Section A-Research paper

PREPARATION AND STANDARDIZATION METHODS OF HERBAL DRUG FORMULATIONS

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Abstract

Today, the utilization of therapeutic plant items in the healthcare system is given increased priority. It satisfies the need for alternative treatment throughout the globe, causing established medical systems to gain popularity. It requires the proper blending of ancient knowledge with cutting-edge scientific methods. Through stability testing investigations, which take into account a number of variables like temperature, humidity, light, oxygen, moisture, other ingredients, microbiological contamination, trace metal contamination, leaching from the container, etc., the quality of herbal products is examined. Because of this, stability studies often comprise a variety

of evaluations, including chemical, physical, microbiological, medicinal, and toxicological research. Herbal medication standardization entails verification of their identification, quality, and purity. The current research involves an analysis of numerous standardization factors and how well they work with high-quality herbal medicines. The current paper also provides an overview of some well-designed procedures, techniques, and tools for the standardization of herbal raw materials and herbal formulations, including chromatography and spectroscopic technology.

Introduction

Herbs having fragrant qualities are often used for flavoring, cuisine, and for medical reasons. However, this excludes plants and vegetables that are high in macronutrients. Typically, we distinguish between herbs and spices when discussing them in relation to cooking. In contrast to spices, which are often dried and are derived from other plant parts including fruits, seeds, roots, and bark, herbs refer to either dried or fresh plant's green leaves or blooming regions. According to Royl Horticulture, "herbaceous plant" is another way to characterize a herb. Herbaceous plants are known for being tiny, having seed without a woody stem, and having their aerial portions (those that are above ground) die back to the ground at the conclusion of each growing season. Herbaceous plants may be annuals, biennials (royl horticulture), or perennials that live for many years before reproducing from seed the next year[1].

n the present era, market of all commodities has become global. Health has been of utmost importance since ancient times for the mankind. Market of health-related products has been active and these products are manufactured at different parts of the world and sold all over. Standardization is necessary to make sure the availability of a uniform product in all parts of the world. Standardization assures a consistently stronger product with guaranteed constituents.

WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programs, in assessing safety and efficacy, in ensuring adequate supplies, and in the quality control of raw and processed materials.[2] Herbal formulations in general can be standardized schematically as to formulate the medicament using raw materials collected from different localities and a comparative chemical efficacy of different batches of formulation is to be observed. A preparation with better clinical efficacy has to be selected. The routine physical, chemical, and pharmacological parameters are to be checked for all the batches to select the final finished product and to validate the whole manufacturing process.

In India, diabetes is a serious disease due to irrational food habits. Most of the hypoglycemic agents and hypolipidemics used in allopathic practice to treat diabetes mellitus and hyperlipidemia are reported to have side effects in long term use. Hence, there is the need to search for effective and safe drugs for these ailments. Pharmaceutical research across the world shows that natural products are potential sources of novel molecules for drug development.[3]

Based on the above rationale the present study was undertaken with an aim to standardize some herbal antidiabetic drugs based on their physicochemical characteristics and compare them with marketed formulations and in-house developed formulations. The present paper reports the

standardization of herbal antidiabetic drugs based on organoleptic characters, physical characteristics, and physicochemical properties.

Need of Standardization

In the past, vaidyas used to treat each patient individually and manufacture medications based on their needs. The quality control issue has been taken into consideration in practically every traditional medical system from its examination of its Rishis, Vaidyas, and Hakims. In contrast to earlier times when traditional healers produced and evaluated the efficacy of herbal remedies, today's issues are economic ones related to industrial scale manufacturing, shelf life, and long-distance distribution. These have made the creation of contemporary, impartial criteria for assessing the effectiveness, quality, and safety of these medications necessary. The strength and negative effects are also becoming more and more well known. Researchers, producers, and regulatory organizations must use exacting scientific procedures to assure the quality and consistency of traditional herbal medicines from lot to lot in order to win the public's confidence and integrate them into the mainstream of today's health care system.

