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Standardization of Dhanvantari gutika: An ayurvedic formulation

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Abstract
According to an ancient and historical approach on medicine mainly performed in the Indian origin is Ayurveda. Ayurveda, one of the oldest medicinal systems from India, uses natural ingredients like herbs, plants, and shrubs to balance physiological doshas and promote healing. Dhanvantari gutika, is an Ayurvedic formulation described in Sahasrayoga which is useful in conditions such as Gastritis, cough, vomiting. The gutika was standardized by different parameters such Organoleptic evaluation like colour, odour, taste, shape; Physicochemical evaluation like Ash value, Extractive value, pH, Loss on drying, Foaming index; Phytochemical evaluation like Phytochemical Screening, TLC, HPTLC; Pharmaceutical evaluation like Hardness, Friability, Weight variation, Disintegration time and Toxicological evaluation like sterility test by pour plate method and heavy metal determination by atomic absorption spectroscopy. All these parameters were well within the norms specified by the Indian Ayurvedic Pharmacopeia with respect to a tablet. These parameters can determine the quality of product as Gutika.

Keywords: Dhanvantari Gutika, standardization, HPTLC, evaluation, quality control

Introduction
Herbal medicine is also known as herbalism or phytomedicine. It is the study of use of medicinal plants. It includes modern standards of testing of herbs and medicines derived from natural sources, few high-quality clinical trials and standards of purity [1]. Ayurveda is one of the traditional medicinal systems with an established history of many centuries. The primary focus of Ayurvedic medicine is to promote good health and prevent illness, rather than fight disease [2]. Dhanvantari Gutika is prepared as per the original recipe with the right herbs to support different dimensions of health. *Ajamoda*, *Asali* and *Jeeraka* in the tablet promotes a hassle-free digestion and are good in addressing acidity, bloating and gas related issues. A perfect companion for pregnant ladies, Dhanvantari Gutika helps relieve digestion related issues and respiratory discomfort during the period. The presence of Cardamom, Camphor etc. in the composition also assists in maintaining optimum respiratory health. When administered with proper adjuvants like cumin seed water, appropriate *Kashayas* etc., Dhanvantari Gutika promotes overall metabolism and ensures proper functioning of *Vata dosha* in the body.

Fig 1: Dhanvantari Gutika

Review of literature

Keerthy S Namboodiri *et al*., (2020) carried out Formulation and Standardization of Trivrutadi Gutika - An Ayurvedic Formulation. Trivrutadi gutika, is an Ayurvedic formulation described in Chakradatta which is useful in conditions such as Udavarta (reverse movement of vata), Vibandha(constipation)etc. Gutika(tablet) is standardised by different physicochemical parameters and chromatographic examination including HPTLC fingerprinting. All the parameters like uniformity of weight, loss on drying, disintegration time, friability, hardness, total ash, water soluble ash and acid insoluble ash were well within the norms specified by the Indian Ayurvedic Pharmacopeia with respect to a tablet.

Vijay Gupta, at *et al*., (2019) carried out Standardization and detailed aspects of Marichadi Gutika. Newer guidelines for standardization, manufacture, quality control and scientifically rigorous research are necessary for traditional medicines. Gutika was standardized by different parameters like physicochemical parameters include Ash value, extractive value, LOD, Physical parameters like hardness, friability, disintegration and chromatographic examination include HPTLC fingerprinting, quantification of marker. And also evaluated by various parameters like, Hardness, weight variation and disintegration time. All parameters were passed from its standard limits.

Rahul Raj Surisetty *et al*., (2014) carried out Standardization of Marketed Churna an Ayurvedic Polyherbal Formulation. Standardization is the need of the hour in Ayurvedic system of medicine. The present work aims to standardize a polyherbal churna called Surya Sakthi Churna available in the market. The churna was procured and standardised for the parameters like organoleptic characters, physical characters, physiochemical properties and phytochemical screening etc. These parameters can determine the quality of the product. The results were found to be within the standards.

Materials and Methods

1. **Organoleptic evaluation [3]:** The formulation was studied for its preliminary characters like colour, odour, taste, shape and size.
2. **Physicochemical evaluation:** The gutika was subjected for Ash value, Extractive value, pH, Loss on drying and Foaming index.

