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Standardization of traditionally designed neutraceutical

*Deepa Padmaja[1], Nagappan Kannappan[2]

[1]*Research Scholar, Karpagam University, Eachanari, Coimbatore, India*
Assistant Professor, Department of Pharmacy, Devaki Amma Memorial College of Pharmacy, Chelembra, India
[2]*Research Guide, Karpagam University, Eachanari, Coimbatore, India*
Associate Professor, Department of Pharmacy, Annamalai University, Chidambaram, India

ABSTRACT

Plants are the rich source of a variety of chemicals with nutritive and therapeutic properties. The pharmacological activities of allopathic drugs are now decreased before the herbal potency. Now most of the pharmaceutical companies are focusing in this area. The Indian pharmaceutical industry is definitely looking forward to a tremendous herbal marketing. The traditionally designed neutraceutical is an Ayurvedic herbal formulation; consist of Brahmi, Tulsi, and other ingredients in powder form. This neutraceutical is proved for its antibacterial and antioxidant activity. Standardization of the traditionally designed neutraceutical is compared with the marketed product. The uses of medicinal plants are increased in developing nations. Nations like India, is a rich source of Ayurvedic plants. Government of India is also promoting the member states to formulate national policies on traditional medicine. Quality assurance is an integral part of traditional medicine. A comprehensive specification must be developed for each herbal drug preparation based on recent scientific data.

***Key Words*:** Neutraceutical, total ash, crude fiber, water insoluble ash, acid insoluble ash.

INTRODUCTION

In developing countries, about 80% of the indigenous population follows traditional system of medicine. In healthcare system, medicinal plants play as a primary role. According to the WHO, Herbal medicine are plant derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or animals. Traditional medicine plays a very important role in health care. Herbal medicines are of great importance to the health of individuals and communities, but the quality assurance needs to be developed.

Quality control of ayurvedic drug is important now-a-days. Quality control monitoring ensures the safety, efficacy and quality of herbal medicines and their preparation. Neutraceutical was defined by Zeisel are directory supplements that deliver a concentrated form of a presumed bio-active agent from a food presented in a non food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods. Traditionally designed neutraceutical consist of *Zingiber officinalis* (zingiberaceae, dried rhizome), Brahmi (scophuleraceae, dried whole plant), Tulsi (labiateae, dried leaves) and Amla (Euphorbiaceae, dried fruit). There are numerous mechanism by which neutraceutical might be expected to exert favorable influence on pathophysiological process. This paper reports the standardization of traditionally designed neutraceutical by physicochemical, microbiological parameters.

***Corresponding Author**:
Deepa Padmaja, Assistant Professor
Department of Pharmacy
Devaki Amma Memorial College of Pharmacy
Chelembra, India.
E-mail: *deepa_kuttie@yahoo.com*
Contact No.: +91-9447631829

MATERIALS AND METHODS

The entire chemicals used in the experiment were analytical grade. All the markers were procured from Mumbai, India. All the Plants are collected from different parts of Kerala, India and they are

dried according to the specifications and the dried parts were ground to coarse powder, passed through sieve no. 24 and stored in air tight containers and used for further formulations. The plants were identified and authenticated by A. K Pradeep Kumar, Herbarium Curator, Calicut University, Calicut. Voucher specimens (Voucher No. 95942, 95944, 95945, 121346) were deposited in the Department of Botany, Calicut University, Kerala.

The method for formulation is under process and cannot be revealed at this stage. One marketed sample and the prepared sample were compared. Both of them are standardized by physical and physicochemical properties. As a point of physiochemical analysis ash value of marketed sample and traditionally designed one are calculated.

Physicochemical analysis

Total Ash Determination

For calculating total ash for prepared sample heat silica crucible to red heat for 30 minutes. Then allowed to cool in a desiccators and weighed.1 gm sample was evenly distributed on the crucible. Dry at 100°C to 105°C for one hour and ignite to constant weight in muffle furnace at 600±25°C. Allow the crucible to cool in a dessicator after each ignition. The material should not catch fire. On prolonged ignition a carbon free ash cannot be exhausted the charred mass with hot water, then collected the residue on an ashless filter paper. Incinerate the residue and filter paper until the ash becomes white. Then calculate the % of ash with reference to air dried drug by using following equation. Weight of the sample taken minus weight of the ash divided by weight of the sample taken. For the marketed preparation the total ash obtained is 10.65 gm and for the formulated one it is 12.23 gm. A high ash value indicates contamination, substitution, adulteration or carelessness in preparing the crude drug. The total ash usually consist of carbonates, phosphates silicates and silica which includes both physiological ash which is derived from the plant tissues itself ad non physiological ash which is residue of the adhering to the plant surface .

