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An concise overview on standardization research of *ASU*-TAM herbal formulated and single drugs, products

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Abstract
Traditionally utilization of ASU herbal drugs as medicine used is the since ancient time for health, wellness and cures to mankind and it is used in all cultures all over history The primeval and tribal's, hillers persons learned by trial and error basis to identified valuable potent medicinal plants. In modern scenario more demand increasing towards herbal medicinal ASU products because ASU and TAM herbal products having low hazardness and toxicity as compare to other medicine and there is increasing awareness and general acceptability of herbal drugs in today's medical practice on global demand basis. In present, It is however a known fact that over 80% of the world population used herbal medicines and product for healthy living. As well as globally demand of herbal products increases the need of herbal quality assurance for customer satisfaction also increases. For the prevention of drug substitution, adulteration and developed abuse herbal products, WHO have been released guidelines on global requirement and public domain to all herbal industries. The identification of this highly active moiety is an important requirement for Quality assurance, quality control and dose determination of plant related dugs. Standardization research of ASU. Herbal drugs means confirmation or validation of its identity, purity, quality and efficacy. It is mandatory for every ASU herbal industry. It must need for collection, handling, processing and production of ASU herbal medicine as well as employ such parameters in ensuring the quality, safety, efficacy and compulsory need for the global ASU. TAM. Herbal market. In this overview all guidelines which are required for development of ASU and TAM herbal products at global scale.

Keywords: ASU and TAM, herbal formulated and single drugs, standardization research, quality assurance, quality control

Introduction
According to the WHO, the quantity, quality, safety and efficacy data on traditional alternative medicine (TAM) and Ayurvedic, Siddha and Unani (ASU.) are not sufficient to meet the criteria needed, so some of the major policy challenges include safety, efficacy, quality, and enlightened the perception for the use of TM. Various policy measures have been applied for a cleareyed view of the use of TM, in order to increase its safety, efficacy and acceptability (G. Bodeker; G. Burford, 2007 and Sagar *et al*, 2023) [2, 14].

World Health Organization (WHO) has defined herbal medicines as finished labeled medicinal product that contain active ingredients, aerial or underground parts of the plant or other plant material or combinations. (Florey *et al*, 1949; Wani *et al*, 2021) [39, 3].

In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection of itself Rishis, Vaidyas and Hakims. However, in modern concept it require necessary changes in their approach by that way concrete method of quality control in terms development of modern methodologies. Thus today quality assurance is thrust area for the evaluation of traditional used medicinal plants and herbal formulation.

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants

and animals for their primary health care. Alternative medicine is the needs of the day. Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector WHO recommends and encourages the use of traditional herbs/remedies because huge amount of raw material is easily available. They are comparatively safe because of their low toxicities. Till today most of the villagers relays on herbal remedies as these have psychological effect on the common man in mind that it will spared of the side effect of allopathic drugs and will magically cured. However, plants are very complex in their composition and their therapeutic activity depends on their chemical constituents, these according to age, geographical location and harvesting processes. Also improper authentication of herbs, adulterations by microorganism, pesticide residue, has made standardization of herbal drug of primary importance. (Wani *et al*, 2021) [3] The quality assurance and quality control of ASU. Herbal crude drugs and formulated products are important in justifying their acceptability in modern system of medicine. Hence it is required to conduct the research on drugs standardization and product validation to provide effective, curable and safe drugs to the needy mass suffering from various ailments. (Sagar *et al*., 2020; 2023) [5, 2] In the past decades there is a sense of awareness among the developing world population, about the importance of traditional system of medicines such as Unani, Ayurveda and Siddha in maintaining health without the side or adverse effects. Due to this scientific awareness a scenario has created to research activities like quality standardization and drug validation of traditional medicines and development of scientific methods for the manufacture of quality medicines. (Ramasamy *et al*., 2009; Sagar *et al*., 2023) [13, 2]. As there is increase demand of herbs and herbal products especially Unani medicinal products, run across many problems like non-availability of good quality of raw materials, proper methodology for standardization. In consequence to ensure and develop the quality, authenticity of Unani formulations, the standardization of single as well as compound drugs on modern analytical parameter is basic requirement for drugs. Before studying pharmacological activity of any drug physico-chemical characteristics is necessary for its authenticity (Naaz A *et al*., 2021; Sagar *et al*., 2020; 2023) [4, 5, 2].