Here is a summary of quality assurance and standards for herbal products:

1. Standardization concepts and technology were quite different when traditional medicines were being produced.
2. The identity of plant material may have altered during the last 1,000 years due to a dynamic process of evolution.
3. The availability of true raw materials has become difficult due to commercialization.
4. Time and environmental variables may have changed the properties of botanicals. The identity of the plants, seasonal variation (which affects the time of collection), ecotypic, genotypic, and chemotypic variations, drying and storage conditions, and the presence of xenobiotics are among the important factors that can cause significant variation in the herbal raw material.

Significant variation in product quality and plant chemical concentrations can be caused by environmental factors like sunlight, rain, altitude, temperature, soil, storage conditions, different harvesting techniques, timing, and method of collection, manufacturing processes like selecting, drying, purifying, and extracting, and genetic variability. Secondary metabolites and, in turn, the chemical makeup of the plant may be affected by ecological factors including insect feeding and microbial diseases. Additionally, the chemical elements in the roots, stem, and leaves of the same plant are concentrated differently. Likewise, seasonal shifts and diurnal variations (such as paclitaxel and opium alkaloids) can contribute to variance in herbal remedies. Depending on the plant portion employed and the state of maturity, a plant's medicinal or poisonous components change [4]. Products from various manufacturers vary greatly, and it is impossible to monitor every aspect that affects the chemical makeup of plants [5, 6] because of complexity and inherent. Modern analytical techniques are anticipated to aid in resolving this issue. Due to diversity of the components of plant-based medications, it is challenging to set quality control parameters. Furthermore, it is usually unclear or only partially understood what components are behind the purported therapeutic benefits. The majority of herbal preparations, particularly the older formulas of traditional medicine, include many herbs. There are a lot of liquid or semisolid preparations. It is quite challenging to set up criteria for quality control for such compositions.

Not even formal standards are offered. The distinct manufacturing processes used to create these medications transform the individual medications into very complex mixtures from which it is exceedingly difficult to separate, recognize, and analyze the constituent parts.

The standardization of herbal products can be broken down into two categories: an active constituent's extract, where known biochemical principles have therapeutic benefits, and a marker extract, where the active principle is unknown and a distinguishing compound is used as a marker to determine the presence of other therapeutic biochemical compounds [7]. Because only isolated components are taken into account and the herb's whole contents, which may have synergistic or buffering properties to lessen the negative effects, are ignored, standardization has limits.

Overview of the conventional medical system

Ayurveda, which translates to "science of life," is a 1500-year-old medical practice that has its roots in India. In the first millennium BC, the Charak and Sushruta Samhitas described over 700 different plants. The earliest written records of herbal medicine date back to 2600 BC and show that Mesopotamia had a working medical system that used over 1000 plant-derived products [3]. Egyptian medicine began to emerge about 2900 BC, but the "Ebers Papyrus" (1550 BC), which contains over 700 medications, provides the most helpful record. Traditional Chinese Medicine has also been well documented throughout the years [8]. Ancient Greek literature serves as the foundation for our understanding of the medical uses of herbal plants. A few notable compendia were written in the first century AD by the Greek physician, Pliny the Elder, a Roman, and Galen, a Roman in the second century AD. The Arabs kept Greco-Roman knowledge between the fifth and the twelfth centuries, fusing it with customary Chinese and Indian plants according to their medical expertise[9]. Herbal plants were utilized at this time without precise understanding of their active ingredient or pharmacological action, or one might argue that the usage of the plants was based only on observation. Investigation of toxic plants like aconite, colchicum, and others throughout the 18th century led to the logical development of curative herbal plants. In the 21st century, more than 50% of professionally prescribed medications are herbal. The government is now making every effort to increase public awareness of the ancient medical system known as AYUSH (Ayurveda, Yoga, Unani, Siddha, and Homeopathy). As suggested by the phrase "traditional" use of herbal treatments, many of the products sold as "traditional herbal medicines" have a lengthy history of use. In many poor countries, a significant portion of the population relies on traditional healers and their arsenal of medicinal plants to meet their healthcare needs. Due to historical and cultural causes, the use of herbal treatments often continues to be widespread even when modern medicine coexists with such antiquated traditions. Commercially speaking, these products are now easier to get, especially in developed countries[10]. Sometimes in the current setting, drugs are advocated for uses that were never considered in the traditional therapeutic systems from which they originally came. One example is the use of ephedra (also known as Ma huang) to reduce weight or enhance athletic performance. The manufacturing process is rigid.