Water Insoluble Ash Determination

For determining the water insoluble ash the total ash obtained is boiled with 25ml water and filtered through an ash less filter paper (whatmann 41). It is followed by washing with hot water. The filter paper is ignited in the silica crucible, cooled and water insoluble matter from the total ash. The percentage of water insoluble ash is calculated by weight of water insoluble ash divided by weight of sample multiply with 100.

Acid Insoluble Ash Determination

Acid insoluble ash can be obtained by boiling for five minutes with 25ml of 2M hydrochloric acid and filtered through an ashless filter paper. The filter paper is ignited in the silica crucible, cooled and then acid insoluble ash is weighed. Weight of acid insoluble ash divided by weight of sample multiply with 100 gives the percentage of acid insoluble ash. Acid insoluble ash value was less than 2 gm% for both. This ash values particularly indicates contamination with silicious material.

Water Soluble Ash Determination

Water soluble ash is a good indicator of either previous extraction of water soluble salts in the drug or incorrect preparation. It is a part of total ash. About 5 grams of the powder is macerated with 100ml of distilled water in a closed flask for 24 hrs. Shake frequently during 6 hrs and allow the same for standing for 18 hrs. It is filtered rapidly and 25ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive is calculated with reference to the air dried powder. The marketed preparation shows the value of less than 37 and for the prepared formulation it is less than 36.

Alcohol Soluble Extractive Method

Two formulations of five grams were macerated with 100ml of alcohol in a closed flask for 24 hours, shaking frequently during six hours and allowed to stand for 18 hours. It was then filtered rapidly .25ml of each filtrate was evaporated to dryness in a tarred flat bottom shallow dish at 105°C to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug and is calculated in percentage. For marketed sample it shows the value less than 12 and for the prepared formulation it is less than 16.

Determination of Moisture

The presence of moisture also affects the drug. Insufficient drying may cause spoilage to the product. Rate at which the moisture is removed or the conditions under which it is removed having equal importance to ultimate dryness. Loss on drying is the loss of mass expressed as percent w/w. Weigh about 1 gm of the powdered crude drug in to a weighed flat and thin porcelain dish. Dry in the oven at 100-105°C for half an hour. Cool and weigh the contents. Keep back the contents in oven and repeat the drying at 100-105°C for half an hour. Cool the contents and check its weight. Repeated the process to get the concordant value. The presence of moisture results in microbial growth and it may affect the stability. The marketed formulation shows the moisture content of about less than 5 percent and for the newly formulated preparation about less than 2 percent.

Crude Fiber Estimation

Crude fiber plays major role. Estimation of crude fiber denotes the measurement of the content of the cellulose, lignin cork cell in the plant tissue and excess indicates adulteration. The fiber content was determined by the following method. The formulated preparation gives the value of less than 4 percent and for the marketed preparation is of about less than 3 percent.

About 2 gm of drug was weighed accurately and transferred to a porcelain dish. 50ml of 10% nitric acid is added and boiled for 30 seconds with constant stirring and filtered through fine mesh cotton cloth. The residue is washed with 5ml of boiled water. The material from the cloth is collected in a porcelain dish and boiled with 50ml of 2.5% caustic soda. Then the liquid is filtered by using the fine mesh cotton cloth. The residue is washed with 100ml of boiling water. Then the fiber is collected in a dried and weighed crucible. The crucible is then placed at 105°C for 2 hours. It is then placed in desiccators and cooled. The cooled crucible is weighed. From the weight of the residue crude fiber content was calculated.

Detection of Heavy Metals

It is very much important to detect the presence of heavy metals in the sample solution. For determining the heavy metals we can either use flame photometry or atomic absorption spectroscopy. Atomic absorption is capable of determining metal concentration ranging from ultra trace to major constituent levels. The fine powder is used for dry ashing. During this time avoid metallic contamination in any form. Pre-cleaned silica crucibles are maintained at 600°C. Until the weight of the crucible is constant 5gm of powdered material are taken in a silica crucible and maintained in a muffle furnace at 600°C for 6 hrs. The crucible are then taken out and cooled at room temperature by keeping it in a dessicator and the ashes valued are measured. Then the ash is dissolved in 100ml of 5% HCL. The dissolved ash solutions are filtered through Whatmann filter paper no. 40 and are stored in a tightly capped plastic bottles. The prepared solutions are directly used for the determination of various elements. The prepared formulation shows arsenic, mercury and lead of about 0.1 ppm and the marketed formulation, shows that the arsenic and mercury of about 0.1 ppm and the content of lead is 1.0 ppm.

