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MODERN ANALYTICAL METHODS FOR STANDARDIZATION OF CLASSICAL POLY HERBAL FORMULATIONS: A REVIEW

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Abstract

The establishment of standards for the quality and purity of raw materials, quality control throughout the drug manufacturing method, development of a high-quality finished product, storage and distribution to preserve the quality of the finished product are all aspects of standardization in ayurvedic formulations. It is crucial for developing Ayurvedic, Siddha and Unani medicine quality control procedures. Standardization in Ayurveda, Siddha, and Unani has been thoroughly established and recorded in both classical and modern writings; nonetheless, these texts were written with an individualistic goal rather than for an industrial or commercial purpose.

Materials and Methods: Considering the most recent standardization techniques, careful consideration of the ancient texts of Ayurveda, Siddha, and Unani among others was conducted. The present WHO standards on the standardization of herbal pharmaceuticals were also studied and examined.

Discussion: This article aims to highlight historical references to standardization while incorporating the most recent scientific methodologies to create, analyse standardized Ayurveda, Siddha, and Unani medications.

Conclusion: According to the review, standardization in Ayurveda, Siddha, and Unani is a continuous process, and in order to examine fine alchemical techniques and the intermediate compounds created, one must be exceptionally watchful of new scientific approaches while also being cognizant of the traditional principles of the practice.

Keywords: Standardisation, Ayurveda, Siddha, Unani, GCMS, HPTLC

Introduction

The World Health Organization (WHO) estimates that 70–95 percent of the earth's population, mainly in emerging economies, uses traditional, complementary, alternative, or nonconventional medications for their healthcare and wellness. Also, in line with the general pattern of people going back to natural therapies, the use of herbal medications has dramatically expanded. The public's increasing usage of

botanicals and phytochemicals are driving efforts to evaluate the efficacy of these agents' potential health benefits and to create manufacturing and quality standards.

Ayurveda, Siddha, and Unani are the three primary branches of the Indian medical system, which is one of the oldest holistic management systems with well-researched treatments. Ayurveda, which is a component of Indian culture, is well-known for its originality and widespread acceptance because it provides all-natural solutions to treat illnesses and advance healthcare. Regrettably, when it comes to the creation of Ayurveda medications, standardisation and quality control are still undefined concepts. Most Ayurveda, Siddha and Unani compositions lack specified quality control measures and an evaluation mechanism as of yet (1). The Rigveda, Atharvaveda, Charaka Samhita, Sushruta Samhita, Astanga Hridaya, and Sangraha are among the ancient texts that explain many formulations and their use against various ailments. The substantial hazardous side effects of western medications have caused modern society to focus increasingly on Ayurveda, Siddha, Unani preparations for alternative treatments (2). Ancient herbal preparations are those that are made in accordance with the instructions provided in the ancient texts of holistic healthcare systems as Charaka Samhita, Sushruta Samhita, Bhaishajyaratnavali etc. The producers of traditional herbal formulations standardise their products using the same procedure as laid out in the approved monographs. Contrarily, private or non-classical preparations are created using the maker's own formula, and frequently, the ingredients and additives used are not listed in the conventional literature. The majority of commercial herbal remedies are made up of an intricate blend of several substances (3). Botanical materials make up a sizeable component of the worldwide medicine business and are used in both developed and developing countries as over-the-counter medication products, pharmaceutical industry raw materials, and home remedies. Thus, it is crucial to create widely accepted standards for evaluating their quality. Although if some herbs have gained popularity throughout time, the wider populace, healthcare professionals, and the media still have limited knowledge about the safe and efficient use of herbal therapy. The risks of using some of these plants carelessly are becoming clearer. The reality is often obscured by media hype, incompletely understood research, and overblown claims. Given the widespread adoption of herbal remedies, it is now imperative that they be standardised.

Necessity of Standardization

Drug’s identities are confirmed and their quality and purity are determined through standardization. The majority of countries utilize standardized herbal formulations of plants called phyto-therapeutic agents to treat a variety of ailments. According to WHO, a herbal medication is one that contains plant components, whether they are in their raw or processed form, together with a few active ingredients, solvents, diluents, or preservatives. To ensure the validity of clinical studies and to provide positive therapeutic results, standardized herbal preparations of consistent quality and with clearly specified ingredients are needed. The phytochemical components found in a herbal composition determine its pharmacological characteristics. There are two kinds of standardization:

1. Genuine standardization: Identifies a specific phytochemical or collection of ingredients recognized to have action.
2. Pseudo standardization: This method relies on producers ensuring the presence of a particular proportion of marker chemicals, which are not signs of the herb's medicinal potency or purity (4).

As the risks and shortcomings of modern medicine become increasingly obvious, there is a global shift toward the use of medicines of natural origin. The regulatory agencies' primary duty is to make sure that patients receive medication that is guaranteed to be pure, safe, potent, and effective. UN recommendations the standardization of natural drugs is a vast and complex topic. The WHO's recommendations can be summed up as follows:

1. An allusion to the drug's name. Examination of the botany, sensory characteristics, foreign organic matter, microscopy, evaluation of the histology, evaluation of the histochemistry, quantitative measurements, etc.
2. Discusses the drug's physicochemical makeup. Physical and chemical identity, fingerprints left by the chromatography, ash and extractive values, moisture content, tests for volatile oils and alkaloids, quantitative estimation techniques, etc.
3. A mention of the biological activity profiles, bitterness values, haemolytic index, astringency, swelling factor, foaming index, etc.
4. Information on toxicity, including pesticide residues, heavy metals, total viable count of microorganisms, and pathogens including E. coli, Salmonella, P. aeruginosa, S. aureus, and Enterobacteria, among others.
5. Microbiological taint.
6. Radiation exposure (5).