In recent years, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics. There are around 6000 herbal manufacturers in India. More than 4000 units are produced Ayurveda medicines. Due to lack of infrastructures, skilled manpower reliable methods,

and stringent regulatory laws, most of these manufacturers produce their product on the very tentative basis.

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs (3). "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations (4). "Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration[11].

PHYSICAL EVALUATION

1. Organoleptic Evaluation:

Organoleptic evaluation refers to evaluation of individual drugs and formulations by color, odor, taste, texture, etc. The organoleptic characters of the samples were carried out based on the method as described by Wallis. For determining the odor of an innocuous material, small portion of the sample was placed in the beaker of suitable size, and examined by slow and repeated inhalation of the air over the material. If no distinct odor was perceptible, the sample was crushed between the thumb and index finger, between the palms of the hands, using gentle pressure or if the material was known to be dangerous, by other suitable means such as pouring a small quantity of boiling water onto the crushed sample placed in a beaker. First, the strength of the odor was determined (none, weak, distinct, strong) and then the odor sensation (aromatic, fruity, musty, mouldy, rancid, etc.) was studied. Taste was distinctively classified as aromatic, pungent, sweet, sour, astringent, mucilaginous, or bitter.

This includes evaluating herbal drugs based on size, shape, color, odour, taste, and specific characteristics such as touch, texture, and so on. This is a qualitative evaluation technique related to the study of morphological and sensory reports of whole drugs. Fractured surfaces in cascara, cinchona, and quillia bark, as well as quassia wood, for example, are essential characteristics.

2. Macroscopic and Microscopic Examination: Medicinal plant materials are classified based on sensory, macroscopic, and microscopic characteristics. An investigation to decide these attributes is the first step in determining the purity and identity of such components, and it should be performed before any further tests are performed.

3. Physical Evaluation: Each monograph contains the detailed botanical, macroscopic, and microscopic descriptions, as well as detailed drawings and photographic images that would provide studies that look of precisely identified material. A microscopy ensures the material's identity and serves as a primary screening test for impurities.

4. Ash determination: The ash remaining after the ignition of herbal plant products is determined using three different methods that quantify total ash, acid insoluble ash, and water-soluble ash. 5.

Extractable matter determination: This method calculates the amount of active components derived with solvents from a set quantity of herbal plant material [12] . a) Extractives that dissolve in water b) Extractives soluble in alcohol c) Extractives soluble in ether 6. Assessment of Foreign Substance: Medicinal herbs should be compiled from the plant's confirmed part. They must be completely free of insects and moulds, as well as visible and excreta contaminants such as stones, sand, detrimental and toxic foreign matter, and chemical residues. CHEMICAL EVALUATION The majority of drugs contain specific chemical constituents that influence their biological and pharmacologic activity. A qualitative chemical test that is used to determine the quality and purity of a drug. 1. Chromatographic Fingerprint recognition and Marker Compound Analysis: A chromatographic fingerprint of a herbal medicine (HM) is a chromatographic pattern of some common active compounds of pharmacologically active and or chemical properties.

5.TLC

Thin-layer chromatography is abridged as TLC. It is a popular and straightforward chromatographic technique for compound separation.

All the procured and authenticated individual drugs were dried in shade and cleaned by hand sorting. The individual drugs were then crushed using willing grinder and passed through mesh no. 40. The individual drugs were then weighed as per the quantity required. The drugs were mixed geometrically using a double cone blender. The mixed formulation was unloaded, weighed, and packed in labeled glass bottles.

6.Extractive values[13]

6.1. Water soluble extractives

Five grams of coarsely powdered air-dried drug was macerated with 100 ml of water in closed conical flask for 24 hours, shaken frequently for the first 6 hours and allowed to stand for 18 hours. This was filtered through Whatman filter paper grade no.100. Twenty-five milliliters of the filtrate was evaporated to dryness in petri dish, dried at 105°C, and weighed. Percentage of water soluble extractive with reference to air-dried material was calculated.

