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# Physicochemical Analysis of Dashanga Agada – An Ayurvedic Formulation

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***ABSTRACT:***
***Background:*** *Standardization of herbal formulation is essential to assess the quality of drugs. This article reports on standardization of Dashanga Agada, an Ayurvedic formulation indicated in various poisonous and non-poisonous conditions by Ayurvedic as well as traditional Keralian Visha Vaidyas.*

***Aims:*** *Physicochemical standardization of Dashanga Agada.*

***Methodology:*** *Dashanga Agada was prepared as per classics in Gullika (Tablet) form. In-house preparation has been standardized on the basis of organoleptic characters, physical characteristics, Physico-chemical properties, High performance thin layer chromatography (HPTLC) and Thin layer chromatography (TLC) methods.*

***Results:*** *pH of Dashanga Agada at 5% aqueous solution was 4.58%w/v, Loss on drying at $110^0$C 10.57% w/w, Total Ash 8% w/w, Acid Insoluble Ash 2.45% w/w, Water Soluble Ash 23.85% w/w, Alcohol Soluble Extractive 15.87% w/w, Methanol Soluble Extractive 23.66% w/w. Fluorescence analysis results indicated no fluorescent material in formulation. Microbial limit test (MLT) showed there was no growth of organisms after 24hrs of incubation as per IP. Thin Layer chromatographic analysis (TLC) showed 10 and 11 picks at 254nm and 366nm respectively.*

***Conclusion:*** *The set parameters can be used as reference standards for the quality control.*

***KEYWORDS:*** *Dashanga Agada, High performance thin layer chromatography, Standardization.*

## I. INTRODUCTION

Dashanga Agada (DA) an Ayurvedic formulation consists of 9 herbs and 1 mineral in *Gullika* (Table1) form. It is one of the most widely used Ayurvedic formulation indicated in *Kita Visha* (Insect bite).[1] by Ayurvedic classics as well as Traditional Kerala Visha Vaidya's.

**Table 1: Ingredients of DA**
WHO collaborates and assists health ministries in establishing mechanism for the introduction of traditional

| Sr No | Drug Name | Latin Name | Part Used | Quantity |
|---|---|---|---|---|
| 1. | Vacha | Acorus calamus Linn. | Kanda (Rhizome) | 1 part |
| 2. | Hingu | Ferula narthex Boiss. | Niryasa (Gummy resin) | 1 part |
| 3. | Vidanga | Embelia ribes Burm. | Phala (Fruit) | 1 part |
| 4. | Saindhava | Rock salt | ---- | 1 part |
| 5. | Gajapippali | Scindapsus officinalis Schott. | Phala (Fruit) | 1 part |
| 6. | Patha | Cissampelos pareira Linn. | Mula (Root) | 1 part |
| 7. | Prativisha | Aconitum heterophylum Wall. | Kanda (Rhizome) | 1 part |
| 8. | Shunthi | Zingiber officinale Rosc. | Kanda (Rhizome) | 1 part |
| 9. | Maricha | Piper nigrum Linn. | Phala (Fruit ) | 1 part |
| 10. | Pippali | Piper longum Linn. | Phala (Fruit ) | 1 part |

WHO collaborates and assist health ministries in establishing mechanism for the introduction of traditional plant medicines into primary healthcare programs, in assessing safety and efficacy, in the quality control of raw and processed materials.[2] The need of quality control for *Ayurvedic* drugs is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of *Ayurvedic* pharmacy in present era.[3] The present study carried out to develop standardization of *Dashanga Agada*.

## II. MATERIALS AND METHODS

### 2.1. Collection and Identification of plant materials

The raw drugs used for preparation of DA were procured from KLEU's GMP certified Ayurved Pharmacy, Belgaum, Karnataka, India and authenticated by AYUSH approved Drug Testing Laboratory, KLEU's Shri. BMK Ayurved Mahavidyalaya and Research Centre, Belgaum, Karnataka.

### 2.2. Preparation of Dashanga Agada

All the authenticated drugs were powdered separately, passed through 80 # sieve and then mixed together in specified proportions (Table 1) to get uniformly blended C*hurna.* The rolled *Gullika* (Tablets) were prepared after getting desired consistency, shade dried and was packed in a tightly closed glass containers for further use.

### 2.3. Chemicals

Solvents and chemicals of analytical grade were procured from E. Merck and S.D. fine chemicals, Mumbai.
Test Solution: Methanol extract of DA.

