂) maintained at its supercritical state i.e., above its critical temperature and pressure. The SFC mobile phase has low viscosity, approximating that of a gas, and high diffusivity, between those of capillary gas chromatography and liquid chromatography.

Capillary electrophoresis: The methodology of CE was introduced to evaluate one drug in terms of specificity, sensitivity and precision, and the results were in agreement with those obtained by the HPLC method. Is more than 100-fold less [61]. Moreover the analysis time of CE method. The hyphenated CE instruments, such as CE-diode array detection, CE-MS and CE-NMR, have been utilized, whereas, there are some limitations are being in CE hyphenations with respect to reproducibility were reported [62].

Role of genetic markers in the standardization of herbal drugs

A DNA marker is a term used to refer a specific DNA variation between individuals that has been found to be associated with a certain characteristic. These different DNA or genetic variants are known as alleles. DNA marker testing or genotyping introduces which alleles an animal is carrying for a DNA marker. DNA tests for simple traits have been on the market for several years and include

those for certain diseases, such as DUMPS (Deficiency of Uridine Monophosphate Synthetase) and BLAD (Bovine Leukocyte Adhesion Deficiency), coat colour, and horned status. It can be described as a variation, which may arise due to mutation in the genomic loci that can be observed. Some of the commonly used of genetic markers are: RAPD (Random amplification of polymorphic DNA), RFLP (Restriction fragment length polymorphism), AFLP (Amplified fragment length polymorphism), Micro satellite polymorphism SNP, (Single nucleotide polymorphism), SFP (Single feature polymorphism), STR (Short tandem repeat).

Random Amplification of Polymorphic DNA

RAPD is a type of PCR reaction, but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction. RAPD markers are decamer (10 nucleotide length) DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way. It is used to analyse the genetic diversity of an individual by using random primers. Unlike traditional PCR analysis, RAPD does not require any specific knowledge of the DNA sequence of the target organism the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers sequence. For example no fragment is produced if primers annealed too far apart of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel.

RFLP (Restriction fragment length polymorphism)

This polymorphism consists of the presence or absence of a restriction site for a bacterial restriction enzyme. This is an enzyme

which breaks strands of DNA wherever they contain they contain a certain sequence of half-a- dozen or so nucleotides. The locus of interest could be probed using a radiolabelled piece of DNA with the same sequence as a part of the test locus. This would selectively hybridise to the restriction fragment derived from the test locus. The whole process consisted of: Extracting DNA from white blood cells, digesting the DNA with a restriction enzyme into restriction fragments, using gel electrophoresis to separate the fragments by size, denaturing the DNA so that the two strands of each fragment separate, blotting the single –stranded DNA onto a filter to immobilize it, washing off excess probe.

DNA fingerprinting technique

DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remain the same irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment [63]. The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples [64]. Proper integration of molecular techniques and analytical tools generated a comprehensive system of botanical characterization that can be applied in the industry level to ensure quality control of botanicals. DNA markers are helpful to identity cells, individuals or species as they can be used to produce normal, functioning proteins to replace defective ones. Moreover, these markers help in treatment of various diseases and help in distinguishing the genuine herb from adulterated drug [65].

ISSR (Inter-Simple Sequence Repeat)

ISSR, a PCR- based application is unique and inexpensive popular technique of DNA finger printing which include the characterization of genetic fingerprinting, gene tapping, detection of clonal variation, phylogenetic analysis, detection of genomic instability, and assessment of hybridization. Cannabis sativa and Arabidopsis thaliana L. Heyne have been differentiated from their adulterated species by using ISSR markers [65]. Molecular characterization by sequence-

characterized amplified region (SCAR) markers allows effective and reliable authentication and discrimination of herbs from their adulterants. In addition, morphological similar plant species can be differentiated using SCAR markers [66].

Phytosomes/pharmacosomes: A novel drug delivery system for herbal drugs

Pharmacosomes commonly known as phytosome are drug- phospholipid complexes having active ingredients of the herb and can be formulated in the form of solution, suspension, emulsion, syrup, lotion, gel, cream, aqueous microdispersion, pill, capsule, powder, granules and chewable tablet [66,67]. Plants namely *Silybium marianum*, *Ginkgo biloba* and Ginseng showed better efficacy than conventional herbal formulations [68]. In addition, the clinical trials of phytosomes have shown increased bioavailability in comparison to conventional herbal formulations generally containing polyphenols and flavonoids in humans [69]. Several phytosomal herbal drug delivery systems have been reported [70]. Phytosomal herbal drug delivery systems are mainly used; to deliver systemic antioxidant, useful in treatment of the disease like blood pressure, liver disease, cancer, skin disease and helps in protecting the brain lining [71].