Approaches to Standardization

Many scientific investigations on classical compositions are carried out to demonstrate their medicinal efficacy for various disorders as mentioned in the classics. To guarantee uniformity and safety of decoction efforts, this dose type's standardization, which is formed of a number of characteristics, was created. Three characteristics can be used to define the standardization efforts. • Methods for standardizing raw materials; • Methods for standardizing processes; • Methods for standardizing products/goods (6).

Standardization and quality control of herbal crude drugs – Processes and procedures : Standardization and quality control of herbs and plants, according to WHO (1996a and b, 1992), is the process involved in the physical and chemical evaluation of crude drugs covering aspects, such as selection and handling of the raw material, safety, effectiveness, and stability assessment of the finished product, documentation of safety and risk based on experience, provision of product information to the consumer, and product promotion. Typically, attention is given to quality indicators like:

1. Macro and microscopic analysis: To determine the proper variety and look for adulterants.
2. Elimination of foreign biological materials is necessary in order to obtain the medicine in its purest form.
3. Ash values: These standards are used to determine the identity and purity of unprocessed drugs. Ash in all forms, sulfated ash, and water-soluble ash
4. Moisture content: By examining the moisture content, inaccuracies in estimating the real weight of medicinal material can be minimized. Reduced dampness signifies higher product longevity against product deterioration.
5. Extractive values: These are approximate weights of the ingredients of crude drugs that can be extracted under various solvent environments.
6. Crude fiber: This is a measure for determining purity and helps to identify the woody material component.
7. Qualitative chemical assessment: This includes identifying and classifying crude drugs in terms of their phytochemical constituents. It uses various analytical methods to find and isolate the active ingredients. Identification of the botanical material, extraction using the appropriate solvents, purification, and characterization of the pharmaceutically significant active ingredients are all steps in preliminary phytochemical approaches.
8. Chromatographic analysis: Involve identifying raw drugs using their primary chemical components as markers.
9. Quantitative chemical analysis: To calculate the concentrations of the main ingredient classes.

10. Toxicological studies: These serve to identify pesticide residues, potentially toxic substances, safety tests on animals like the LD50, and microbial assays to assess whether potentially damaging bacteria are present or absent.

Analytical methods:

The requirement for adequate analytical procedures for establishing identification, quality, and comparative potency is essential to compliance with any monograph requirement. Many analytical techniques are available. Chromatography is one of the well-known analytical tools that is essential in the standardization of monographs, while it is frequently challenging to determine which is the most suited to utilize.

Chromatography:

The science of chromatography examines the separation of molecules based on differences in their content and/or structure. Generally speaking, chromatography entails transferring a test preparation of the elements to be segregated over a stationary support. Different interactions between the test preparation molecules and the stationary support will cause similar compounds to be separated. Test compounds with tighter contacts will often travel through the substrate more slowly than sample molecules with weaker interactions. By doing this, as molecules pass through the support material, they can be kept apart from one another.

Immobilized silica on glass plates (thin layer chromatography), highly sensitive HPTLC, volatile gases (gas chromatography), paper (paper chromatography), and liquids that may contain hydrophilic, insoluble molecules are just a few examples of the supports that can be used for chromatographic separations (liquid chromatography). For the examination of botanical materials' quality, high performance thin layer chromatography (HPTLC) is a useful quality assessment tool. It makes it possible to analyze many different substances quickly and affordably. Moreover, multiple samples can be run in a single analysis, significantly cutting down on the amount of time required for analysis. The same study can be viewed collectively with HPTLC in a variety of light wavelengths, offering a more comprehensive profile of the plant than is generally seen with more specialized sort of examination.

The equipment for UV-Visible measurements are simple to use, and the validation processes are easy to follow while yet being accurate. Although measurements can be performed quickly, sample preparation can be laborious and is only effective for simpler samples and substances having UVvisible absorbance. For the quantitative examination of increasingly complicated combinations, HPLC is the method of choice. Even though HPLC can be used to separate volatile substances like fatty and essential oils, GC or GCMS are preferable. Recent advancements in analytical instrumentation have made it simple to determine components quantitatively. Recent developments in the isolation, purification, and structure elucidation of naturally occurring metabolites have made it possible to develop suitable methods for assessing quality and standardizing herbal products. Chemotaxonomy is the classification of plants and other living things based on their chemical makeup.

The homogeneity of a plant extract can be assessed using LC, HPLC, GC, quantitative TLC (QTLC), and high performance TLC (HPTLC). OPLC, MS, GC, liquid chromatography (LC), nuclear magnetic resonance (NMR), and electrophoretic techniques, particularly by hyphenated chromatographic techniques, are powerful tools that are frequently used for standardization and to control the quality of both the raw material and the finished product.