Determination of Ash Values [4]

The ash value is useful to determine the quality and purity of the crude drugs.Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium,potassium, magnesium, calcium etc., sometimes inorganic variables like calciumoxalates, silica, carbonate content of the crude drug affects total ash value.

- **Determination of Total ash:** Incinerate about 3g accurately weighed drug powder in a tarred platinum or silica dish at temperature not exceeding 4500C until free from carbon, cool and weigh. If the carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at temperature not exceeding 450^0C. Calculate the percentage of ash with reference to the air-dried drug.
- **Determination of Acid insoluble ash:** To the crucible containing total ash, ash 25ml of dilute HCl. Collect the insoluble matter on a ashless filer paper and wash with hot water until the filtrate is neutral.Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 min and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.
- **Determination of Water-soluble ash:** Boil the total ash for 5 min with 25 ml of water; collect the insoluble matter in aashless filter paper, wash with hot water, and ignite for 15 min at temperature not exceeding 450^0C. Substarct the weight of insoluble matter from the weight of ash;the difference in weight represent the water soluble ash. Calculate the percentage of water-soluble ash was calculated with reference to the air-dried drug.
- **Determination of Sulphated ash:** One gram of powdered drug was accurately weighed and taken in a tared silica crucible which was previously ignited and weighed. The drug was ignited gently at first until the substance was thoroughly charred. It was then cooled, the residue was moistened with 1 ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at $800^oC \pm 250^0$C until all black particles havedisappeared. The crucible was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

Determination of Extractive values [4]

These are used to determine the amount of matter soluble in the solvents used, whichinclude alcohol soluble extractives and water-soluble extractives. Percentage oalcohol and water-soluble extractives were calculated and used as standards.

- **Determination of alcohol-soluble extractive:** Five gram of the air dried coarsely powdered drug was macerated with 100 ml ofalcohol in a closed conical flask for 24hours, shaking frequently during six hours and allowing to stand for eighteen hours. This was filtered rapidly, taking precautions against loss of solvent evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105℃ to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.
- **Determination of water-soluble extractive:** Five gram of the coarsely powdered air-dried drug was macerated with 100 ml ofwater in a closed flask for twenty-four hours, shaking frequently for six hours andallowing to stand aside for eighteen hours. It was filtered rapidly, taking precautionsagainst loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarredflat-bottomed shallow dish and dried at 105℃ to constant weight and weighed.
- **Determination of ether-soluble extractive:** Five gram of the coarsely powdered air-dried drug was macerated with 100 ml ofether in a closed flask for twenty-four hours, shaking frequently for six hours andallowing to stand aside for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish and dried at 105℃ to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Loss on Drying [4]

Accurately weighed drug samples were placed (without

preliminary drying) in atarred evaporating dish. They were dried at 105℃ for 5 hours, and weighed;percentage loss on drying was calculated with reference to initial weight.The percentage of loss on drying was calculated.

Foaming index [4]

1g of coarsely powdered drug and transferred to 500ml conicalflask containing 100ml of boiling water maintained at moderate boiling at 80-90^{0}Cfor about 30 mins then made it cold, filtered into a volumetric flask and addedsufficient water through the filter to make the volume up to 100ml. Cleaned 10 stopper test tubes were taken and marked with 1-10. The successive portions of 1,2ml up to 10ml drug was taken in separate tubes and adjusted remaining the volume with liquid up to 10ml in each. After closing the tube with stoppers, shake them for 15sec and allow to stand for 15min then measure the height.

Determination of pH [5]

5 g of the sample was dissolved with water and covered it with Aluminum foil. Allow to withstand in room temperature for 24 h. After 24h, decanted the supernatant liquid and determined the pH using pH meter.

Phytochemical evaluation

Phytochemical screening, TLC and HPTLC was carried out.

Thin Layer Chromatography [6]

Hydroalcoholic extract of gutika was subjected to TLC as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate marking were made with soft pencil. Micropipette were used to spot the sample for TLC applied sample volume 10-microlitre by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system. after the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365nm.