Standardization of herbal nano medicines

Herbal nanotechnology helps incorporation of the active phytoconstituents to obtain desired therapeutic effect. The increased solubility, stability, bioavailability, pharmacological activity of many popular herbal extracts including Milk thistle, *Ginkgo biloba*, grape seed, green tea, hawthorn, ginseng using nano dosage forms such as polymeric nanoparticles nanospheres and nanocapsules, liposomes, proliposomes, solid lipid nanoparticles, and nanoemulsion has been reported [72]. Other advantage of herbal nanomedicine include protection from toxicity, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation [73]. Nanotechnology patents issue in Chinese herbal medicine has been reported and

CONCLUSION

India can emerge as the major country and play the lead role in production of standardized, therapeutically effective ayurvedic formulation. India needs to explore

proliferation of nano – based Chinese herbal medicine patents in China was due to the illustrations of biomedical technology progress extensively [74].

Bhasma as a nanoherbal medicine technology

Bhasma are the ayurvedic metallic preparations in which metal act as a nanocarrier for drug delivery and are widely recommended for treatment of a variety of chronic ailments and are taken along with milk, butter, honey, or ghee to eliminate the harmful effects of metals and enhancing their biocompatibility in the body [75]. Neutron activation analysis of twenty metallic based bhasmas such as calcium, iron, zinc, mercury, silver, potassium, arsenic, Copper, tin, gemstones, confirmed the purity of these bhasma as the other elements such as Na, K, Mg, V, Mn, Fe, Cu, Zn were found in microg/g amounts and Au and Co in ultratrace (ng/g) amounts [76]. Various techniques like atomic force microscope, scanning electron microscope, transmission electron microscope and energy dispersive spectroscopy have been employed for the estimation and characterization of bhasma. The Kasisa bhasma analyses qualitatively through X-ray diffraction, Fourier transform infrared spectroscopy correspond to inorganic metal (Fe₂O₃) hydrated metal salt or oxide (FeO or Fe₃O₄). The FTIR spectra showed no peak for any organic molecule or bond corresponding it, there by confirming the absence of organic matter and external organic contamination. The AAS study was conducted to determine the concentration of elements present in the formulation. The results showed that the element iron was seen in the major concentration of 85.91%. AFM analysis confirmed the formulation has spherical morphology with an average particle size of 100 nm. The spherical morphology was due to the aggregation of the nanocrystals of the metallic oxides. It has been used in traditional medicines for treatment of anemia, hepatotoxicity [18]

the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization such as UV-visible, TLC, HPLC, HPTLC, GC-MS,

spectro fluorimetric and other methods. These guidelines for the assessment of herbal medicines are intended to facilitate the work of regulatory authorities, scientific bodies and industry in the development, assessment and registration of such products. The assessment should reflect the scientific knowledge gathered in that field. Such assessment could be the basis for future classification of herbal medicines in different parts of the world. Other types of traditional medicines in addition to herbal products may be assessed in a similar way. The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf- life of herbal drugs. The effective regulation and control of herbal medicines moving in international commerce also requires close liaison between national institutions that are able to keep under regular review all aspects of production and use of herbal medicines, as well as to conduct or sponsor evaluative studies of their efficacy, toxicity, safety, acceptability, cost and relative value compared with other drugs used in modern medicine.