These advanced procedures' results give a chemical fingerprint of the types of compounds or contaminants that are present in the plant or extract (WHO, 2002c). The chromatographic fingerprints of herbal medicines can be utilized to address the issue of quality control based on the idea of photo equivalence (7).

Modern Analytical Methods for Standardization of Classical Poly Herbal Formulations
Polyherbal preparations are those that contain two or more than two herbs. Ayurvedic medicine formulation is based on two principles: using more than one drug and using a single drug. Last is referred to as a polyherbal composition. Ayurvedic medicine has a unique concept known as polyherbalism, albeit it can be difficult to define in terms of contemporary standards. A polyherbal composition has been used for medical and therapeutic purposes all over the world. It is also known as herb-herb combination therapy or polyherbal therapy. A review focused on the breadth and prospects of polyherbal medications on a worldwide and local scale, as well as the significance of Ayurveda and polyherbal formulations.

The Sarangdhar Samhita, a work of Ayurveda literature, introduced the concept of polyherbalism to achieve better treatment effectiveness. A polyherbal composition has been used for medical and therapeutic purposes all over the world. It is also known as herb-herb combination treatment or polyherbal therapy. Single plants' active phytochemical components are insufficient to produce the desired therapeutic benefits. When numerous herbs are combined in a precise ratio for polyherbal and herbo-mineral formulations, the medicinal effect is boosted and the toxicity is reduced. The active ingredients taken from a single plant are insufficient to provide a therapeutic activity. There is evidence to suggest that crude plant extracts frequently have higher potencies than individual ingredients. Instead of isolated components, whole plants or combinations of plants are employed in herbal and folklore medicine.

Advantages of Poly herbal Formulation over single herb–

1. There is a long tradition of using herbal remedies, and patients are more accepting of them.
2. The only source of consistent deliveries of less expensive medications for the expanding population of the world is the medicinal herb.
3. There is no shortage of medicinal plants, especially in developing nations like India with its diverse agro-climate, cultural, and ethnic landscapes.
4. Traditional medicines and herbal remedies are grown and processed in an environmentally sustainable manner.
5. Prolonged and seemingly uneventful usage of herbal medications may attest to their effectiveness as well as safety.

Worldwide, herbal remedies have contributed many of the most potent medications to the extensive drug library at the disposal of modern medical science, both in their raw state and as pure components upon which medical treatment is based.

7. They have no adverse side effects and do not cause allergic reactions.
8. They blend in seamlessly with skin and hair.
9. They are much more effective in little amounts than synthetic cosmetics.
10. Plant extract from different sources delivers suitable therapeutic action and reduces the bulk property of cosmetics products.
11. There are many different and many options that are readily available.
12. Low cost and simple to make.

Limitations of Poly herbal Formulation-

Combinations of plants containing these elements may exhibit more activity than separate extracts when combined. Yet, the existence of numerous ingredients may also cause chemical incompatibility,

which could cause instability. Although the Medicines and Cosmetic Act was established to regulate the production and quality assurance, the manufacturing of Ayurveda herbal preparations is subject to rather less rigorous regulations in India, where the majority of ayurvedic or indigenous PHFs are developed and exported. Toxicology studies and clinical trials on herbal formulations are not required, per acceptable clinical standards, for the filing of patents and granting of commercial production licenses to the maker of Ayurveda herbal formulation or preparations.

Standardization of classical polyherbal formulation by using modern analytical techniques

Sitopaladi Churna

Vishvnath. G *et al*.,2011 explained- A simple and reproducible UV- spectrophotometric method for the quantitative determination of piperine in *Sitopaladi churna* (STPLC) were developed and validated in the present work. The parameters linearity, precision, accuracy, and standard error were studies according to Indian Herbal Pharmacopeia. In this present study a new, simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of piperine in market and laboratory herbal formulation of *Sitopaladi churna*, which were procured and purchased respectively from the local market and they were evaluated as per Indian Herbal Pharmacopoeia and WHO guidelines. The concentration of piperine present in raw material of PSC was found to be 1.45±0.014 w/w in piper longum fruits. Piperine has the maximum wavelength at 342.5 nm and hence the UV spectrophotometric method was performed at 342.5 nm. The samples were prepared in methanol and methods obeys Beers law in concentration ranges employed for evaluation. The content of piperine in ayurvedic formulation was determined. The result of analysis has been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of piperine in crude drug and its herbal formulation (9).

Nellikai Lehyam

Sudha. V *et al*.,2014 explained- Plants play a major role in all alternate systems of medicines like Siddha, Ayurveda, Unani and Chinese. In the recent years there has been considerable researches going on traditional siddha medicines focusing towards validation and standardization aspects. In the present work, an immuno-modulatory drug-*Nellikai lehyam* was subjected to physicochemical standardization and in vitro cytotoxic studies. A preliminary phytochemical screening was carried out to identify the nature of phytoconstituents present. The yield of tannins, flavonoids, phenols, vitamin C, total fatty matter, were estimated as per standard procedures. Percentage of iron was also estimated and was found to be 77.49 %. Finger printing profile of flavonoids in *Nellikai lehyam* was carried out by HPTLC technique using toluene: ethyl acetate: formic acid (5:4:1 v/v/v) as mobile phase. The bio active constituents in lehyam were also identified through GCMS and LC-MS analysis. In vitro antioxidant activity was carried out using DPPH and reducing assay. This drug possesses strong antioxidant activity which was exposed by its ability to scavenge the stable free radical DPPH. The cytotoxic activity was tested against cells using MTT assay which revealed strong activity. This is the first report on chemical standardization, antioxidant and anticancer activity of this unique siddha formulation *Nellikai lehyam* (10).

