Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (4), 2022;

Physicochemical and Phytochemicals investigation of Dooshivishari Agada by HPLC

Research Article

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Abstract

Background: *Dooshivishari Agad* (DA) is a herbo-mineral formulation which has been reported in Indian Traditional Medicine for the treatment of latent poisoning. Objective: to investigate active components from DA using HPLC-UV technique. Methods: Physicochemical characterisation including Loss on drying, Acid insoluble Ash, Water soluble ash, Total ash value, Water & Alcohol soluble Extractive Values were tested and evaluated. Phytochemical analysis was performed by RP-HPLC. Observation: from collective analytical study it confirmed that the Loss on drying 2.54%,Water soluble Ash 1.84%, Acid insoluble Ash 0.5% , Total Ash value 5.20% Water Soluble Extractive Value 16.89%, Acid Soluble Extractive Value 23.94%, pH 5.6.Preliminary data of phytochemical screening revealed that it is rich in phenol compound and terpenoids Conclusion: This study provide the data for standardisation & phytochemical characterisation of DA for its pharmacological action.

Key Words: *Dooshivishari Agad,* Physicochemical , Phytochemical, HPLC.

Introduction

Several literatures have revealed that approximately seventy percent of the world population is still rely on medicinal plants as their primary source of medicines. Ayurveda, the oldest traditional system of India reveals that ancient Indians had a rich knowledge of medicinal value of different plant species. To counter-act the action of *poison* ancient *Acharyas* have mentioned antidotes in ancient text, these antidotes have broad spectrum of activities (1). *Dooshivishari Agada* (DA) is a herbomineral formulation that is described as detoxifying property (2). It is a potent remedy widely used in Ayurvedic practice. This formulation has a potential to cure allergic & chronic disorder in human being (3,4,5) .This DA is combination of ten herbs and two minerals. In addition in the manual under DA, all selected herbal species are often reported in Ayurveda practices for treating different disorders.

Nevertheless the lack of documentation and quality control standards for alternative medicines, despite the reality that vast ancient literatures offer evidence of their therapeutic value, has limited their acceptance in comparison to their modern counterparts. Hence, it becomes crucial to understand more about the standardisation of reported plant material used in DA .Hence the present study reflects to understand its physicochemical parameters, including extractive values, total ash, acid insoluble ash, and water-soluble ash, moisture content, pH values as well as its phytochemical valuation by RP-HPLC technique.

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Material and methods

Materials

All raw plant materials were purchased from local market and Drugs identification and authentication was confirmed from Dravyaguna department of M.G.A.C.H.RC, Wardha. All chemicals and solvents were of HPLC and AR grade purchased from Merck and S.D. fine chemicals (Mumbai, India).

Ingredients of D. A depicted in (Table no 1).

Table 1: Contents of *Dooshivishari Agad*

Sr. No	Name of Drug	Latin /English name	Part Used	Quantity
1	Pimpali	*Piper longum*.Linn	Fruit	50gm
2	Dhyamak	*Cymbopogon Martini*.Roxb.Wats.	Leaf	50gm
3	Jatamansi	*Nardostachys jatamansi*. DC.	Root	50gm
4	Ela	*Elattaria Cardamomum*.Mat on	Seeds	50gm
5	Lodhra	*Symplocos racemosa* .Roxb.	Bark	50gm
6	Katunatam	*Oroxylum indicum*.Vent	Root bark	50gm

7	Tagar	*Valeriana wallichi*.D.C	Root	50gm
8	Kuth	*Saussurea lappa*.C.B.Clark.	Root	50gm
9	Mulethi	*Glycyrrhiza glabra* Linn.	Root	50gm
10	Chandan	*Santalum album*.Linn	Heart wood	50gm
11	Suvarchika	Potassium Nitrate		50gm
12	Gairik	Red ochre		50gm

Methodology

Preparation of *Dooshivishari Agada*

All selected herbal ingredients were thoroughly cleaned, dried and pulverized to get fine powder. All pulverized ingredients were sieved (Sieve no 80) and were mixed and ensured uniform distribution of all contents. Furthermore the red ochre (purified in Goghrut) and the potassium nitrate were added in the mixture. Finally the prepared sample of DA was stored in air tight container.