High performance thin layer chromatography [7]

10 µl of hydroalcoholic extract was applied on the HPTLC plate. HPTLC was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried. Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

Pharmaceutical evaluation

Hardness, friability, weight variation, and disintegration test were carried out.

Hardness [8]

Pfizer's tablet hardness tester was used to independently test the randomly chosen tablets. The tablet was held vertically between the hardness tester’s jaws. By gradually applying more pressure to the tablet's edge while pressing the jaws with the aid of a hand, the tablet eventually broke. The reading was taken, and each group's average hardness was determined independently.

Friability [9]

The friability test is conducted in the Roche friability apparatus by taking 20 tablets. This consists of a plastic drum that revolves at 25rpm, dropping the tablets through six inches in the friabilator to undergo shock, which is then operated for 100 revolutions. The tablets are reweighed.

Weight variation [5]

Cross. Weighed 20 tablets and then its average weightis calculated. Values are compared with the standard.

Disintegration test [5]

The tank of the disintegration apparatus was filled with distilled water up to the mark. 750ml of distilled water in each of the 1000ml beaker is taken. The timer of the instruments was set for 60 minutes. The temperature of water in beakers to 37°C was maintained. 1 tablet was introduced into each tube added a disk to each tube. The assembly was suspended in the beaker containing water in it after that apparatus is operated. The time duration at which the tablets disintegrate was noted.

Toxicological evaluation

Sterility test by Pour plate method and heavy metal determination by Atomic absorption spectroscopy was performed.

Heavy metal determination by Atomic absorption spectroscopy [11]

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard Preparation

As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO3

Sterility test by Pour Plate Method [12]

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{0}C added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37^{0}C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

Results and Discussion

Dhanvantari gutika, is an Ayurvedic formulation described in Sahasrayoga which is useful in conditions such as various health benefits, including promoting digestive health, enhancing immunity, and supporting respiratory function. The gutika were taken and subjected to various standardization parameters. The organoleptic evaluation (Table 1) provides the simplest and quickest means to establish the identity and quality of a particular sample which are useful in judging the material in its entirety and in powder form. It include parameters like colour, odour, taste, shape.

Physicochemical evaluations (Table 2) like ash value, moisture content, extractive value, loss on drying, pH, foaming index was carried out and confirmed the consistency and stability of the Gutika formulation. Extraction of powdered gutika was done by maceration using hydroalcoholic as solvent. The extract was then subjected to

phytochemical screening, TLC, HPTLC analysis. The Phytochemical evaluation (Table 4) gives the information about phytoconstituents present in the formulation. In TLC, the chromatogram shows the separation of compounds in the Hydroalcoholic extract of Dhanvantari Gutika. The result is typically presented as a series of spot on the plate, with each spot representing the presence of different Phytoconstituents. It was further confirmed by HPTLC. HPTLC fingerprinting reveals the presence of 10 prominent peaks corresponds to the presence of versatile phytocomponents present with in it. The major Rf value of the peaks ranges from 0.04 to 0.8.

Pharmaceutical parameters (Table 3) such as hardness, friability, weight variation, disintegration test and Toxicological evaluation such as Test for heavy metals by atomic absorption spectroscopy, Sterility test by pour plate method was carried out. Sterility test by pour plate techniques results that there is no growth / colonies were observed in any of the plates inoculates with the test sample. Heavy metal determination test by atomic absorption spectroscopy is conducted and the results of the present investigation have clearly shown that the sample has no traces of heavy metal such as Cadmium were as the sample evident the presence of Lead, Arsenic and Mercury at 2.546, 1.577 and 0.298 ppm levels as listed in the table.