Katakakhadiradi Kashayam

Jessica.A *et al*.,2016 explained- *Katakakhadiradi kashayam* is an ayurvedic formulation for the treatment of diabetes, skin and urinary tract ailments. This is made up of 12 types of plants. The present study deals with the GCMS analysis of this kashayam to know the presence of different bio molecules present in it. It was observed that it contained molecules like Ethyl acetate, 3Trifluoroacetoxytridecane, 3- Trifluoroacetoxypentadecane, Dodecane, 1,2-dibromo-, 4Trifluoroacetoxytridecane, Trichloroacetic acid pentadecyl ester, Trichloroacetic acid hexadecyl ester, E-14-Hexadecenal, E-11,13-Tetradecadien-1-ol 6- and Tridecene, (Z)- which have various

medicinal activities. Further work is in progress to evaluate these molecules and correlate their activities to *Katakakhadiradi kashayam*. This study will help in better understanding of the mechanism of action of this medicine (11).

Kesari Lehyam

Jeganathan N.S. *et al*.,2016 explained- Any medicine to have international acceptance should have unequivocal proof of its safety, efficacy and quality. In many cases the efficacy and quality are interrelated. Hence, utmost importance needs to be given to prove the quality of herbal drugs. The proposed work involves the development and validation of the selected siddha formulation – *Kesari lehyam* (KL) by determining various physicochemical & phytochemical parameters. The individual drugs were subjected to organoleptical/morphological screening in order to ascertain their authenticity. KL was subjected to systematic phytochemical screening in order to ascertain various phytochemical constituents present in KL formulation. HPTLC fingerprint profiles for the extracts were established and co-chromatography was carried out by using marker compound, piperine. For establishing fingerprint profiles, the methanolic and ethyl acetate extracts of standard formulation and commercial formulation of *Kesari lehyam* (KL) were resolved by using the selected solvent system. The finger print profile of the chromatogram of various extracts can be considered as a reference to characterize the presence of other constituents in the formulation of KL.

The research findings will help to provide quality product to the consumers and to expand the market for the herbal products to international level and thereby we can popularise our indigenous medicine globally (12).

Balaguloochyadi Kashayam

Khan. A.T *et al*.,2016 explained- The present study reports the physicochemical, microbial and analytical (marker based) standardization of an Ayurvedic marketed formulation- *Balaguloochyadi kashayam* (Rajah Ayurveda). Physicochemical standardization was performed by determining organoleptic properties, pH, specific gravity and total solid content. Total bacterial count and total fungal count studies were performed for microbial standardization. Marker based standardization involved development and validation of a High-Performance Thin Layer Chromatography (HPTLC) method for quantitative determination of a chemical marker viz. ephedrine present in Bala, major herb of *Balaguloochyadi kashayam* as per the standard protocols. The physicochemical and microbial tests were performed in triplicate and average results were calculated. A new, accurate, precise and robust HPTLC method for estimation of ephedrine in *Balaguloochyadi kashayam* was developed on Camag Linomat-5 using silica gel 60 GF254 as stationary phase and toluene: chloroform: n-propanol: ethanol: formic acid (6: 3: 1: 2: 1, v/v/v/v/v) as mobile phase. The validation studies were performed as per International Conference on Harmonization- Quality (ICH-Q2 (R1)) guidelines. The results of these experiments can be used by manufacturers as a reference for controlling the quality of *Balaguloochyadi kashayam* formulations prior to release for marketing (13).

Thulasi Ennai

Rani S.Y *et al*.,2018 explained- The present study deals with the Gas Chromatography Mass Spectrometry (GCMS) analysis of *Thulasi ennai* (THULASI OIL - TO), a polyherbal siddha formulation. *Thulasi ennai* said to cure soolikanam which is mentioned in classical siddha textbook balavagadam. The symptoms of soolikanam are continuous cough, wheezing, tightness of chest, shortness of breath, abdominal bloating, loss of appetite. These symptoms are correlated with childhood asthma. Though the individual herbs used in this formulation have the previous record of standardization, there is no evidence of the molecules present in the physical form of the study drug TO and hence this study was aimed. TO is an oil form of medicine (Ennai) and it is a combination of 11 types of different drugs. All the ingredients were produced from reputed raw drug store and botanically authenticated by the medicinal botanist of National Institute of Siddha, Chennai.

Purification was done individually as per the siddha classical literature and the formation was prepared as per the procedure mentioned in sastric siddha text Balavagadam. The prepared drug was subjected to analysis. The derived GCMS analysis result was indicated that the presence of eighteen bio molecules in the formulation (14).