Stationary Phase: Silica gel $GF_{254}$ for TLC plates with aluminium sheet support (0.2mm thickness) (E. Merck) were used. Mobile Phase- Toulene:Ethyl acetate (7:3v/v) was selected as solvent system through trial and error method. The developed plates were visualised under visible day light, short UV (254nm), long UV (366nm) and RF values were recorded.

HPTLC - Instrumentation and Chromatographic conditions - The samples were spotted (10 µL) in the form of bands of width of 6 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 $F_{254}$ (5 cm X10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm X 0.45 mm and scanning speed of 20 mm/sec was employed. The linear ascending development was carried out in 10 cm X 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Ethyl Acetate (7:3 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 340 nm for all developments operated by WINCATS software version 1.4.2.

### 2.4. Physico chemical evaluation

DA was subjected to various analytical parameters as follows –
Organoleptic parameters: *Rupa* (colour), *Rasa* (Taste), *Gandha* (odour), *Sparsha* (Touch), [4] Physico-chemical Parameters: pH% w/v of aqueous solution.[5] Loss on drying at 110°C.[6] Ash value.[7] Acid insoluble ash.[8] Water soluble extractive.[9] Hydro alcoholic soluble extractive, methanol soluble extractive.[10]
Quantitative test for Gullika: Weight variation test. [11] Tablet hardness test. [12] Tablet disintegration time. [13] Friability.[14]
Qualitative test for various functional groups. [15-16]
Microbial limit Test was carried out for Fungal and Bacterial study. [17]
Fluorescence analysis: The powdered sample of DA was exposed to UV light at wavelength of 254nm and 366nm. Results were recorded. [18]
Physical Characteristics: Bulk densities, Tap density, Haussner Ratio, Carr's index were determined and recorded. [19-20]

## III. RESULTS AND DISCUSSION

Organoleptic characters for finished product of DA shows - Surface of DA was uniform and without any cracks, was grey in colour, DA was pungent, bitter in *Rasa* due to more *Bhavana,* DA having characteristic *Gandha* (odour) due to the specific ingredients as well as *Bhavana Dravya*, DA was harder in *Sparsha* because of reduction in particle size due to more *Mardana* (Trituration). [Table2]

**Table 2: Organoleptic characters of DA**

| Sr. No. | Parameters | DA |
|---|---|---|
| 1 | Colour | Grey |
| 2 | Odour | Characteristic |
| 3 | Taste | Pungent, Bitter |
| 4 | Consistency | Hard |

Physico chemical analysis – pH value represents alkalinity and acidic nature of formulation, pH of DA was weak acidic. Acidic nature of DA was due to *Bhavana dravya* (Triturating media) given for more duration. Loss of drying indicates the moisture content, in DA it was 10.57%w/w. Presence of inorganic substances in the formulations indicated by determination of Ash value, which plays important role in standardization, more ash value denotes higher inorganic substances, in present sample Ash value was 8%w/w. Various components have different solubility media, present formulation solubility was seen in water and methanol, Water and methanol soluble extractive value of DA was 23.85%, 23.66% respectively which shows that DA having more bioavailability in water media than methanol.[Table 3]

**Table 3: Physicochemical properties of DA**

| Sr. No. | Parameters | DA |
|---|---|---|
| 1 | pH at 5% aqueous solution (% w/v) | 4.58 |
| 2 | Loss on Drying at $110^0$C (% w/w) | 10.57 |
| 3 | Total Ash (% w/w) | 8 |
| 4 | Acid Insoluble Ash (% w/w) | 2.45 |
| 5 | Water Soluble Ash (% w/w) | 4.96 |
| 6 | Water Soluble Extractive (%w/w) | 23.85 |
| 7 | Alcohol Soluble Extractive (%w/w) | 15.87 |
| 8 | Methanol Soluble Extractive (%w/w) | 23.66 |

Average Weight, Disintegration time, Hardness and Friability of DA were given in [Table 4], the weight variation is +/- 2%, by this proper fixation of therapeutic dose can be achieved. Hardness and Disintegration of DA is more due to more *Mardana* duration, friability was 0.764 %, helps to carry easily with less percentage of breakage.

**Table 4: Quantitative parameters of DA**

| Sr. No. | Parameters | DA |
|---|---|---|
| 1 | Wt. Variation Test | +/- 2% |
| 2 | Tab. Disintegration Time (min) | 28 |
| 3 | Hardness ($Kg/cm^2$) | 11 |
| 4 | Friability (%) | 0.764 |

Qualitative analysis shows presence of Carbohydrates, reducing sugar, alkaloids, proteins, amino acids, fats and oils, steroids, Flavonoids, Saponins was present given in Table 5 and 6 respectively.