The standardization of herbal drugs includes 1. Firstly Authentication of herbal drugs which includes collection, parts of the plant collected botanical identification by botanist. 2. Removal of all Foreign matter (Herbs collected should be free from soil, insect parts or animal excreta, etc.) 3. Determination of organoleptic evaluation of herbal drugs as (Sensory characters - taste, appearance, odor, feel of the drug, etc.) 4. Tissues of diagnostic significance present in the drug powder. 5. Determination of ash values and extractive values. 6. Determination of volatile matter 7. Determination of moisture content 8. Determination of Chromatographic and spectroscopic evaluation. TLC, HPLC, UPLC, HPTLC, GC-MS/ LC-MS, UV-Visible and AAS with Graphite furnace, ICP-OES methods will provide qualitative and semi quantitative information about the main active mineral, metals and active phyto-chemical constituents present in the crude or formulated drugs as chemical markers in the TLC fingerprint evaluation of herbals (FEH). The quality of the drug can also be assessed on the basis of the chromatographic fingerprint. 9. Determination of heavy metals-e.g. cadmium, lead, arsenic-by AAS with Graphite furnace, ICP-OES, etc. 10. Determination of pesticide residue - by GC-MS etc. according to WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbal sources. These pesticides are mixed with the herbs through the time of cultivation. Mostly pesticides like DDT, BHC, toxaphene, aldrin source serious side-effects in human beings if the crude drugs are mixed with these agents. 11. Microbial contamination - generally medicinal plants having bacteria and molds are coming from soil and atmosphere. Determination of the limits of *E. coli* and molds clearly throws light towards the harvesting and production practices. Determination of the Limits *Escherichia coli*, *Salmonella typhai Spp*. *Staphylococcus aurous* author pathogenic contamination as well as these substance known as aflatoxins - B1, G1 and B2 G2 will produce serious side-effects if taken along with the crude drugs (Patil *et al*., 2011) [24].

The World Health Organization (WHO) has a provided of specific Guidelines for the evaluation of the safety, efficacy and Quality of herbal medicinal drugs or herbal medicines. WHO find out that 80% of the world people now use herbal medicine or drugs, except in some countries herbal medicinal drugs may also used by traditional, natural or in form of inorganic active constituents. Those are the not directly obtained from plant. An herbal medicinal drug is a main constituent in usual medicine and a common ingredient in ASU- TAM, Homeopathic, Ayurvedic, and Naturopathic and in other medicine system. Herbs are regularly measured as safe toxicity, side effects of allopathic drugs, has led to more increased in numeral of herbal drugs manufacturers. For the past few years, herbal drugs have been mostly used by the citizens with no prescription, Leaves, stem, bark, flower, seeds, roots and extract of all these have been used in herbal medicinal drugs over the thousands of their use (Bhutani *et al*., 2003) [11]. Official standards the identification, purity and quality of herbal drugs are determined by reference given in a pharmacopoeia. Pharmacopoeia prescribes like Analytical, physical and structural standards for the herbal drugs. A significant identification and examination of crude drugs is important in processes of herbal formulation because of more diversity and changes in their chemical nature or characters. To reduce this problem all pharmacopeias have certain standards. Specific test for specific plant material are given below. Alkaloids content dragendorff test, Fat content Acid value Iodine value, saponification value molish test carbohyadrates Millon tests Amino acid Volatile oil Hemolytic activity Assay for Phosphate/Aluminium/ Camphor /Potassium /Lead/Iron/Gold/Calcium (Nikam *et al*., 2012; Pandey *et al*., 2016; Bodhisattwa *et al*., 2011) [18, 19, 12].

The main objective of these guidelines is to contribute to the quality assurance of medicinal plant materials used as the source for herbal medicines, which aims to improve the quality, safety and efficacy of finished herbal products and minimize the risks involved in any pharmaceutical production that cannot be eliminated through testing the final product (WHO 2002). Diagrammatic presentation of various standardization and evaluation parameters, using for the herbal products quality control as per WHO guidelines given in figure 1. Morphological or Organoleptic evaluation It includes the evaluation of herbal medicinal drugs by size,

shape color, odor, taste and particular characteristics like touch, texture etc using sense of organs. This is a method of qualitative evaluation related to the study of morphological and sensory report of whole herbal drugs.eg. Fractured surfaces in cascara, cinchona, and quillia bark and quassia wood are essential individuality. Umbelliferous fruits have aromatic odour and liquorice have sweet taste are the example of this type of evaluation (Kokate *et al*., 2010; Ansari *et al*., 2011; Pandey *et al*., 2013) [14, 10, 21].

Validation of pharmacopoeial standards by experimentation and observations provides a set of characteristics to a particular herbal medicine. Therefore, Scientific Validation of Unani Formulations is an important tool used in the standardization process (Kunle, 2012) [17]. Historically, herbal medicines have played a significant role in the management of both minor and major medical illness (Bahuguna *et al*., 2014) [20]. The Standardization and Validation of ASU herbal Drugs is not an easy challenge as various factors influence the bio efficacy and reproducible therapeutic effects. In order to obtain assured quality based herbal products, care through pharmacovigilance and care should be taken right from the proper identification of plants, season and area of collection, grading, drying, extraction, purification process and rationalizing the combination in the case of poly-herbal drugs. (Patel *et al*., 2006) [42], the subject of standardization of herbal drugs is massively wide and deep. There are many seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. (Yadav *et al*., 2011) [23].