Alcohol soluble extractives Five grams of air-dried and coarsely powdered drug was macerated with 100 ml of 70% ethanol in a closed conical flask for 24 hours, shaken frequently during the first 6 hours, and allowed to stand for 18 hours. This was filtered rapidly taking precaution against loss of ethanol. Twenty-five milliliters of the filtrate was evaporated to dryness in a petri dish, dried at 105° C, and weighed. Percentage of alcohol soluble extractive was calculated with reference to air-dried drug.

6.2 Ether soluble extractives

Five grams of air-dried and coarsely powdered drug was extracted with ethyl ether in a soxhlet extractor for 20 hours. The ether extract was transferred in a petri dish and allowed to evaporate. It was dried at 105° C to constant weight. Percentage of ether soluble extractive was calculated with reference to air-dried drug.

Physicochemical properties Physical characteristics like moisture content, bulk density, tap density, angle of repose, Hausner ratio, and Carr's index were determined for different formulations.

Moisture Content. The shade-dried drug was grounded in a mixer grinder. The powder passed through #40 and retained on #120. Accurately weighed 10 g of # 40/120 drug powder was kept in a tared evaporating dish. This was dried at 105°C for 5 hours in tray drier and weighed. The drying was continued and weighing was done at one-hour interval until difference between two successive weighings corresponds to not more than 0.25 percent. Drying was continued until a constant weight was reached with two successive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator was showing not more than 0.01 g difference.

7.Bulk Density and Tapped Density[10]

In the present study, we had taken the weighed quantity (30 gm) of shade-dried and presieved (#40/120) different drugs, marketed and in-house formulation powders and carefully added them to a cylinder with the aid of a funnel without any losses. The initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

7.1Carr's Index

Carr's index has been used as an indirect method of quantifying powder flowability from bulk density; this method was developed by Carr. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and stability, and is calculated according to following equation.

Carr's index (% compressibility) = 100 × (1 - Db / Dt)

Where Db = Bulk density, Dt = Tapped density

Hausner Ratio Hausner ratiohas been also used as indirect method of quantifying powder flowability from bulk density. Hausner ratio = Dt / Db. Where Db = Bulk density and Dt = Tapped density.

8.pH of suspension of the drugs

pH of freshly prepared 1% w/v suspension and 10% w/v suspension in distilled water was determined using simple glass electrode pH meter.

9.Ash values[7,11]

Total ash Two grams of grounded air-dried material was accurately weighed in a previously ignited and tared silica crucible. The drug was gradually ignited by raising the temperature to 450°C until it was white. The sample was cooled in a desiccator and weighed. The percentage of total ash was calculated with reference to air-dried drug.

9.1Acid Insoluble ash

The ash was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water Soluble ash The ash was boiled with 25 ml of water for 5 minutes, the insoluble matter on ash less filter paper collected, washed with hot water, ignited, cooled in a desiccator, and weighed. The weight of the insoluble matter from the weight of the total ash was subtracted; the difference represents the water soluble ash. The percentage of water insoluble ash was calculated with reference to the air-dried drug[14].

10.Gas Chromatography

Gas chromatography also known as gas liquid chromatography, Its is a technique for separation of mixtures of mixtures into components by a process which depends on the redistribution of the components between a stationary phase or the support material in the form of a liquid, solid or combination of both and a gaseous mobile phase. It is well-known that many pharmacologically active components in herbal medicines are volatile chemical compounds. Thus, the analysis of volatile compounds by gas chromatography is very important in the analysis of herbal medicines. The GC analysis of the volatile oils has a number of advantages. Firstly, the GC of the volatile oil gives a reasonable "finger print" which can be used to identify the plant. The composition and relative concentration of the organic compounds in the volatile oil are the characteristic of the particular plant and the presence of impurities in the volatile oil can be readily detected. Secondly, the extraction of the volatile oil is relatively straight forward and can be standardized and the components can be readily identified using the GC-MS analysis. The relatively quantities of the components can be used to monitor or assess certain characteristics of the herbal medicines. Changes in composition of the volatile oil may also be used as indicators of oxidation, enzymatic changes or microbial fermentation. The advantages of GC clearly lie in its high sensitivity of detection for almost all the volatile chemical compounds. Thus, over the past decades, GC is a most popular and useful analytical tool in research field of herbal medicines. Especially, with the use of hyphenated GC-MS instrument, reliable information on the identity of the compounds is available as well.