REFERENCES

1. Satheesh Madhavi NN, Kumud Upadhya, Asha bishti. Phytochemical screening and standardization of poly herbal formulation for Dyslipidemia. *Indian journal of physiology and pharmacology*, 3(3), 2011.
2. Sunita Panchawat, Kamal Singh Rathore, Ssisodia Nema RK. Standardization and evaluation of herbal drug formulations. 2010.
3. EMEA. Quality of herbal medicinal products. Guidelines. European Agency for the Evaluation o Medicinal products (EMA), London, 1998.
4. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organisation, Geneva, 1992.
5. WHO. The International Pharmacopeia, Vol.3: Quality Specifications for Pharmaceutical Substances, Excipients, and Dosage forms, 3rd edn. World Health organization Geneva, 1988.
6. De Smet. PAGM. *Drug Information Journal*, 33, 1999, 717-724.
7. WHO. Guidelines for the appropriate use of Herbal Medicines. WHO Regional publications, Western pacific series No 3, WHO Regional office for the Western Pacific, Manila, 1998.
8. WHO. Guidelines for the Assessment of Herbal Medicines. WHO Technical Report Series, No863. World Health Organization, Geneva, 1996.
9. Watson DG. *Pharmaceutical Analysis*. Churchill Livingstone, Edinburgh, 1999.
10. WHO. Quality Assurance of Pharmaceuticals –A Compendium of Guidelines and Related Materials. 1, 1997.
11. ICDRA. 6th International Conference on Drug Regulatory Authorities, World Health Organization, 1991.
12. TDR. Operational Guidance: Information Needed to Support Clinical Trials of Herbal Products, UNICEF/ UNDP/ WORLD Bank/WHO special program for Research and Training in Tropical Diseases (TDR) 2005.
13. Neeraj Choudary and Bhupinder Singh Sekhon. An overview of advances in the standardization of herbal drugs. *Journal of Pharmacognosy*, 2, 2011, 55-70.
14. *Indian Herbal Pharmacopoeia*, Indian Drug Manufacturers Association, Mumbai, 2002.
15. *Quality Control Methods for Medicinal Plant Materials*, WHO, Geneva, 1996.
16. Mosihuzzaman M, Choudary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine. *Pure Applied Chemistry*, 80(10), 2008, 2195-2230.
17. Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standard can be achieved. *Journal of Drug Information*, 32, 1998, 101-110.
18. Arun Rasheed, Anvesh Mari. Formulation, Characterization and Comparative Evaluation of Kasisa

- Bhasma. A Herbo-Mineral Indian Traditional Medicine. *Journal of Complementary and Integrative Medicine*.
19. Arun Rasheed, K.V. Satyanarayana. Chemical and pharmacological standardization of Ashwagandhadi lehyam: An ayurvedic formulation. *Journal of Complementary and Integrative Medicine*.
 20. Arun Rasheed, Roja C. Formulation, standardization and pharmacological evaluation of a poly herbal traditional remedy-Ashwagandharishtam. *Oriental Pharmacy and Experimental medicine*. 2012, 51-58.
 21. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. *Journal of Pharmacognosy research*, 3(1), 2011, 49-56.
 22. Meena AK, Rao MM, Panda P, Kiran, Yadav A, Singh U, et al. Standardization of ayurvedic polyherbal formulation, Pancasama Churna. *International Journal of Pharmacognosy and Phytochemistry Research*, 2(1), 2010, 11-14.
 23. Sanjay J, Sweta S, Rakesh B, Praveen K. Standardization of „Dashamularishta“: A polyherbal formulation. *Journal of Pharmacognosy*, 1(3), 2009, 54-57.
 24. Kumar T, Chandrasekhar KS, Tripathi DK, Nagori K, Pure S, and Agarwal S. Standardization of “ Gokshuradi Churna” An ayurvedic polyherbal formulation. *Journal of Chemical and Pharmaceutical Research*, 3(3), 2011, 742-749.
 25. Meena R, Meena AK, Khan SA, Mageswari S. Standardization of Unani polyherbal drug-Jawarish-e-Darchini. *Journal of Pharmacognosy research*, 7(3), 2010, 11-12.
 26. Rajini M, Kanaki NS. Phytochemical standardization of herbal drugs and polyherbal formulations. *Bioactive Molecules and Medicinal Plants*, 2008, 349-369.
 27. Ahmad I, Aqil F, Owasis M. Turning medicinal plants into drugs. *Modern Phytomedicine*, 384, 2006, 67-72.
 28. Rajurker S, Rekhe DS, Maini S, Ravikanth K. Acute toxicity studies of polyherbal formulation. *Veterinary World*, 2(2), 2009, 58-59.
 29. Pattanaya P, Jena RK, Panda SK. HPTLC fingerprinting in the standardization of sulaharan yoga: An ayurvedic tablet formulation. *International Journal of Pharmaceutical Sciences Review and Research*, 3(2), 2010, 33-36.
 30. Chandrakant K., Dere Pravin J., Honde Bharat S.,Kothule Sachin Kote Amol P. An overview of supercritical fluid extraction for herbal drugs. *Pharmacologyonline*, 2, 2011, 575-596.
 31. VyasN, Khan MY, Panchal S, Butani A, Kumar V. Supercritical fluid technology- an unlimited frontier in herbal research. *International Journal of Pharmaceutical Sciences and Nanotechnology*,1(4), 2009, 303-307.
 32. Bertucco A, Franceschin G. Supercritical fluid extraction of medicinal and aromatic plants: Fundamentals and applications in: *Extraction technologies for medicinal and aromatic plants*. International Centre for Science and High Technology Trieste, 2008.
 33. Trease and Evans. *Text book of pharmacognosy*. 15, 138.
 34. Chaudary RD. Regulatory requirements. *Herbal Drug Industry- A practical approach to industrial pharmacognosy*. Eastern publishers, New Delhi. 1, 1996, 537-546.
 35. Wir-Ferenc A, Biziuk M. solid phase extraction technique_ Trends, oppurtunities and applications. *Journal of Environmental Studies*, 15(5), 2006, 677-690.
 36. Yan Z, Feng D, Li S, Zhao Y, Yang H. Determination of organochlorine pesticide residues in nine herbs by solidphase extraction and capillary gas chromatography. *National centre for Biotechnology information*, 23(3), 2005, 308-311.
 37. Lee MC, Tsao CH, lou SC, Chuang WC, Sheu SJ. Analysis of aristolochic acids in herbal medicines by LC/UV and LC/MS. *Journal of Speration Science*, 26, 2003, 818-822.
 38. Trease and Evans. *Text book of*