Advantages of Herbal Medicine

1. Herbal medicine have long history of use and better patient tolerance as well as globally acceptance.
2. Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity.
3. Medicinal plants have a renewable source, which is our only hope for sustainable supplies of cheaper medicines for the world growing population.
4. Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
5. The cultivation and processing of medicinal herbs and herbal products is environmental friendly.
6. Throughout the world, herbal medicine has provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form and as a pure chemical upon which modern medicines are structured.

Expanding global market makes AYUSH a booming business

AYUSH has witnessed strong traction amid efforts to boost traditional medicines' global reach. As a result, India's wellness market, valued at over US$10 billion, is expected to grow to US$21 billion by 2020, AYUSH has witnessed strong traction amid efforts to boost ASU-TAM herb-mineral, traditional medicines' global reach and globally acceptability these ASU-TAM boost up on demand. As a result, India's wellness market, valued at over US$10 billion, is expected to grow to US$21 billion by 2020. Export from the sector was worth US$1.1 billion in 2016-17. To drive export growth, AYUSH Premium Mark, a quality certification scheme, is ensuring the efficacy of ASU. Indian herbal products.

Limitations of ASU/ TAM Herbal Medicines

Like any other branch of science and technology, present scenario of herbal medicine has its own limitations arising out of its own technical constituents. The prominent limitations of herbal medicines can be summarized as follow

1. Ineffective in acute medical care

As may be observed, herbal medicines are not varying effective to treat any acute illness. As most of the medicines are designed to work at molecular level of physiology, the drug takes its time to deliver the results. However there are few herbal medicines which works instantly in acute conditions like diarrhea. On the other hand, modern system of medicine has adequate paraphernalia for management of acute conditions. It has already been established by virtue of its efficacy. It may be a futile exercise to investigate and discover such methods of acute medicinal care within the framework of herbal medicines.

2. Inadequate standardization and lack of quality specifications

This is the most often criticized aspect of herbal medicines. One important fact is that a herbal medicines. One important fact is that a herbal preparation is administered for its holistic value. Each herbal ingredient in the herbal preparation has an array of chemical constituents with complex molecular formulae. This each herbal preparation is a source of poly-pharmacy within itself.

As results, standardization of herbal preparation or its ingredients become a highly complex issue. Standardization of herbal drugs by known marker compounds may not be complete answer. Despite it is major limitation, pharmaceutical industry strives hard to have in house specifications based on the quantification of marker compounds. Therefore a consensus is being arrived at to incorporate the qualitative finger-printing together with other physicochemical parameters of quality protocols for herbal medicines is an ongoing process and this shortcoming could be overcome shortly.

3. Lack of scientific data of ASU/ TAM Herbal Medicines

Literature on herbal medicines, lack of Lack of scientific data in support of the medicinal activity claimed and their safety and efficacy assumed. Hence there is a need to incorporate certain parameters of the pharmacological evaluation of moderns on modern lines. Who guidelines clearly direct that it is not necessary to carry out detailed toxicological evaluation of herbs and herbal preparation originating from ASU/ TAM traditional system medicine.

Need of Standardizations of ASU/ TAM Herbal Medicines

In recent years there is a spurt in the interest regarding survival of ASU/ TAM Herbal Medicines forms of medication. In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine have started getting more apparent, majority of ASU/ TAM Herbal Medicines formulation are prepared from herbs.

It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, which guarantee. Purity, safety, potency and efficacy. This duty is discharged by the regulatory authorities by rigidity following various standards of quality prescribed for raw materials and finished products in pharmacopoeias controlling manufacturing formulate through the use of formularies and manufacturing operation through statutory imposed "Good manufacturing practices". All these procedure logically would be apply to all type of medication whether included in modern system of medicine or one of the traditional system such as ASU/ TAM Herbal Medicines system of medicine. Unfortunately the ASU. Pharmacopoeias and the formulary have been exempted from the standard attained by present day. Modern medicine, which is continuously undergoing metabolic changes and improvements in the standard of purity, safety and efficacy. Thus maintaining the quality of ASU/ TAM Herbal Medicines medication becomes the sole responsibility of the manufacture.

ASU/ TAM Herbal Medicinal product has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the ASU/ TAM Herbal Medicines formulation is the lack of standard quality control profile. The quality of herbal medicine i.e. the profile of the constituents in the final product has implication in efficacy and safety. Due to complex nature and inherent variability of the constituents of plant based drugs, it is difficult to establish quality control parameter and modern analytical technique are expected to help in circumventing this problem.

The quality control and quality assurance and drug standardization research of crude drugs and ASU/ TAM Herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations.