Pathyashadangam Kwath

Abraham. A *et al*.,2018 explained- *Pathyashadangam kwath*, a classical ayurvedic polyherbal formulation is used for the treatment of cluster head ache, migraine, upper respiratory diseases, ear ache and night blindness. Review of literature suggested that characterization parameters of *Pathyashadangam kwath* are not reported. To report characteristic parameters of *Pathyashadangam kwath* to confirm quality and purity. The fruit pericarps of Haritaki, Bibhitaki and Amalaki, aerial parts of Bhunimba, rhizome of Haridra, stem bark of Nimba and stem of Guduchi were the ingredients of *Pathyashadangam kwath*. Three batches of the kwath were prepared as per standard procedures. The kwath was evaluated for organoleptic, physical, phytochemical and chromatographic parameters as per standard methods. HPTLC analysis revealed that Toluene: Ethyl Acetate: Formic acid (2.5: 2.0: 0.5) was a suitable mobile phase for characterization of the kwath. HPLC analysis revealed that andrographolide was a suitable marker for standardization of the kwath. The characterization parameters presented in this paper may serve as standard reference for quality control analysis of *Pathyashadangam kwath* (15).

Panchabakiya Choornam

Indumathy. V *et al*.,2019 explained- Siddha system of medicine is a traditional medicinal system being followed mainly in South India. It is better known as one among the AYUSH medicinal system. The present study aims to standardize the siddha polyherbal formulation *Panchabakiya chooranam* (PBC), which is mentioned in the siddha classical text Anubogavaidya Brahma Rahasyam indicated for stomach burn, burning sensation in anus and diarrhea. Accordingly, PBC was studied through Inductively Coupled Plasma Optical Emission Spectrometric analysis (ICP-OES) and Fourier Transform Infrared Spectroscopy (FTIR) techniques to identify the metals and functional groups present in it. Results showed that the sample contains elements such as calcium, iron, sodium, potassium, and phosphorous. Among the detected elements phosphorus was present in a maximum concentration, and iron was in lowest concentration. Moreover, the toxic metals namely mercury, arsenic, cadmium, nickel, and lead were in below detectable level, which ensures that the drug is safe to consume. Besides, functional groups such as alcohols, alkanes, amides are identified using IR spectrum (16).

Sirinigipaerathi Choornam

Vijaya. R N *et al*.,2019 explained- The aim of the study is to standardize the siddha poly herbal drug "*Siringipaerathi chooranam*" through the scientific method FTIR. Siddha system of medicines plays an important role in treating acute and chronic diseases through the herbal preparation. *Siringipaerathi chooranam* is the herbal formulation which has been indicated for jaundice in siddha classical literature. Thus, this trail drug was standardized through FTIR and the results were noted. The instrumental analysis of *Siringipaerathi chooranam* through FTIR shows the presence of alcohol, amine, alkane, acid, alkene, aromatic, alkyl halide, nitro groups, ether, esters. The functional group analysis of siddha drug *Siringipaerathi chooranam* will provide the good information for the biological activity (17).

Drakshadi Kashayam

Narayanan. G *et al*.,2019 explained- The present work deals with the gas chromatography (GC) mass spectrometry (MS) analysis of *Drakshadi kashayam*, which is used in the treatment of panduroga (anemia), jaundice, and diseases caused due to imbalance in pitta (bile) consistency. *Drakshadi kashayam was* bought from a standard ayurvedic vendor at Chennai and subjected to GC

MS analysis by standard procedures. The medicinal roles of the biomolecules indicated in the GCMS profile were screened for their various medicinal roles using Dr. Duke's phytochemical and ethnobotanical data and other data. GCMS profile of *Drakshadi kashayam* indicated the presence of important biomolecules such as carbonic acid, pentyl phenyl ester, bisphenol C, histamine, N-benzoyl-2-cyano-, o-Methoxy-. alpha. -phenethylamine, benzenethanamine, 3,4dimethoxy-. alpha. -methyl-, cis-Z-. alpha. -Bisabolene epoxide, and 1,3-Dimethoxy-5-(1-methylheptyl)-benzene which have medicinal roles supporting the efficacy of *Drakshadi kashayam* as a liver tonic. It is concluded that *Drakshadi kashayam*, which is an important ayurvedic medicine, does contain some very important molecules showing its efficacy. Further research is required for a better understanding of the medicinal roles of this medicine (18).

Mahamanjishtathi Kashayam

Nandhini. E et al.,2019 explained- Standardization of siddha formulaion drugs is very essential to order to justify their acceptability in the modern system of medicine. A siddha herbal drug "*Mahamanjishtathi kashayam*" from the siddha text have vital importance in standardization which will encompass the entire field of study from the cultivation of medicinal plants to its clinical application. Here in standardization parameters like organoleptic characters, physicochemical analysis, heavy metal analysis, TLC and HPTLC analysis, phytochemical analysis and sterility test are carried out as per AYUSH guidelines. The outcome of this study clearly proves the quality, purity, safety and potency of the drug which will help the medicine to survive and succeed in future researches on both clinically and economically (19).