Table 1: Organoleptic evaluation

Colour	Black
Odour	Characteristic Odour
Taste	Astringent
Weight	0.22
Shape	Round

Table 2: Physicochemical Evaluation

Total ash	16.5%w/w
Acid insoluble ash	2.4%w/w
Water soluble ash	9%w/w
Sulphated ash	8.2%w/w
Water soluble extractive value	51.2%w/w
Alcohol soluble extractive value	39.46%w/w
Ether soluble extractive value	26.66%w/w
Loss on drying	12%w/w
Ph	5.59
Foaming index	a=5

Table 3: Pharmaceutical evaluation

Hardness	9.6kg/m^2
Friability	0.678%
Weight variation	0.678%
Disintegration	47 min

Table 4: Phytochemical evaluation

Alkaloids	1ml Alc. Extract + 1ml conc. HCl + Hager's Reagent	Yellow PPT	+
Carbohydrates	1ml extract + 1ml Fehling A + 1ml Fehling B	Blue colour	+
Proteins and free amino aacids	1ml extract + 1ml 4% NaOH + few drops 1% $CuSO_4$	Violet or pink colour	+
Phenolic compounds and tannins	1ml extract + drop wise $FeCl_3$	Violet colour PPT	+
Flavonoids	1ml extract+ 1ml Dil. ammonia solution + Conc. H2SO4	Yellow colour disapperar	+
Fixed oils and fats	1ml extract +alcoholic potassium + phenolphthalein	formation of soap	-
Gums and mucilages	1ml extract+ Ruthenium red solution	Pink colour	-
Glycoside	1ml extract + 0.5ml Glacial Acetic acid + few drops of Dil. FeCl3 till colourless + 1ml Dil. H2SO4	Brown ring	+
Tritherpinoids	1ml extract +0.5ml $CHCl_3$+ 1ml Conc. H_2SO_4	Yellow colour	+
Saponins	1ml extract + Few drops of olive oil+ Shake vigorously	Froth	+