The task of lying down standards for quality control of ASU/ TAM Herbal Medicines or herbal crude and their formulation involves biological evaluation for a particular disease area, chemical profiling of the material and lying down specification for the finished product. Therefore, in case of herbal drugs and product, the word "Standardization" should encompass entire field of study from cultivation of medicinal plant to its clinical application.

Plant material and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern technique and by applying suitable parameters and standards. In order to overcome certain inevitable shortcoming of the Pharmacopoeial monograph other quality control measures must be explored. Quality control has wide connotation and covers; many aspects of drug manufacture, distribution and sale is not restricted to final product analysis either regulatory or otherwise, while engaging in this task, it must be realized that some of the Quality control practices that work excellently either modern drug may not be appropriate with ASU drug. (Wani *et al*, 2021) [3].

Current Regulations for Standardization research of ASU/ TAM Herbal crud drugs

Natural medicinal plants are after unable to meet demand for popular of patent, sheastra oushadhee, preparatory ASU. Herbal products as well as used as Alternative and Traditional medico care therapy in ISM. Populations of many species have limited distribution in their natural habitats, requiring conservation strategies for protection (Kokate *et al*, 2010) [14]. Unavailability of such medicinal plants has led to arbitrary substitution and adulteration in raw drug market (Tewari NN, 1991; Bisset WG, 1984) [31, 38]. Adulteration it is a practice of substituting the original crude drug partially or fully with other substances which is either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled or spurious drugs or entirely different drug similar to that of original drug substituted with an intention of enhancement of profits (Bisset, WG., 1984; Sunita, G., 1992; Sagar, PK., 2014) [38, 37, 9].

Patent proprietary ASU/ TAM Herbal Medicines are sold over the counter in pharmacies. These products appear to represent a major share of branded traditional medicine in India. Nevertheless systems like ASU/ TAM Herbal Medicines still need to gain an empirical support of modern medical sciences to make them credible and acceptable for all. An innovative research effort to define the advantage of traditional system of medicine with respect to their safety and efficacy could result in a better utilization of these complementary systems of medicine. Internationally several pharmacopoeias have provided monographs stating parameter and standard of many herbs and some product made out of these herbs. No today demand boost up of uniforms and develop a Pharmacopeial harmony on National basis. Several pharmacopoeias like.

- Pharmacopoeia Committee.
- Chinese Herbal Pharmacopoeia.
- United States Herbal Pharmacopoeia.
- British Herbal Pharmacopoeia.
- British Herbal Compendium.
- Japanese Standards for Herbal Medicine.
- The Ayurvedic Pharmacopoeia of India (API).
- The Unani Pharmacopeia of India (UPI).
- The Siddha Pharmacopeia of India (SPI).
- The Homeopathic Pharmacopoeia of India (HPI).

Lay down monograph for herbs and herbal products to maintain their quality in their respective nations. Government of India too has brought out Ayurvedic, Unani, Siddha, and Homeopathic Pharmacopoeia of India, which recommends basic quality parameters for several common ASU/ TAM Herbal crud and formulated herbo-mineral drugs.

Drug Standardization research, Quality Control and Quality Assurance of ASU/ TAM Herbal formulated drugs, Herbal Crude Drugs: (Ritch *et al*., 2000; Wani *et al*, 2021) [27, 3]

Accounting to WHO it is the process involving the physicochemical evaluation of crude drug covering the aspects, as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.

- **Macro and Microscopic Examination:** For Identification of right variety and search of adulterants.
- **Foreign Organic Matter:** Remove of matter other than source plant to get the drug in pure form.
- **Ash Values:** It is criteria to judge the identity and purity of crude drug – Total ash, sulfated ash, water soluble ash and acid insoluble ash etc.
- **Moisture Content:** To check moisture content helps prevent degradation of product.
- **Extractive Values:** These are indicating the approximate measure of chemical constituents of crude drug.
- **Crude Fiber:** To determine excessive woody material Criteria for judging purity.
- **Qualitative Chemical Evaluation:** It covers identification and characterization of crude drug with respect to phytochemicals Constituent.
- **Chromatographic Examination:** Include identification of crude drug based on use of major chemical constituent as marker.
- **Qualitative Chemical Evaluation:** Criteria to estimate amount the major class of constituents.
- **Toxicological Studies:** Pesticide residue, potentially toxic elements, and Microbial count approach to minimize their effect in final product.

Details description please may see in Table-1 and Graphical Abstract details description may see in Figure-1 respectively.