Standardization of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in the case of polyherbal drugs.

The Standardization of crude drug materials includes the following steps-

Authentication: - Each and every step has to be authenticated.

a) Stage of collection.
b) Parts of the collected plant.
c) Regional status.

Botanical identity like phytomorphology, microscopical and histological analysis (characteristic of cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibers, vessels etc.) (6). Various histological parameter studies are:

a) Leaf constant: - Palisade ratio, Vein islet number, Vein termination, Stomatal number and

Stomatal index.

b) Trichomes.
c) Stomata.
d) Quantitative microscopy.
e) Taxonomical identity.
f) Foreign matter.
g) Organoleptic evaluation.
h) Ash values and extractive values.
i) Moisture content determination.
j) Chromatographic and spectroscopic evaluation.
k) Heavy metal determination.
l) Pesticide residue.
m) Microbial contamination.
n) Radioactive contamination.

In general, the herbal formulation may be standardized graphically such that the medication is made using raw materials gathered from various locations, and different batches of the formulation should be compared for chemical effectiveness. The most clinically effective formulations should be chosen. To choose the final completed product and to confirm the whole production process, routine physical, chemical, and pharmacological criteria must first be verified for all batches[15].

The following are the physical, chemical, and microbiological stability characteristics for herbal formulations:

Physical characteristics include viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, flocculation, sedimentation, settling rate, and ash values. Other characteristics include color, odor, appearance, clarity, and viscosity.

Chemical parameters include assays, tests, and limit tests, among others.

Herbals may be analyzed chromatographically using a variety of methods, including fluorimetry, GC, UV, GC-MS, TLC, HPLC, and HPTLC.

Total viable content, total mold count, total enterobacterial count, and their totals are all examples of microbiological criteria. Limiters may be used as a quantitative or semi-quantitative instrument to measure and manage a variety of impurities, including chemicals employed during the abstraction of different herbs, contaminants originating directly from the production vessels and from the solvents, among others[16].

1. Herbal Drug Standardization Guidelines[18]

The standardization of natural drugs is a vast and complex topic. The WHO's recommendations may be summed up as follows:

1. An allusion to the drug's name. Botanical examination techniques include sensory characteristics, foreign organic matter, microscopic, histological, and histochemical analysis, among others.

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, hemolytic 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 contaminant.

6. Radioactive taint.

Monographs on contemporary ayurvedic herbs[19]

Modern herbal Ayurvedic monographs describe the standardization criteria in great detail. The following is a list of the quality control methods from the contemporary Ayurvedic monograph:

The synonyms, plant-related publications, plant components, and analytical techniques.

Descriptive analysis: Drug description, phytomorphology, microscopy, organoleptic analysis, and foreign materials, among other things.

Botanical: Sensory assessment, foreign objects, and microscope analysis.

Ash, extractable matter, water content and volatile matter, and volatile oils make up the physicochemical TLC.

Pharmaceutical properties include astringency, bitterness value, hemolytic activity, sterling index, and foaming index.

Toxicological: Metals, arsenic, and pesticide residue. Pathogens, aflatoxins, radioactive contamination, and total viable count are examples of microbial contamination.

The standardization of herbal products and drugs

Due to the rising demand for medicinal plants, the commercial production of herbal medicines and their commerce is now the sector of the economy that is developing the quickest. This negatively affects the supply chain, which results in adulteration and the replacement of fake pharmaceuticals for real ones.

1. Fluorescence quenching:

Fluorescence quenching, which is directly correlated with extract concentration, occurs when a plant extract is spotted on a layer of fluorescent silica gel and subjected to UV radiation, causing

the extract to appear as a spot on a fluorescent backdrop. The GF silica gel plate served as an adsorbent for dampening fluorescence. Hexane toluene, ether, ethyl acetate, butanol, methanol, and water were used as solvents [20].