**Table 5: Qualitative parameters of DA – Organic test**

| Sr. No. | Parameters | Test | DA | | |
|---|---|---|---|---|---|
| | | | Aq | A/L | M |
| 1 | Carbohydrates | Molish | + | + | - |
| 2 | Reducing Sugar | Benedict's | + | + | + |
| 3 | Non reducing sugar | Benedict's | - | - | - |
| 4 | Proteins | Biurets test | + | - | - |
| 5 | Amino Acids | --- | + | + | - |
| 6 | Fats and Oils | --- | + | + | - |
| 7 | Volatile oils | --- | - | - | + |
| 8 | Steroids | --- | + | + | - |
| 9 | Glycosides | Cardiac Glycosides | - | - | + |
| 10 | Saponins | --- | + | + | - |
| 11 | Flavonoids | --- | + | - | - |
| 12 | Alkaloids | Dragandroff's | + | + | + |

+ Present, - Absent, Aq – Aqueous, A/L – Alcoholic, M - Methanolic

**Table 6: Qualitative parameters of DA - Inorganic test**

| Sr. No. | Parameters | DA |
|---|---|---|
| 1 | Carbonate | + |
| 2 | Calcium | - |
| 3 | Magnesium | - |
| 4 | Potassium | - |
| 5 | Iron | + |
| 6 | Sulphate | - |
| 7 | Chloride | + |
| 8 | Nitrate | + |
| 9 | Sodium | + |

+ Present, - Absent

Findings of Physical characteristics, Fluorescence analysis, Microbial limit test (MLT) is given in Table 7-9 respectively. Tapped density gives information on consolidation of powder. The Hausner ratio and Carr's index are both measures of the flow properties. Fluorescence analysis results indicated no fluorescent material in formulation. MLT showed there was no growth of organisms after 24hrs of incubation as per IP.

**Table 7: Physical characteristics of DA**

| Sr. No. | Formulation | Bulk Density (gm/ml) | Tap Density (gm/ml) | Angle of Repose | Hausner Ratio | Car's Index (%) |
|---|---|---|---|---|---|---|
| 1 | DA | 0.714 | 0.869 | 40.596 | 1.217 | 21.70 |

**Table 8: Fluorescence analysis of DA**

| Sr. No. | Materials | DA | | |
|---|---|---|---|---|
| | | DL | UV 254nm | UV 366nm |
| 1 | Powder As such | LG | DGR | LG |
| 2 | P + N. NaOH | BR | DBR | GR |
| 3 | P + Picric Acid | BR | DBR | DGR |
| 4 | P + Acetic Acid | BR | DBR | DGR |
| 5 | P + 1N. HCL | BR | BL | DGR |
| 6 | P + 1N. $HNO_3$ | BR | BL | DGR |
| 7 | P + Iodine 5% | DBR | DBL | DBR |
| 8 | P + 5% $FeCL_3$ | BR | DBL | GR |
| 9 | P + 50% $HNO_3$ | BR | DBL | DGR |
| 10 | P + Methanol | BR | BL | GR |
| 11 | P + Methanol + NaOH | DBR | DBL | DGR |

LG: light green, BR: brown, DBR: dark brown, DGR: dark green, BL: black, DBL: dark black, GR: green

**Table 9: Microbial limit test of DA**

| Sr. No. | Pathogens | Limits (As per IP) | Results |
|---|---|---|---|
| | | | DA |
| 1 | E coli | Absent | Absent |
| 2 | S aureus | Absent | Absent |
| 3 | P aeruginose | Absent | Absent |
| 4 | S abony | Absent | Absent |

TLC analysis – Rf values and TLC plate photograph is shown in Table10 and Fig.1, 2 respectively.