Table: 1 (Ritch *et al*., 2000; Wani *et al*., 2021) [27, 3]

QC, QA, DSR. Parameters	Details parameters	Mandatory Regularity Guidelines required for ASU. herbal products / drugs
Macro and Microscopic Examination	Organoleptic characteristics, powder microscopy identification and pharmacognosy, microscopy identifications	For all ASU herbo-mineral Classical and Patents Formulated and single products / drugs as per WHO./IPC/ AYUSH. Guidelines
Foreign Organic Matter	Removal of all kind Foreign matter	As above
Ash Values	Total ash, sulfated ash, water soluble ash and acid insoluble ash	As above
Moisture Content	LOD/ moisture content helps prevent degradation of product	As above
Extractive Values	Water Soluble Extractive matter, Alcohol Soluble Extractive matter, Chloroform Soluble Extractive matter, Hydro-Alcohol Soluble Extractive matter, Ether Soluble extractive matter and Water Soluble Successive Extractive matter, Alcohol Soluble Successive Extractive matter, Chloroform Soluble Successive Extractive matter and Hydro-Alcohol Soluble Successive Extractive matter, Ether Soluble Successive extractive matter	As above
Crude Fiber	Detect excessive woody material Criteria for judging purity	As above
Qualitative Chemical Evaluation	Detect and identification of phytochemicals Constituent eg.- Alkaloids, Glycosides, Flavinoides, Terpinoides, Flaveonles, Glycogens, Steroids, Sterols, Tannins etc,	As above
Chromatographic Examination	Detect and qualitative identification of major active phyto-chemical constituents as marker using by TLC/ HPLC/UPLC/ HPTLC/ GC-MS/LC-MS Advance sophisticated techniques, applying required Reference Standard.	As above
Qualitative Chemical Evaluation	Detect and qualitative identification of estimate amount the major class of active phyto-chemical constituents and active minerals and metals as marker using by TLC/ HPLC/UPLC/ HPTLC/ GC-MS/LC-MS and ICP-OES etc. Advance sophisticated techniques, applying required Reference Standard.	As above
Toxicological Studies	Heavy Metals- Lead (Pb), Arsenic (As, Cadmium (Cd), Mercurry (Hg), Pesticide residues- Organo chlorine, pesticides, Organo phosphorus pesticides, Pyrethroids etc., potentially toxic elements, Aflatoxins, B1, G1 and B2 G2 in ppm or ppb levels and Microbial load contaminations - TBC/TFC detect in cfu/gm. *Escherichia coli*, *Salmonella typhai Spp. Staphylococcus aurous* author pathogenic contamination should be absent / nil.	As above

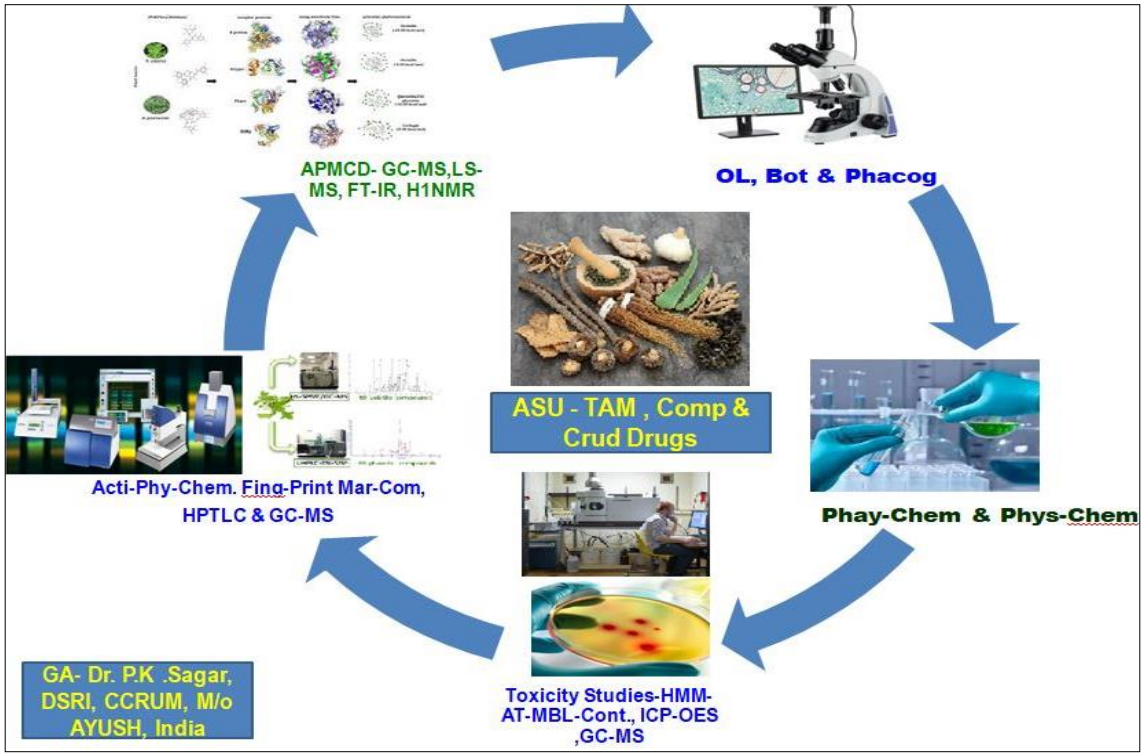

Fig 1: Graphical Abstract of DSR, QC & QA of ASU-TAM Herbal Drugs

Physical evaluation

Each monograph contains detailed botanical, macroscopic and microscopic descriptions of the physical characteristics of each plant that can be used to insure both identity and purity. Each description is accompanied by detailed illustrations and photographic images which provide visual documentation of accurately identified material. Organoleptic characteristics, powder microscopy identification and pharmacognosy, microscopy identifications