Physicho-chemical studies of Dooshivishari Agada (6,7,8)

Analytical study was done to establish the basic quality standards of *Dooshivishari Agada* .The formulation was first tested for organoleptic character such as odour , colour and taste . (Table no 2). Physicochemical analysis includes loss on drying at 105^{0}C, total ash, Acid insoluble ash, Alcohol soluble extractives, water soluble extractive, pH (Table no 3). Microbial specifications were tested to validate its safety for internal as well as external use. Enterobacteriaceae, Total fungus count, E-coli, Salmonella, Pseudomonas aueruginosa performed (Table no.4).

Determination of Total Ash

The 2gm sample was placed in a weighted dish and heated for 3 hours in a muffle furnace at 450°C. Continue heating was done until a constant weight was obtained. The dish was cooled in a desiccators and weighted. Then percentage of ash with reference to air dried sample was calculated as:

Total Ash = 100* (weight of Ash)/weight of sample obtained for test.

Water Soluble Ash

The total ash was boiled for 5 minutes in 25 cc of water; the insoluble material was then collected in an ash-free filter paper, washed with hot water, and ignited for 15 minutes at a temperature no higher than 450^{o}C. By deducting the weight of the insoluble material from the weight of the ash, the water-soluble ash is represented by the difference in weight. With reference to the air-dried medication, the proportion of water-soluble ash was calculated.

Acid Insoluble Ash

Using 25 ml of diluted hydrochloric acid, boil the complete ash for 5 minutes. Then, collect the insoluble material in ashless filter paper, wash with hot water, and ignite to a consistent weight. With reference to the air-dried medication, the proportion of acid-insoluble ash was calculated.

Water soluble extractive

A 5gm coarsely powdered, air dried sample was transferred into a glass-stoppered, 250ml reflux conical flask, followed by 50ml of boiled water. The flask was shaken vigorously before being put aside for 10 minutes. It was filtered and cooled. The filtrate was transferred to a 7.5cm diameter evaporating dish, where the solvent was evaporated on a water bath, then allowed to dry for 30 minutes before being dried in an oven and the residue weighed.

Alcohol soluble extractive

In a closed flask, 5 gm. of dried samples was macerated with 100 ml of alcohol, shaking frequently for the first 6 hours, and then left to stand for 18 hours separately. Following that, it was quickly filtered in order to minimize methanol loss. In a tared flat bottom shallow dish dried at 105^{0}C and weighted, evaporate 25ml of filtrate to dryness. With the air dried samples as a reference, the percentage of alcohol soluble extractive was calculated.

Loss on drying

10 g of the drug was placed in a tared evaporating plate it after accurately weighing. Then dried it at 105°C for 5 hrs & weighed. Continue drying and weighing per hour until the difference in weight between two consecutive measurements is no greater than 0.25 percent. When there is less than a 0.01 g difference between two successive weightings after drying for 30 minutes and cooling for 30 minutes in desiccators, constant weight has been reached.

Determination of pH

10%aqueous solution of DA was taken in a beaker and reading was noted by digital thermometer .

Determination of microbial specification

1ml sample of DA was dissolved in 15ml liquefied casin Soyabean digest agar and add that mixture on a petridish 9-10 cm in diameter. Incubate at 30^0 to 50^0 for 24 hours. After incubation sample was tested for Staphylococcus aureus,Pseudomonas aueruginosa, Salmonella, Ecoli. For fungi Sabouaraud dextrose agar was used.

Phyto-chemical Analysis by HPLC

HPLC analyses were performed on a Shimadzu, LC-20AT system (model SCL10-AVP SHIMADZU corporation, Kyoto, Japan) equipped with LC-10AT tertiary pump, detector, and interface. The data were acquired and processed using Lab Solution software.