2. Chemical and chromatographic methods

May be employed to assist in the identification of a plant substance or extract. These methods include fingerprinting and marker chemicals for the identification and standardization of botanical medications. For fingerprinting, chromatographic techniques like HPLC, TLC, GC, and capillary electrophoresis as well as spectroscopic techniques like IR, NMR, and UV may be utilized. Numerous species have made extensive use of DNA fingerprinting, such as Panax species and their adulterants [9]. In order to standardize botanical preparations at all stages of production processes, identify herbal materials, specify standards for raw materials, and acquire stability profiles, marker compounds may be utilized.

3. Aescin was identified using densitometric thin layer chromatography in a herbal medicine that included dried extracts of Asculum and Vitis. The total saponin content, commonly known as the aescin content, of a herbal medicine including two dry extracts in capsules is examined using a TLC technique. The materials are purified by C (18) solid phase extraction, and then they are examined on a silica gel HPTLC plate with the top layer of the mobile phase being a solution of acetic acid, water, and butanol (10/40/50v/v/v). Anisaldehyde reagent is sprayed on the plate, which is then heated for 5–10 minutes (100–105°C) and monitored at a wavelength of 535 nm to reveal the spots[21].

4. Using HPLC and evaporative light scattering detection, stigmasterol, beta-sitosterol, and stigmastanol concentrations in oral dose forms: For the examination of two sterols, stigmasterol, beta-sitosterol, and a stanol discovered to be widespread in various herbal formulations and dietary supplements, a verified and reproducible HPLC technique using online evaporative light scattering was devised. Using this technique, it was possible to test commercially available items that were made into oral dose forms and claimed to include stanol, sterols, and African potato.

5. Neutron activation analysis using the kO standardization technique for the elemental analysis of herbal remedies: Market-purchased medicinal plant mixtures that were recommended for certain therapeutic objectives were examined using instrumental neutron activation analysis with kO standardization. Liquid chromatography was used to analyze the samples after they had been palletized under six tones of pressure and irradiated for about six hours at a thermal flux of 2.29 x 10 (12) n/cm2/s (13). Sitosterol and stigmasterol in soy bean oil were characterized by UV determination and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry: For the measurement of stigmasterol and sitosterol, two of the most prevalent naturally occurring phytosterols in vegetable oils, a narrow bore HPLC-UV technique was created. The technique allowed for quantification of sitosterol and stigmasterol at concentrations of 0.52 and 0.54 g/ml, respectively, and allowed for detection of the compounds at a concentration of 0.42 g/ml [22].

6. Capillary GC-FID simultaneous determination of cinnamaldehyde, eugenol, and paeonol in traditional Chinese medicinal preparations: Wei Tong Ding tablet (WTDT) and Guifu Dihuang pill (GDHP) were the two traditional Chinese herbal medicinal preparations that were subjected to the simultaneous determination. The tests used nitrogen as the carrier and a FID detector in a 30m x 0.53mm capillary column with a preset temperature GC. Over the ranges of 0.45-0.452mg/l CNMD, 0.31-0.625mg/l EL, and 0.30-610mg/l PL, respectively, good linearity was attained [15].

These are significant Ayurvedic formulations used for perinatal care of mother and child health. 7. HPTLC fingerprinting of marketed formulation containing Shankhpushpi. Thin layer chromatography, phytochemical analysis, qualitative organic and inorganic analysis, UV-visible spectrophotometer, and HPLC fingerprint investigations were used to standardize churnas. Both churnas included alkaloids, steroids, phenols, tannins, glycosides, resins, saponins, and flavonoids, according to a qualitative organic study [23].

5. Evaluation of Herbal Products and Drugs

1. Biological parameter (bioassay):

Some drugs have specific biological and pharmacological activity which is utilized for their evaluation. Actually this activity is due to specific type of constituents present in the plant extract. For evaluation the experiments were carried out on both intact and isolation organs of living animals. With the help of bioassays, strength of drug in its preparation can be evaluated.

It is well known that the biological potency of herbal constituents results from a combination of bioactive plant constituents rather than just one, and that the relative properties of a single bioactive compound can change from batch to batch while the biological activity stays within the desired limits (1). Some instances include: Evaluation of herbal preparations' adaptogenic activity profiles: Adaptogens improve overall health and function while assisting the body in coping with stress. AVM is a natural medicine. Composition: *Piper longum, Emblica officinalis, Withania somnifera, Asparagus racemosus, Ocimum sanctum,* and *Withania somnifera*. AVM is a prospective adaptogen since it has strong antistress, immunomodulatory, and anabolic properties in many animal models.