Microscopic evaluation

Full and accurate characterization of plant material requires a combination of physical and chemical tests. Microscopic analyses of plants are invaluable for assuring the identity of the material and as an initial screening test for impurities. Most manufacturers of herbal products lack the quality control personnel to accurately assess plant identity and purity microscopically. The Ayurvedic Herbal Pharmacopoeia (AHP); API; UPI; SPI and HPI fully characterize herbal products against the literature and AHP. Verified trade mark authenticated materials to assure identity of test materials. Ideally, submitted materials should be in their whole or semi-whole (Cut) form for microscopic assessment. However, much information can be discerned from microscopic evaluation of powders as well.

Chemical evaluation

A chemical method for evaluation covers the isolation, identification and purification. Chemical analysis of the drug is done to assess the potency of vegetable and animal source material in terms of their active principles. Detect and identification of phytochemicals Constituent eg.- Alkaloids, Glycosides, Flavinoides, Terpinoides, Flavones, Glycogens, Steroids, Sterols, Tannins etc., The chemical tests include colour reaction test, these tests help to determine the identity of the drug substance and possible adulteration.

Biological evaluation

Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. All living organism are used, these assays are known as Biological assays or Bioassay.

Analytical Methods

Critical to compliance with any monograph standard is the need for appropriate analytical methods for determining identity, quality, and relative potency. There are a plethora of analytical methods available. However, it is often difficult to know which is the most appropriate to use. The primary goal of AHP; API; UPI; SPI and HPI is to provide multiple methods of identification and testing by which all aspects of the botanical can be appropriately assayed.

Chromatographic Characterization

Chromatography

Chromatography is the science which is studies the separation of molecules based on differences in their structure and/or composition. In general, chromatography involves moving a preparation of the materials to be separated the "test preparation" over a stationary support. The molecules in the test preparation will have different interactions with the stationary support leading to separation of similar molecules. Test molecules which display tighter interactions with the support will tend to move more slowly through the support than those molecules with weaker interactions. In this way, different types of molecules can be separated from each other as they move over the support material. Chromatographic separations can be carried out using a variety of supports, including immobilized silica on

glass plates (thin layer chromatography), very sensitive volatile gases (gas chromatography), paper (paper chromatography), and liquids which may incorporate hydrophilic, insoluble molecules (liquid chromatography). High Performance Liquid Chromatography (HPLC.), Ultrafast Performance Liquid Chromatography (UPLC) and High Performance Thin Layer Chromatography (HPTLC),

Purity Determination

Each monograph includes standards of purity and other qualitative assessments which include when appropriate: foreign matter, ash, acid-insoluble ash, moisture content, loss of moisture on drying, and extractives.

High Performance Liquid Chromatography (HPLC.), Ultrafast Performance Liquid Chromatography (UPLC) and High performance thin layer chromatography (HPTLC), while in HPTLC is valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed sing different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses. UPLC and HPTLC, GC-MS and LC-MS define very important and major role for the detection of purity and quality as well quantity, Rf values, area percentage and mass determination percentage of active phyto chemical marker compounds and constituents using and applied with their reference standards samples corresponding with run test samples.

Quantitative Analysis

When applicable, the most appropriate quantitative analytical method with accompanying chromatograms shall be provided. The primary goal of the method (s) is to provide validated methods to be used for the quantization of the compound(s) most correlated with pharmacological activity or qualitative markers as determined by the primary pharmacological literature, constituent declaration in product labeling, and a survey of experts. The method (s) will be selected from the primary analytical literature by a Methods Selection Committee with priority given to compendial methods when available. In this context, validation consists minimally of a two-lab validation using the same procedures, samples, and reference standards.