Habb-e-Sara Khas

Sagar P.K *et al*.,2020 explained- Standardization is used to describe all measures under taken during the manufacturing process and quality control of drug assuring its reproducible quality. Most of the traditional medicine are effective but still they lack in its standard parameters. Therefore, we need to develop standard techniques to standardize and validate herbal formulations. The drug *Habb-eSara Khas* is therapeutically useful in the treatment of Sara Khas (Epilepsy and Infantile epilepsy). The drug *Habb-e-Sara Khas* was prepared in three different batches as per the guidelines of National Formulary of Unani Medicine (Part-VI), present study is aimed to evaluate the pharmacopoeial standards using physico-chemical parameters; HPTLC fingerprints, quality control and assurance parameters, using WHO guideline to ascertain the quality of drug. The physico-chemical data showed that the drug contain moisture (1.89%), total ash (5.43%), acid insoluble (1.06%), alcohol and water-soluble extractive matter (19.39%) and (60.34%), pH (1% solution) (5.93), pH (10% solution) (5.67), ASSE (18.07%) and CSSE (17.43%), bulk density of granules (0.4989) and the TLC/HPTLC finger prints showed various spots at 254nm, 366nm and visible light (V-S reagent). The quality control study revealed the absence of microbial load, aflatoxins, heavy metals and pesticide residues, The evaluated standards will very much useful for laying the pharmacopeial standards of *Habb-e-Sara Khas* and also in providing the quality medicine to needful human beings (20).

Qurs-e-Safa

Beg.B.M *et al*.,2021 explained- The unani system of medicine has been practised since centuries for the treatment of a range of diseases. In spite of their efficacy, they have been widely criticised due to the lack of standardization and poor-quality control. Standardization of unani medicine is a valuable issue at the present because they are very prone to contamination, deterioration, adulteration and variation in composition due to biodiversity as well as careless collection. To standardize and development of HPTLC fingerprinting of a polyherbal unani formulation *Qurs-e-Safa*. The conventional and modern analytical techniques were used to standardise *Qurs-e-Safa*. The study was carried into three different batches of *Qurs-e-Safa* prepared with its ingredients. The parameters studied are organoleptic, microscopic, physicochemical parameters, phytochemical screening, TLC, HPTLC profile, aflatoxin, microbial load and heavy metal analysis. *Qurṣ-e-Safa* is dark yellow in

colour and aromatic smell. Uniformity of diameter and weight variation were found to be 13 ± 0, and 524.7 ± 1.72 mg. friability, hardness and disintegration time of all 3 batches were found to be (0.0615 ± 0.004, 0.0885 ± 0.0047 and 0.0725 ± 0.0058), (3.5 ± 0.2886, 3.67 ± 0.1674 and 3.67 ± 0.1674) and (16 to 17 minutes). Extractive value was found to be maximum in distilled water (38.488 ± 0.20, 37.3824 ± 0.38 and 39.8177 ± 0.13) followed by alcohol (27.5406 ± 0.54, 27.5656 ± 0.32 and 26.9229 ± 0.25). Loss of weight on drying, pH, total ash, acid insoluble ash, qualitative test was set in. Phytochemical screening revealed the presence of carbohydrates, phenols, resins, proteins, steroids, fixed oil and flavonoids. The microbial load was found absent and heavy metals were with in permissible limits. The data evolved from the study may serve as a reference to validate and also help in the quality control of other finished products in future research (21).

Guduchi Satva

Katara. A *et al*.,2021 explained- *Tinospora cordifolia* stems (*T. cordifolia*), commonly known as Guduchi, is an effective ayurveda drug used for diabetes management and various other disorders and ailments. *Guduchi satva* is classical ayurvedic medicine being used for the treatment of diabetes and a variety of other disorders. The present study was conceded to determine the aqueous extract of *T. cordifolia* and *Guduchi satva* formulation for their relative identification of secondary metabolites using physicochemical parameters, Fourier-Transform infrared spectroscopy (FTIR) fingerprint, and GCMS profiling. In vitro enzyme inhibition assay on α amylase and α glucosidase were also evaluated for both the extracts, in which *Guduchi satva* possess enhanced inhibition of enzymatic activity over the aqueous extract of *T. cordifolia*. The phytochemical and FTIR investigations were confirmed for the presence of alkaloids, tannins, carbohydrates, flavonoids, steroids, aromatic, hydroxy, and nitrogen-containing compounds. The gas chromatography mass spectrometry (GCMS) analysis revealed the existence of stigmasterol (1.2%), stigmastane3, 6 dione, -5a (2.2%), betulin (8.4%), eicosanoic acid (1.09%), glycerol 1 palmitate (20.72%), ascorbyl palmitate (0.92%) and triamcinolone acetonide (0.89%) in both the extracts and effective in diabetes management (22).

Naaga Chendooram

Janani. S *et al*.,2022 explained- The current study is pointed at the characterization of the physicochemical analysis of the traditional indian siddha medicine, *Naaga chendooram* (NAC). It is a metal-based medicine which cures especially breast cancer. A literature survey revealed that a scientific study was lacking on this drug. *Naaga chendhooram* was made as per the Veeramamunivarsiddhar method. The ingredients were zinc, potassium nitrate, *Zingiber officinale*, *Curcuma longa* and *Carum copticum* and *Aspera bidentate*. The prepared drug was analysed in Fourier Transform Infrared (FTIR) for its chemical properties which help in the identification and localisation of chemical species, scanning electron microscopy (SEM). FTIR spectroscopy has been used to study the presence of organic substances, physiochemical, organoleptic and biochemical were analysed. Loss on drying at 105 °C 1.053 ± 0.1747%, total ash 91.6 ± 3.7360%, acid insoluble ash 0±0%, water soluble extractive 6.367 ± 2.779%, alcohol soluble extractive 1.46± 0.73%., pH 7.5. It has the acid radical-carbonate and sulphates and basic radical- mercury and in the instrumental analysis, it has arsenic group. Findings divulge that samples need more studies to standardize the drug and to determine the importance of the siddha drug preparation technique, which may disclose the scope for chemical modulation by traditional methods in succeeding years (23).