Clinical investigation to compare the safety and effectiveness of the allopathic drug "Diclofenac sodium" with the natural medication "Dysmo-off" for the treatment of primary dysmenorrhoea: the clinical trial on primary dysmenorrhea to contrast-examine the real allopathic medication "Diclofenac sodium" with the coded herbal drug formulation "Dysmo-off". There was a random controlled clinical experiment. To determine the rate of analgesic effects on dysmenorrhoeic pain, these assessments were based on a verbal rating scale. The patients received Dysmo-off powder twice daily for 4 days (5g one day prior to and three days after the menstruation) as the test treatment, and Diclofenac sodium tablets twice daily for 4 days (50mg one day prior to and three days after the menstruation) as the control treatment, which was randomly assigned in a ratio of 1:2 to the patients. Four successive menstrual cycles were treated. ESR, ultrasonography, and hemoglobin were all assessed at study baseline. Clinical research was done on each subject [23]. scientifically. In the cases that were suitable for Keishi-bukuryo-gan, the so-called Keishi-

bukuryo- gan Sho, a significant skin temperature rise was observed in the upper half of the body after the intake of Keishi-bukuryo-gan. In a case that was suitable for Hochuekkito, a marked elevation of skin temperature spread through the upper trunk. It suggested that thermography is useful for an objective evaluation of Sho in Kampo medicines, and for identification of the action site of the herbal formulation [24].

Biochemical evaluation

Most of the herbal drugs are a mixture of a number of ingredients. Their cumulative effect increases the efficacy of the drug in curing the diseases. Muthu Marunthu is an herbal formulation comprising of eight various plant ingredients and has been claimed to possess anticancer effect. It was observed that the growth rate in rats was normal and there was no change in blood parameters such as glucose, urea, proteins, cholesterol and also in the activities of pathophysiological enzymes such as lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline and acid phosphatase after Muthu Marunthu administration. The tumor weight was found to be reduced in methylcholanthrene induced fibrosarcoma rats after Muthu Marunthu treatment [25].

Evaluation of Kutaj

Ghanavati for alkaloidal principles- Kutaj-Ghanavati is a reputed Ayurvedic preparation used in dysentery and diarrhea. It contains a water extract of Kurchi bark and fine powder of aconite roots. It was evaluated quantitatively and qualitatively employing TLC and titrimetric method. In the TLC study no interference of Kurchi and Aconite alkaloids with one another in their respective solvent systems. The formulation was found to contain all alkaloids of Kurchi and Aconite [26].

Organoleptic evaluation: Organoleptic evaluation of food products plays an important role in judging the censoring acceptability or rejection of food items in the market. Effect of various treatments (blanching, pricking, and lye treatment), sugar concentration (50%, 60%, 70%) and storage on the color scores; flavor scores; texture scores of intermediate moisture apricots. The overall acceptability of the products was significantly higher in 70% sugar syrup but these scores decreased as the storage period advanced.

Conclusion

The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function.

For the purpose of research work on standardization of herbal formulations, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance[27].

Even when the chemical composition of a plant extract is known, the pharmacologically active moiety may not be. Environment, climate and growth conditions influence the composition, as does the specific part of the plant and its maturity. Monographs detailing standardization of active ingredients would improve the marketplace. Even if an herbal product is standardized to, for

example, 4% of a constituent, the remaining 96% of ingredients is not standardized and may affect the product's solubility, bioavailability, stability, efficacy and toxicity. Just as controlled trials are necessary to establish safety and efficacy, manufacturing standards are required to ensure product quality.

Nowadays newer and advanced methods are available for the standardization of herbal drugs like fluorescence quenching, the combination of chromatographic and spectrophotometric methods, biological assays, use of biomarkers in fingerprinting etc. Bioassay can play an important role in the standardization of herbal drugs and can also become an important quality control method as well as for proper stability testing of the product[28].

India can emerge as the major country and play the lead role in the production of standardized, therapeutically effective Ayurvedic formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization such as UV- visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods [29].

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