Primary factors for considering a method as appropriate include accuracy of the findings, speed, basic ruggedness, applicability to a large segment of the manufacturing community, and avoidance of the use of toxic reagents and solvents. In an attempt to promote harmonization, primary consideration is given to those methods which are already accepted in official pharmacopoeias or by AOAC International. When necessary, comparative tests shall be conducted to determine which of the available method(s) is most appropriate. The validation process minimally includes: standard precision, linearity, sample precision using replicate samples, sample linearity, selectivity (co-elution, sensitivity to analyte degradation), retention times, and limits of detection. Other methods which may be of value to the industry may be included or cited in the monograph but are not required for compliance with the monograph. (Wani *et al*, 2021) [3]

Determination of Total Ash Value: Used for determination of quality and purity of a crude herbal drug, using ash of herbal drug because they contains inorganic radicals like phosphates, carbonates and silicates of sodium, Potassium, Magnesium and calcium. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug like affects 'Total ash value'. Such variables are then removed by treating through acid (as they are soluble in hydrochloric acid) and them acid insoluble ash value is determined e. g. Rhubarb, Liquorice etc. The residue after incineration is the total ash content of the crude herbal drug, which simply represents inorganic salts, naturally found in drug or adhering to it or deliberately added to it, in the form of adulteration of herbal drugs. Two types of total Ash value mainly:

1. Water soluble Ash value.
2. Acid-insoluble Ash value.

Determination of Extractive Values: The herbal extracts obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. The varieties of solvent are used for determination of extractives. These are classified as fallows.

1. Water soluble extractives values.
2. Alcohol Soluble extractives values.
3. Chloroform soluble extractive values.
4. Ether Soluble extractives values. Etc.

Determination of heavy Metals: In general, quantitative and limit tests correctly determine the concentration of heavy metals in the form of impurities and contaminants. The heavy metals like Arsenic, mercury, lead, thalium, and cadmium have been exposed to be contaminants of few herbal ingredients. A simple determination of heavy metals, using pharmacopeias methods and it is based on color reaction with special reagents such as diethyl dithio carbonate or thio acetamide and amount is determined by comparison with a standards. The methods usually used for analysis are inductive coupled plasma (ICP) (ICP-OES), Netron activation analysis (NAA), Atomic Absorption Spectrophotometer (AAS) with Graphite furnace etc. (Watson *et al*., 1999; WHO *et al*., 2007; Mehta *et al*., 2018) [29, 36, 8].

Determination of Radioactive contamination: The microbial growth in herbal medicinal drugs is usually avoided by irradiation. Dangerous contamination may be the result of a nuclear accident. The WHO, in close support with several other international organizations, has developed guiding principle in the event of a wide spread contamination by radio nuclides resulting from major nuclear accidents. The nuclear accident in chernobyl and Fukushima may be severe and depend on the specific radionuclide, the stage of contamination and the quantity of the contaminant taken. Examples of such radio nuclides include extended lived and short lived fission products, actinides and activation products (AOAC *et al*., 2005; De Smet PAGM *et al*., 1992; Mehta *et al*, 2018) [10, 16, 8].

Determination of pesticides residue: Determination of pesticides residues are any particular substance in food, agriculture commodities or animal feed resulting from the use of pesticides. Herbal medicinal drugs are prone to hold pesticide residue, which gather from agricultural practices,

such as Spraying, behavior of soil during cultivation and addition of fumigants during storage. The Pesticides mainly contain chlorine in the molecules, which can be determined by analysis of chlorine; insecticides containing phosphate can be identify by measuring total organic phosphorus. The varieties of methods are used to measure pesticides by GC, MS, OR GC-MS. Some simple methods are also published by the WHO and European pharmacopeia has in general limits for pesticides residue in herbal medicine (Bajaj *et al*., 2012; Mehta *et al*, 2018) [40, 8].

Determination of specific optical rotation (Shukla *et al*., 2013; Mehta *et al*, 2018) [22, 8]

Determination of Specific rotation formula -D25= 100 × φlc Where: φ = corrected observed rotation in drug at-25° D = d line of sodium light l = length of the polarimeter tube in done. c = concentration of substance in percent w/v.

Methods of Standardization Research of ASU. TAM medicines products.

1. Raw material standardization.
2. In process standardization.
3. Finished product standardization.

1. Raw material identification and standardization under this process following parameters considered carefully. Area of the collection, parts of the plant, the regional situation, botanical identity, microscopic and histological analysis, taxonomic identity, Foreign matter, Loss on drying, swelling index, foaming index, ash values, extractive values, Chromatographic, spectroscopic evaluation, Determination of heavy metals, pesticide residues, Microbial and Radioactive contamination proper way.

2. In during process standardization In during process standardization is the key method for the development of quality herbal products. So the standard operating process should be pursuing strictly, if other substances are added in through manufacture in order to adjust the herbal formulations. A method for qualitative, quantitative and where possible, the assay of the plant preparation should be added. If identification of a bioactive principle is not possible, it should be sufficient to recognize a characteristic substance or mixture of herbal medicinal substances to ensure consistent quality of the product.