Thirinethira choornam

Vajahath A.A et al.,2022 explained- *Thirinethira chooranam* is a multi-herbal formulation indicated as hematinic and appetizer in siddha literature. The aim of this study is to standardize *Thirinethira chooranam* to ensure its quality, purity and safety through its physicochemical, microbiological, chromatographic, biochemical parameters and analysis of heavy metals, pesticide residue and aflatoxins. This study determines the physicochemical parameters that are loss on drying, total ash,

acid insoluble ash, water soluble extractive, alcohol soluble extractive and pH as 2.187%, 6.9 %, 0.18%, 14.17%, 4.567% and 5.5 respectively denotes purity and quality of drug. From the study results it is ensured that the test drug *Thirinethira chooranam* is free from microbial contamination, heavy metal traces, Pesticide residue toxicity and aflatoxin toxicity. Qualitative preliminary phyto-chemical analysis reveals that presence of major phytochemicals alkaloids, flavonoids, steroids, triterpenoids, coumarins, phenol, tannin, sugar and betacyanin. High performance thin layer chromatography finger printing analysis of the test drug reveals the presence of seven eminent modes relevant to presence of seven different phytocomponents present with in it. Rf value of the modes ranges from 0.01 to 085. Bio-chemical analysis reports the presence of carbonate, sulphides, phosphate, ferrous and magnesium. The obtained results of all the analyses of this study provide data on pharmacognostic (physical, chemical and biochemical) properties of siddha polyherbal formulation *Thirinethira chooranam*. In future these data can be utilized as references for the standardization of the drug *Thirinethira chooranam* (24).

Conclusion

It can be concluded that the standardization in Ayurveda, Sidha, Unani is an ongoing process where one needs to be strictly vigilant about the new scientific methods to study the fine alchemical procedures and the intermediate compounds formed, but at the same time be aware of the classical concepts of the procedure as well as the drug (25).

Traditional methods of standardization are found to be insufficient to validate these formulations, hence, modern advanced techniques play vital role. Indian ayurvedic formulations could be well accepted by all developed nations world-wide if these are manufactured using standard procedures and standardized using sophisticated modern analytical techniques. Fingerprint profile obtained by various chromatographic techniques play an important role for the standardization of ayurvedic formulations. It is essential to develop advanced hyphenated techniques to serve as rapid and specific tools for herbal drug standardization. The combination of qualitative fingerprint and quantitative multicomponent analysis act as a novel and rational method in the quality control of Ayurvedic formulations.

The standardization protocols using hyphenated techniques such as GCMS, LC-MS, LC-NMR, could be developed and employed for evaluation of Polyherbal formulations where miniscule amount of marker compounds is available. Modern analytical methods of standardization are yet to be developed for most of the other Ayurvedic, Siddha and Unani formulations (26).

It can be concluded from the review that standardization in ayurveda is an ongoing process where one needs to be strictly vigilant about the new scientific methods to study the fine alchemical procedures and the intermediate compounds formed, but at the same time be aware of the classical concepts of the procedure as well as the drug.

References

1. Mukhi Sangeeta, Bose. A, Ray. A, Swain P.K. Analytical standards of *Amrtadi churna*: A classical ayurvedic formulation. Indian J Pharm Sci. 2017;79(2);227-240.
2. Das .C, Ghosh. G, Bose.A and Das.D. Analytical methods for standardization of ayurvedic Asavas and Aristas; A review. Indian J Pharm Sci.2019;81(3);396-405.
3. Silpavathi Lingarkar, Das.M.K and Das.D. Challenges and Prospective Solutions in Nonclassical Herbal Formulations: A Review. Indian J Pharm Sci . 2019;81(6);981-987.
4. Nhawkar Smita V.*, Mullani Ashish K., Magdum Chandrakant S. and D'Souza John I. Quality standardization and toxicity study of ayurvedic formulation. International Journal of Bioassays. 2014; 3(09);3244-3253.
5. Bhusnure Omprakash G., Suryawanshi Shivraj, Vijayendra Swamy S.M., Gholve Sachin B., Girm padmaja S., Birajdar Mahesh J. Standardization and quality evaluation of herbal drug. Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):1058-1063.