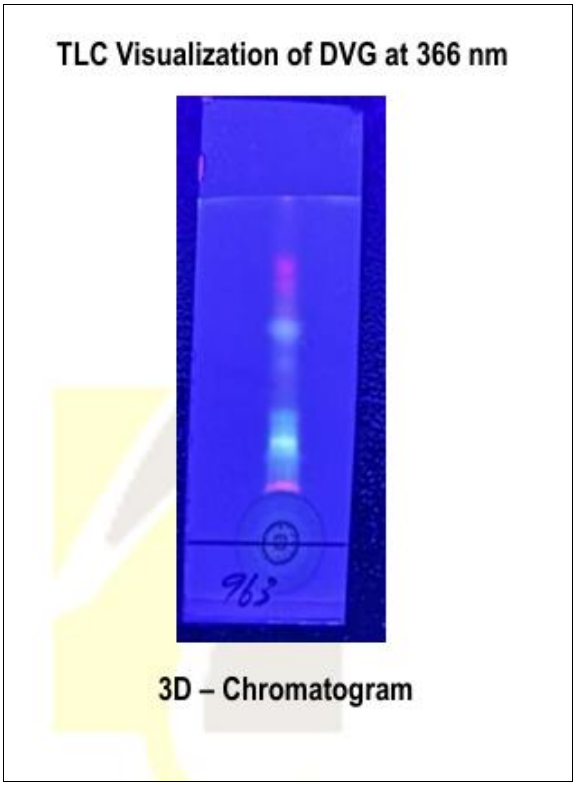

Fig 2: TLC chromatogram

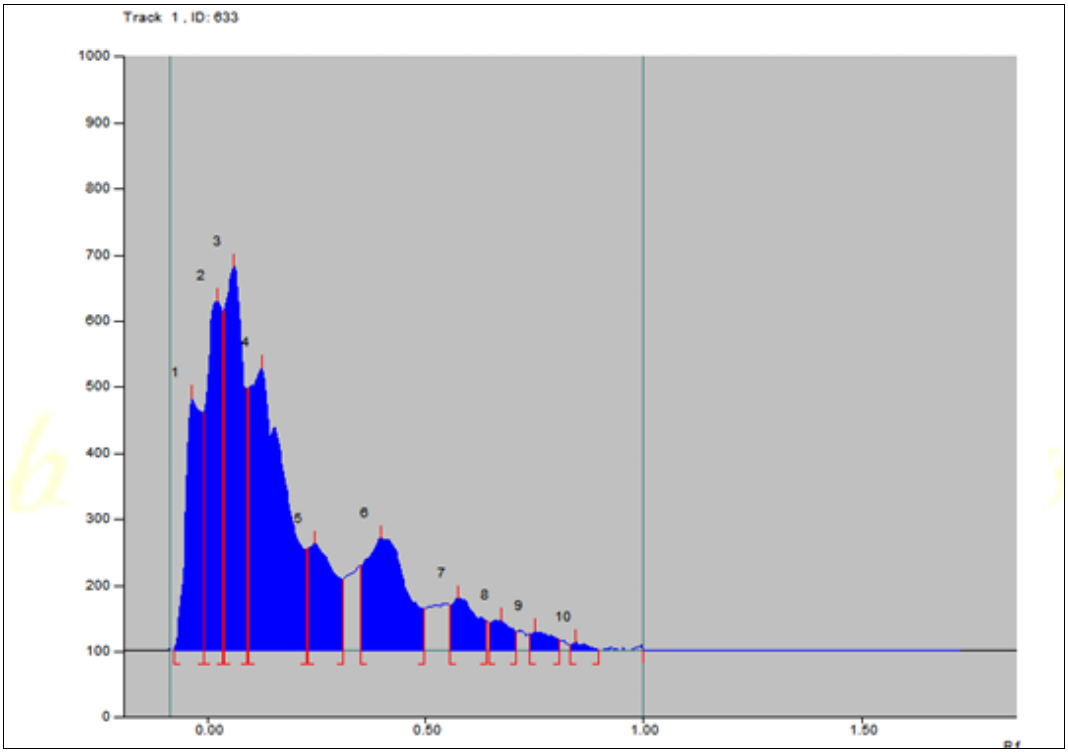

Fig 3: HPTLC peak display

Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.08	1.2	-0.04	381.5	15.81	-0.01	358.5	6387.6	11.17
2	-0.01	360.0	0.02	529.0	21.92	0.04	510.9	8631.0	15.10
3	0.04	513.5	0.06	580.2	24.04	0.09	395.7	10655.8	18.64
4	0.09	396.1	0.12	427.1	17.70	0.23	152.8	15938.5	27.88
5	0.23	153.9	0.25	162.2	6.72	0.31	108.3	4383.4	7.67
6	0.35	127.1	0.40	170.0	7.04	0.50	62.9	7236.2	12.66
7	0.56	68.7	0.57	78.2	3.24	0.64	44.9	2107.1	3.69
8	0.65	41.8	0.67	45.2	1.87	0.71	29.4	990.3	1.73
9	0.74	25.3	0.75	28.1	1.16	0.81	15.9	656.3	1.15
10	0.83	8.1	0.84	11.9	0.49	0.90	0.7	185.1	0.32

Fig 4: HPTLC peak table

Table 5: Result of heavy metal determination

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Result Analysis
Lead	217.0 nm	2.546	10 ppm
Arsenic	193.7nm	1.577	3ppm
Cadmium	228.8nm	BDL	0.3ppm
Mercury	253.7nm	0.298	1ppm

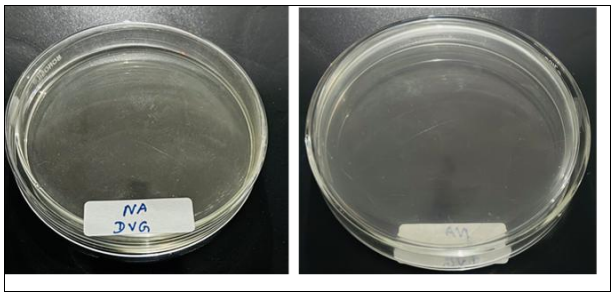

Fig 5: Sterility test by pour plate method

Table 6: Result of sterility test by pour plate method

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Conclusion

From the present investigation, various standardization parameters such as Organoleptic, physicochemical, Phytohemical, Pharmaceutical and Toxicological evaluations were carried out, and it can be concluded that the results obtained were found to be within the permissible limits as per WHO. As the above investigations are not specified in the standard literature such as in pharmacopoeia, the study will be helpful in authentication of Dhanvantari gutika. The result of present study can be served as reference monograph in the preparation of drug formulation

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