Sodium Content Estimation

Sodium content can also be estimated by flame photometry. Sodium chloride content in the marketed formulation and the in house formulation was determined by titration method. 2gm of the sample was dissolved in 25ml purified water against 0.1N standard silver nitrate solution using potassium chr-omate as indicator. Each ml of 0.1N silver nitrate solution is equivalent to 0.005845gm of Sodium chloride.

Microbiological determination

Presence of micro organism also affects the stability of the drug. Sample of 10 gm was weighed and 100ml of sterile distilled water in a sterilized conical flask are prepared for serial dilution. The flasks are kept in a mechanical shaker for five minutes to obtain uniform suspension of micro organism. The dilution is 1-10. From this 1ml of dilution from 10^{-1} sample is taken and transferred in to 9ml, this is 10^{-2} dilution. The procedure is repeated up to 10^{-6} dilution. Transfer 1ml of serial dilution from 10^{-1} to 10^{-6} sterilized petridishes for enumerating pathogens such as *salmonella, E. coli, pseudomonas aureogenesa*.

Two replication is maintained for each dilution, for each group of micro organism. The medium such as nutrient agar, potato dextrose agar, salmonella shigella agar and Eosin methylene blue agar are

added to the sterilized Petri dish with oneml of sample and rotating the plate clock wise and anti clock wise to get a uniform distribution of microbial cells. The medium is allowed to set and the dishes are incubated in inverted positions at 37°C for about 1-2 days for bacteria. The colonies are counted on the plates with the aid of colony counter. The numbers of colonies obtained from two plates are kept for replication. The total numbers of bacterial populations are calculated by taking the average of two dilutions employed.

Physical determinations

Bulk Density

Bulk density is the ratio between the mass of the powder and the bulk volume of the powder. The bulk density depends on particle size distribution, shape and cohesiveness of the particle. For determining the bulk density 60 g is passed through a standard sieve number 20. A weighed amount 50 gm is introduced in to a 100ml graduated cylinder. The cylinder is fixed on a bulk density apparatus and the timer knob is set for 100 tappings. The volume occupied by the powder was noted. Further another 50 taps were continued and the final volume was noted. For reproducible results the process of tappings may be continued until concurrent volume is achieved. Marketed preparation shows the value of about 0.66 g/cc. In house formulation showed the bulk density of about 0.64 g/cc.

True Density

True density is defined as the ratio between the weight of the powder and the true volume of the powder. It depends upon the types of atoms in a molecule, arrangement of atoms in a molecule, arrangement of molecule in the sample. True density is calculated by dividing weight of the powder divided by true volume of the powder. The marketed preparation shows the true density of about 0.81 g/cc and the formulated preparation shows the value of about 0.83 g/cc.

Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. The flow characteristics are measured by angle of repose. Improper flow of powder is due to frictional forces between particles. The frictional forces are quantified by angle of repose. For doing angle of repose a glass funnel is held in with a clamp on any support over a glass plate. The glass plate is placed on a micro-lab jack. About 100g of powder is transferred in to funnel, keeping the orifice of the funnel blocked by a thumb. As the thumb is removed the lab jack is adjusted so as to lower the plate and maintain about 6.4 mm gap between the bottom of the funnel stem and top of the powder pile .When the powder is emitted from the funnel, the angle of the heap to the horizontal plane is measured with a protractor. The height of the pile and radius of the pile is measured with the ruler. The angle of repose is thus estimated. The angle of repose of both the marketed and the sample are compared.

Phytochemical screening

The pharmacological actions of crude drugs are determined by the nature of constituents. It is essential to single out the chemical compounds responsible for the therapeutic effects. The plant drug chemically consists of a complex but organized mixtures of organic and inorganic constituents. Here qualitative chemical analysis was used for determining the nature of constituents in a complex mixture.

In the case of herbal drug formulations where the constituents responsible for the therapeutic activity are unknown, assays or marker substance or other justified determinations are required. The appropriateness of the choice of the marker should be justified. The TLC study of DMSO extract of prepared formulation is compared with individual markers. For the separation hexane: ethyl acetate in the ratio of 8.5:0.5 is used as mobile phase. The separated compounds are collected and it undergoes qualitative analysis for confirming the phyto chemical constituents. Analytes separated on a TLC can be quantified by eluting the relevant chromatographic zone from the sorbent. With the help of micro spatula scratch out the perimeter around the zone of interest. Within the marked area the zone is scraped. The scrapings are transferred to a suitable container containing the extracting solvent. To dissolve the analyte the mixture is agitated and by filtration or by centrifugation the sorbent is removed, then apply the sample on the sorbent layer of the TLC plate by using a capillary tube of 1-2µl with a spot size of 3-4 mm. Micro syringes allowed a variable volume to be applied. It is important to

Table 1: Physicochemical analysis.