3. Finishing product Prepared finished product should have standard nature of characteristics. All information associated to product formula, including the exact amount of recipients, should be described in specify. A finished herbal product specification should be defined to ensure and validated consistent quality of the product. The finished herbal product should fulfill with general requirements for particular dosage forms and quantity. The processes involves wide array of scientific screening like as physical, chemical and biological evaluation employing various analytical methods and tools. The specific object of such investigation in validate herbal quality are as varied as the processes used. Analytical Specifications of Herbal products followed as per requirement according to official monograph (WHO) and form of the medicine. Following mentioned different products specifications are (WHO *et al*., 1998; Calixto *et al*., 2000; Mehta *et al*, 2018) [33, 15, 8]:

- Examination of Colour, Odour.
- Determination of total – ash.
- Determination of acid - insoluble ash.
- Determination of water & Alcohol-soluble extractive.
- Determination of viscosity.
- Determination of refractive index.
- Determination of specific gravity at 250 C.
- Determination of alcohol content Test for methanol.
- Determination of total acidity.
- Determination of no. reducing and reducing sugar.
- Determination of PH.
- Determination of total sugar content.
- Determination of loss on drying at 105 ºC.
- Determination of particle size (80-100 mesh for • Churna; 40- 60 mesh for Kvathachurna).
- Determination of weight variation.
- Determination of disintegration time -Not more than 15 min.
- Identification test of TLC/HPTLC/GLC.
- Determination of Assay.
- Determination of heavy/Toxic metals: Lead, Cadmium, Mercury, Arsenic.
- Determination of microbial contamination: Total bacterial count, Total fungal count Determination of specific Pathogen: *E. coli*, *Salmonella spp. S. aureus*, *Pseudomonas aeruginosa*.
- Determination of pesticide residue: Organochlorine, pesticides, Organo phosphorus pesticides, Pyrethroids etc.
- Determination of aflatoxins (B1, B2, G1, G2) etc.

Advanced techniques and standardization of herbal drugs and its products Quality control of herbal preparation is a tedious and difficult job. Hence in standardization process of herbal medicinal drugs and its prepared formulations are now days analyzing by using hyphenated techniques based. Various advanced these technologies are HPLC, UPLC, HPTLC and HPLC-MS, LC-MS, GC-MS and FT-IR etc. These modern and fingerprint analysis techniques is the most effective tool for quality control of herbal medicines because of its accuracy and more reliability. By these techniques that determines the correct concentrations of potent bioactive chemical constituents and a set of distinctive chemical substances in herbal drugs. Knowing the relative concentrations is a means of assuring the best quality of herbal products have or not. It can serve as an important tool for identification, authentication and quality control of herbal drugs (Usha *et al*., 2010; Mehta *et al*, 2018) [26, 8].

Future prospective Standardization of herbal drugs means confirmation or validation of its purity, quality and identity. It is mandatory for every herbal industry, follow as guidelines of WHO and maintain the quality of products. Its need starts from collection, handling, processing and production of herbal medicine as well as employ such parameters in ensuring the safety and compulsory need for the global herbal market. The global herbal market increasing suddenly and India could play key role. Hence it is mandatory to follow all the factors or rule which affecting the quality of herbal products, international level or export quality. These factors which are responsible for quality of herbal drugs or products should maintain and improve the bioactive potency of products. Who want to lead in market globally. Others consideration keep maintain as related to quality of herbal drugs may need more complete

information on aspects of herbal products. As the variety of seeds, condition of cultivation and harvesting represent an important aspect in producing a reproducible quality of herbal medicinal products. Purity and Quality of ASU/ TAM Herbal formulated drugs, Herbal Crude Drugs -herbal products are depends on physical, chemical, biological factors, hence assessment of these factors should be done, validate and documented of Drug Standardization research, Quality Assurance data's. This plays a very important role in the identification and quality and purity of ASU/ TAM drug. All these parameters are mandatory for the development, validation and export quality of herbal drugs (Mehta *et al*., 2018; Sagar *et al*., 2020; 2023)) [8, 5, 2].

Conclusion

ASU/ TAM Herbal formulated drugs, Herbal Crude Drugs is one of the most famous oldest therapeutic systems since ancient time that has globally nourished and flourished from earliest ages till date. India can come into sight as the major country and playing the lead role in improvement of standardized, therapeutically effective ASU and TAM formulation and development. India may explore to investigate the medicinally importance of ASU and TAM herbal plants. This can be happen, only if the herbal formulated products are proper way evaluated and analyzed in the manner of purity, quality and efficacy by using sophisticated hyphenated modern techniques like as T L C, H P L C, U P L C, H P T L C, G C - M S, L C - M S, spectro fluorimetric, UV-Vis, FT-IR spectroscopy and other methods. The determination of general peaks in a set of chromatographic fingerprints peaks Area, Rf values and active phyto-chemical marker compound mass values, investigated, detect and applied with their reference standards gives useful qualitative and quantitative analytical information, detect purity parentage of active phyto-chemical constituents in the test samples on the distinctive components of ASU and TAM herbal medicines. Consequently chromatographic fingerprint analysis and active marker detection analysis provides potentially useful quality control and quality assurance tool for standardization research of ASU and TAM herbal medicines.

Conflicts of interest: None

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