- Pharmacognosy. 15,140.
39. Wang Y, Xi GS, Zheng YC, Miao FS. Microwave-assisted extraction of flavonoids from Chinese herb Radix puerariae. *U Med Plant Res*, 4(4), 2010, 304-308.
 40. Beatrice K, Philippe C. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. *Phytochemical Analysis*, 13, 2002, 105-113.
 41. Vivekananda M, Yogesh M, Hemalatha S. Microwave assisted extraction- an innovative and promising extraction tool for medicinal plant research. *Journal of Pharmacognosy*, 1(1), 2007, 78-84.
 42. Chimezie A, Ibukun A, Teddy E, Francis O. HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. *African Journal of Pharmacy and Pharmacology*, 2(2), 2008, 29-36.
 43. Saravanan J, Shajan A, Joshi NH, Varatharajan R, Valliapan K. A simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form. *International journal of Chemistry and Pharmaceutical Sciences*, 1(2), 2010, 323-324.
 44. Rao Udaykumar B, Anna NP. Stability-indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. *African Journal of Pharmacy and Pharmacology*, 3(12), 2009, 643-650.
 45. Rathod Shobhen, Patel N.M, Patel P.M. A Review on modification of analytical techniques in herbal research. *International Journal of Research in Ayurveda and Pharmacy*, 2(5), 2011, 1483-1485.
 46. Zhang Q, Ye M. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). *Journal of Chromatography A*, 1216(11), 2009, 1954-1969.
 47. Manisha K, Gharate, Veena S. Kasture. Development and Validation of RP- HPLC method for determination of marker in polyherbal marketed Kankasava formulations. *Scholars research library*, 3(5), 2011,28-33.
 48. Vogel H, Gonzalez M, Faini F, Razmilic I, Rodriguez J and Martin JS. *Journal of Ethnopharmacology*, 97, 2005, 97-100.
 49. Xie P.S., Chromatographic fingerprint analysis – a rational approach for quality assessment of traditional Chinese herbal medicine. *Journal of Chromatography A*, 1112, 2006, 171-180.
 50. Soni K, Naved T. HPTLC – Its applications in herbal drug industry. *The Pharma Review*, 2010, 112-117.
 51. Ashok kumar, Mani Tripathi S.N., Satish K.V. Estimation of gallic acid, Rutin, and Quercetin in Terminalia chebula by HPTLC. *Jordan Journal of Pharmaceutical Sciences*, 3(1), 2010.
 52. Jirge SS, Tatke PA, Gabhe S. Development and validation of a novel HPTLC method for simultaneous estimation of beta-sitosterol- d-glucoside and withaferin A. *International journal of Pharmacy and Pharmaceutical Sciences*, (12), 2011, 227-230.
 53. Shanbhag DA, Khandagale NA. Application of HPTLC in the standardization of a homoeopathic mother tincture of Syzygium jambolanum. *Journal of Chemistry and Pharmaceutical Research*, 3(1), 2011, 395-401.
 54. Kshirsagar VB, Deokate UA, Bharkad VB, Khadabadi SS, HPTLC method development and validation for the simultaneous estimation of Diosgenin and Levodopa in marketed formulation. *Asian Journal of Research Chemistry*, 1(1), 2008.
 55. Kasthuri P. Development of GC-MS for a polyherbal formulation-MEGNI. *International Journal of Pharmaceutical sciences*, 2 (2), 2010, 81-83.
 56. Binit DK, Sunil K, Nayak C, Mehta BK. Gas chromatography mass spectroscopy (GC-MS) analysis of the hexane and benzene extracts of the Piper beetle from India. *Journal of Medicinal Plants Research*, 4(21), 2010, 2252-2255.