Parameter	Formulated preparation	Marketed preparation
Color	Brownish yellow	Cream color
Odor	vanilla	odourless
Taste	Sweet taste	Sweet taste
Total ash	12.23	10.65
Water insoluble ash	0.5	1
Acid insoluble ash (%)	0.9	1.9
Water soluble extractive	36	35
Moisture (%)	3	4
Crude fiber (%)	5.2	4.8
Heavy metal	Arsenic-0.1 ppm Mercury- 0.1 ppm Lead-0.1 ppm	Arsenic-0.1 ppm Mercury-0.1 ppm Lead-1.0ppm
Alcohol soluble extractive	Less than 16	Less than 12
Sodium content (%)	22	12

ensure that a flat ended, preferably PTFE-coated needle was used to avoid disruption of the sorbent layer. On the same way the markers was also applied on TLC plates of the same or another. With the help of different solvent systems and different visualizing agents the retardation factor of the marker is compared with the analyte.

Organoleptic Evaluation

Herbal ingredients must be accurately identified by comparing with authentic material or accurate description of authentic herbs. Here authenticity was obtained from the Department of Botany, Calicut University, Kerala.

RESULTS AND DISCUSSION

Quality control tests include physico-chemical analysis, microbiological determination, physical determination and phyto chemical screening.

Physicochemical analysis

The physicochemical analysis of the formulated and marketed neutraceutical are given in table 1. Total ash and alcohol soluble extractive value for the whole preparation was of about less than 16. Acid insoluble ash content was of about less than 2%. Alcohol soluble extractive value was about also less than 16.Water soluble extractive value is of about less than 39. The preparation shows moisture content of about less than 6%.

Table 2: Comparative physical characteristics of the formulated and marketed neutraceutical.

Parameter	Formulated preparation	Marketed preparation
True density	0.83 g/cc	0.81 g/cc
Bulk density	0.64 g/cc	0.41 g/cc
Angle of Repose	47.4°C	42.5°C
Carr's index	13.10	13.31

Physical determinations

The Comparative physical Characteristics of the formulated and marketed neutraceutical are given in table 2. Physical characteristics of the formulated product were compared with marketed product. Angle of repose for the formulated product was about 47.4°C. The bulk density is of about 0.64 g/cc. True density is of about 0.83 g/cc. All these values are obtained by calculating the average of three readings. Angle of repose for the marketed product was about 42.5°C. The bulk density is of about 0.41 g/cc. True density is of about 0.81 g/cc. All these values are obtained by calculating the average of three readings.

Phytochemical screening

The active constituents present in both formulations are carbohydrate, proteins, flavanoids etc. TLC profile of the marker compound corresponding to the individual ingredients is to authenticate the presence of these ingredients in the formulated sample. The retardation factor of test sample of Brahmi was about 0.91 cm and it complies with the standard. The retardation value for curcumin standard was about 0.83 and it complies with test had the value of about 0.83. Tulsi shows the value of about 0.32 and the standard is of about 0.46. For authenticity the marker compound is compared with the extracted compound for total ash. In the case of amla the total ash value for both is less than 8 % w/w. Ash content for the pepper is of about less than 7% for both. For Brahmi the ash content is of about less than 8 % w/w. Ash content of curcumin is of about 10 %w/w. Ash content of ginger of about less than 8 %w/w for both. The TLC profile of both the marker and the formulation shows uniformity. Phytochemical screening of the formulated and marketed neutraceutical are given in table 3.

Table 3: Phytochemical screening of the formulated and marketed neutraceutical.

Sl. no	Phyto constituents	Formulated preparation	Marketed preparation
1	Carbohydrate	(+)	(+)
2	Proteins	(+)	(+)
3	Lipids	(+)	(+)
4	Saponins	(+)	(-)
5	Coumarin	(+)	(-)
6	Tannins	(+)	(-)
7	Terpenoids	(+)	(-)

Microbiological determination

The formulated product shows that the *E. coli*, *Pseudomonas auregenesa* were absent in the formulation and *Staphylo cocci aureus* was of less than 100 cfu. The marketed formulation also shows *E. coli*, *Staphylo coccus aureus* and *Pseudomonas auregenesa* less than 100 cfu.

CONCLUSION

The need of standardization of Ayurvedic supplement is due to the fact that the preparation of drug according to the ancient method has been reduced due to the wide spread of commercialization in this area. Traditionally designed neutraceutical has been standardized by intervention of modern scientific quality control measures. As a part of standardization the marketed and the formulated product was tested for the relevant physical, Phytochemical, physicochemical and microbiological determinations. The results obtained could be used to lay down a new set of pharmacopeia standards.

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