**Table 10: TLC - Rf values of DA**

| Extract | Solvent System | Spots at UV 254 nm | Spots at UV 366 nm |
|---|---|---|---|
| Methanol | Toulene:Ethyl Acetate (7:3) | 0.09, 0.32, 0.36, 0.42, 0.48, 0.56, 0.74, 0.79, 0.31, 0.87 | 0.10, 0.21, 0.33, 0.46, 0.53, 0.58, 0.64, 0.72, 0.82, 0.89, 0.93 |

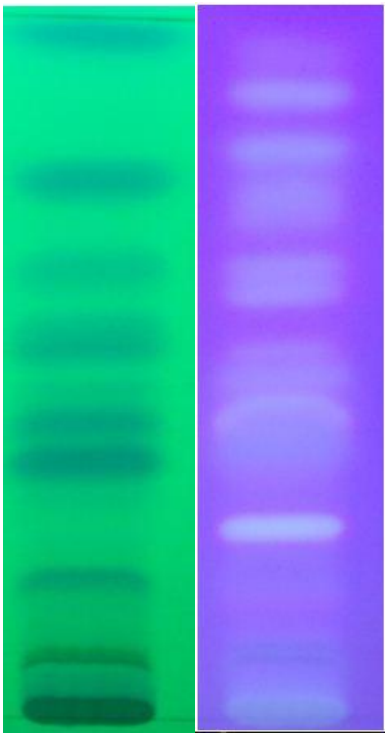

Fig. 1 and 2 - TLC of Methanolic Ext. Of DA at - 254nm 366nm

HPTLC profile results shown in Table11 and Fig. 3 respectively.

**Table 11: HPTLC analysis of Methanolic Extract at 340nm**

| Peak | Rf | Height | Area |
|---|---|---|---|
| 1 | 0.07 | 7.9AU | 4298.3AU |
| 2 | 0.50 | 2.0AU | 378.5AU |
| 3 | 0.57 | 43.9AU | 3363.2AU |
| 4 | 0.70 | 4.1AU | 6805.1AU |
| 5 | 0.96 | 0.0AU | 1426.4AU |

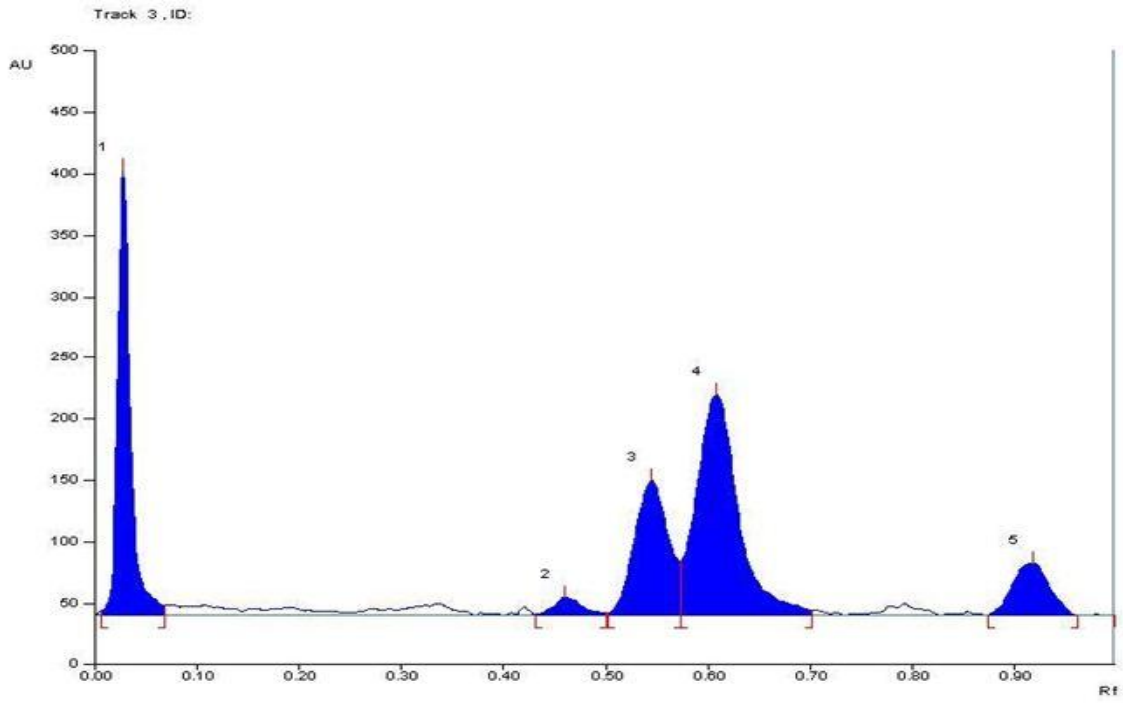


Fig. 3: HPTLC fingerprinting of Methanolic Ext. Of DA

## IV. CONCLUSION

Present work carried out for development of quality standards of Dashanga Agada. Physicochemical, preliminary phytochemical studies, HPTLC and TLC profile have been useful for identity of *Ayurvedic* formulation. The results obtained from this study could be utilised for the standardization of formulations.

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