57. Mike Lee S, Edward Kerns H. LC-MS applications development. Milestone Development services, Pennington, New Jersey, 1999.
58. Ip SP, Zhao M, Xian Y, Chen M, Zong Y, Tjong YW, ET AL. Quality assurance for Chinese herbal formulae: Standardization of IBS-20, a 20- herb preparation. *Journal of Chinese Medicine*, 5, 2010, 8-9.
59. Yu-Tse Wu, Ming –Tsang Wu, Chia-Chun Lin, Chao- Feng Chien, and Tung-Hu Tsai. Pharmacokinetic studies of Chinese Medicinal Herbs Using an Automated Blood sampling system and Liquid Chromatography-Mass spectroscopy. *Journal of Traditional Chinese medicine*, 2(1), 2011, 33-40.
60. Sombra LL, Gomez MR, Olsina R, Martinez LD, Silva MF. Comparative study between capillary electrophoresis and high performance liquid chromatography in guarana based phytopharmaceuticals. *Journal of Pharmaceutical and Biomedical Analysis*, 36, 2005, 989-994.
61. Tistaert C, Dejaegher B, Heyden YV. Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. *Analytica Chimica Acta*, 690, 2011, 148-161.
62. Shikha S, Mishra N. Genetic markers - a cutting -edge technology in herbal drug research. *Journal of Chemical and Pharmaceutical Research*, 1, 2009, 1-18.
63. Lazarowych NJ, Pekos P. The use of fingerprint and marker compounds for identification and standardization of botanical drugs. *Journal of Drug Information*, 32, 1998, 497-512.
64. Jayvant K, Vaibhav S, Abhay H. Application of ISSR marker in pharmacognosy. *Current Sciences*, 3, 2009, 216-228.
65. Usha K, markers: A potential tool for authentication of herbal drugs. *Fitoterapia*, 81, 2010, 969-976.
66. Shalini S,Roy RK. Phytosomes : an emerging technology. *International journal of Pharmaceutcal Research and Development*, 2, 2010, 14-15.
67. Shivanand P, Kinjal P. Phytosomes: Technical revolution in phytomedicine. *International journal of Pharmaceutical Technology and Research*, 2(1), 2010, 627-631.
68. Battachatya S, Ghosh A. Phytosomes: the emerging technology for enhancement of bioavailability of botanicals and nutraceuticals. *International journal of Aesthetic & Antiaging Medicine*, 2(1), 2009, 87-91.
69. Darshan D, Satyaendra S, Shweta K, Gangwal A, Dubey PK. Phytosome: Anovel dosage structure. *International journal of Chemical Sciences*, 1(1), 2007, 132-34.
70. Jagruti P, Rakesh P, Kapil K, Nirav P. An overview of phytosomes as an advanced herbal drug delivery system. *Asian Journal of Pharmaceutical Science*, 4(6), 2009, 363-371.
71. Kidd PM. Bioavailability and activity of phytosome complexes from botanical poly phenols: the silymarin, curcumin, greentea, and grape seed extracts. *Alternative Medicine Review*,14(3), 2009, 226-46.
72. Huang S, Chang WH. Advantages of nanotechnology- based Chinese herb drugs on biological activites. *Current Drug Metabolism*, 10(8), 2009, 905-13.
73. Musthaba SM, Sayeed A, Alka A, Javed A. Nano approaches to enhance pharmacokinetic and pharmacodynamic activity of plant origin drugs. *Current Nanoscience*, 5(3), 2009, 344-352.
74. Dong TP, Sung CH. Exuberence or bubble study of nano- based herbal medicines patients in the PR China. *Journal of Intellectual Property rights*, 16, 2011, 225-234.
75. Kumar A, Nasair AG, Reddy AV, Garg AN. Bhasmas: unique Ayurvedic metallic- herbal preparations, chemical characterization. *Journal of Biological Trace Element Research*, 109, 2006, 231-254.
76. Saper RB, et al. Heavy metal content of ayurvedic herbal medicine products. *Journal of Advanced Medical Research*, 292, 2004, 2868-2873.