6. Bhatt Narendra, Deshpande Manasi, Chaskar Sunetra, A critical review of standardization of ayurvedic dosage form kwatha - Part - II: Approaches and Outcome. International Journal of Ayurvedic Medicine.2020;11 (3);378-386.
7. Kunle, Folashade Oluyemisi , Egharevba, Omoregie Henry and Ahmadu, Ochogu Peter, Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation.2012;4(3);101-112.
8. Kaderkar Dattatray Shrikar , Tamboli Ashpak.M, Review on Concept and Scope of Polyherbal Formulations. International journal of innovative research in Technology. 2020;7(1);282-288.
9. Vishvnath Gupta and Jain U. K, Quantitative analysis of piperine in ayurvedic formulation by UV Spectrophotometry. International Journal of Pharma Sciences and Research (IJPSR).2011;2(2);58-61.
10. Sudha. V, Niraimathi K.L, Lavanya.R, Raj David. C and Brindha. P, Chemical standardization and in vitro cytotoxic studies on *Nellikai lehyam*. Asian Journal of Chemistry.2014; 26(12);3679-3682.
11. Jessica Angelina, Rao M.R.K., Anthony Jacintha, K Prabhu, , WMS Johnson , B. Shanthi Balasubramanian, Lakshmi Sundaram, Shruthi Dinakar, The GCMS study of one ayurvedic preparation *Katakakhadiradi kashayam*. International Journal of Pharmaceutical Sciences Review and Research. 2016; 39(2); 216-224.
12. Jeganathan N.S., Anbazhagan. S, Aruna. P,Deepa. D and Kalaivani. A, Preparation and quality control of a siddha formulation –*Kesari lehyam*. International Journal of Advanced Research in Biological Sciences. 2016; 3(11);180-185.
13. Khan Asif Tabassum, Mallaya Rasmi, Gohel Anjali, Standardization of marketed Ayurvedic formulation, *Balaguloochyadi kashayam*- physicochemical, microbial evaluation and ephedrine content. Journal of Applied Pharmaceutical Science.2016; 6 (12);184-189.
14. Rani S.Yavana, Mirunaleni.P, Sathiyabama.M , Suresh. K, M. Sundaram Meenakshi, Banumathi. V, GCMS Analysis of *Thulasi ennai* – A siddha polyherbal formulation. International Journal of Advanced Multidisciplinary Research.2018;5(7);65-69
15. Abraham Aji, Samuel Sarala, Mathew Lizzy, Phytochemical analysis of *Pathyashadangam kwath* and its standardization by HPLC and HPTLC. Journal of Ayurveda and Integrative Medicine xxx (2018). 1-6.
16. Indumathy. V, Anbarasan. B, Anbu. N and Kanakavalli. K, Standardization of siddha polyherbal formulation *Panchabakiya chooranam* Through ICPOES and FTIR techniques. International Journal of Pharmaceutical Sciences and Research.2019;10(2);783-785.
17. Vijaya.R Nirmala, Abinaya.R, Punithavalli. V, Velpandian. V, Standardization of siddha poly herbal formulation "*Siringipaerathi chooranam*" through a scientific method – FTIR (Fourier Transform Infrared Spectroscopy). International Journal of Current Research in Chemistry And Pharmaceutical Sciences. (2019);6(2);24-37.
18. Narayanan. G, Prabhu. K, Rao Mudiganti Ram Krishna, Kannan Kamala, R. Lakshmi Sundaram, Dinakar Shruthi, N. Vijayalakshmi, Gas chromatography mass spectrometry analysis of one ayurvedic medicine, *Drakshadi kashayam.* Drug Invention Today. 2019;11(10);2652-2655.
19. Dr Nandhini. E, Mohamed Musthafa M. and Nivetha. G, Standardization of siddha herbal drug – "*Mahamanjishtathi kashayam*. World journal of pharmaceutical and medical research. 2019;5(4);120-127.
20. Sagar Pawan Kumar, Murugeswaran. R, Meena Rampratap, Mageswari. S and Devi Sri P.Meera, Khair Shahidul, Standardization and HPTLC. Fingerprinting study of polyherbal unani formulation - *Habb-e-Sara Khas*. International Journal of Traditional and Complementary Medicine. 2020; 5:21.
21. Beg Belal Mirza, ViquarUzma, Mohammad Abdul Rasheed Naikodi, Habiba Suhail, Munawwar Husain Kazmi, Standardization and HPTLC fingerprinting of a polyherbal unani formulation. CellMed.2021;11(1);1-8.

22. Katara Antariksh, Garg Naveen Kumar, Mathur Mukul, Separation and identification of antidiabetic compounds in *Tinospora cordifolia* extract and ayurvedic formulation *Guduchi satva* by GCMS and FTIR study with subsequent evaluation of in-vitro hypoglycemic potential. International Journal of Pharmaceutical Sciences and Drug Research. 2021;13(2);183-189.
23. Janani. S, Megala. K, Saravana Devi M.D., Velpandian. V, Physiochemical and sophisticated instrumental analysis-FTIR and SEM of herbomineral formulation-*Naaga chendooram* through plim guidelines. International Journal of Pharmaceutics and Drug Analysis. 2022;10(2);46-53.
24. Vajahath A Ali, Gobindaraj. B, Saravanadevi M.D., Velpandian V, Standardization of siddha Polyherbal formulation *ThirinethiraChooranam* by modern pharmaceutical analytical techniques. International Journal of Health Sciences and Research. 2022;12(5);187-199.
25. Guleria Praveen, Chandla Anubha, standardisation in ayurveda - ancient vis-a-vis modern perspective. international ayurvedic medical journal. 2017; 5(7) 2668-2680.
26. Das .C, Ghosh. G, Bose.A and Das.D. Analytical methods for standardization of ayurvedic Asavas and Aristas; A Review. Indian J Pharm Sci.2019;81(3):396-405.