The phytochemical analysis was performed on a reverse-phase Zodiac C18 (4.6 mm × 250 mm, 5 µm).

The mobile phase was composed of 30% H_2O with 0.1% and 70% acetonitrile. Prior to HPLC injection, samples were filtered through 0.20µ nylon filters. Exactly 20µl of freshly prepared sample was injected to the column and analysied for 75 min at 28^{0}C with a flow rate of 1 ml/min. Entire separation was monitored at 254nm wavelength.

Observations and Results

Table 2: Organoleptic character of DA

Sr. no.	Organoleptic character	DA
1	Colour	Brownish
2	Odour	Characteristic
3	Taste	Bitter

Table 3: Physicochemical property of DA

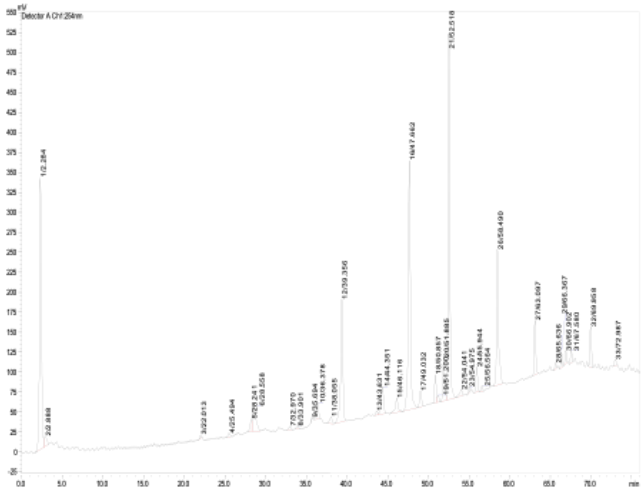
Sr no.	Test parameter	Observation
1	Loss on drying at 105^{0}C	2.54%
2	Water soluble ash	1.84%
3	Acid insoluble ash	0.5%
4	Total Ash Value	5.20%
5	Water soluble extractive	16.89%
6	Alcohol soluble extractive	23.94%
7	pH	5.6

Table 4: Microbial specification

Specification	Pharmacopeia standard	DA
Total Viable count	Maximum 10^5/gm	No growth
Enterobacteriaceae	10^3/gm	Absent
Total fungus count	Maximum 10^3/gm	Absent
E- coli	Maximum 10^3/gm	Absent
Salmonella	None	Absent
Staphylococcus aureus	Absent	Absent
Pseudomonas aueruginosa	Absent	Absent

HPLC observation

Fig. no. 1



The HPLC for Methanol extract of DA monitored at 254nm wavelength Those constituents identified in 2-4 mins in HPLC report (Fig.x) were not considered since they eluted at void volume and hence they are considered unretained components. Importantly those compounds detected in the range of 4-30 mins were collectively considered to have acid/base properties and hence in these ranges of HPLC chromatograph 3 components were identified which have either acid or basic characteristics. Moreover, those compounds retained in 30-55 mins are considered to be polyphenols/flavonoids such as chrysin, gossypin, juglon, plumbagin, galangin, quercetin, naringin, hesperidin, myricetin, rutin etc, which exhibit strong absorbance at 254 nm wave-length in UV detection. In addition, those compounds intersperse between 55-70 mins were considered as terpinoids and after 72 minutes are steroids. Therefore, as displayed in Figure 1; exactly 12 polyphenols/flavonoids were identified between 30-55 mins interval. After polyphenols, roughly 7 potential terpinoids were detected at 254 nm wavelength but no any steroids were detected at 254 nm wavelength.

Table 5 : Determination of Phytochemicals by HPLC

Sr No	Test for Determination	Confirmation	No. of Components	Contribution
1	Aliphatic/Phenolic/ Polyphenolic Acids	+	**2**	**0.36%**
2	Phytoamines/ Alkaloid	-		
3	Polyphenols/ Flavonoids/ Antioxidants	+	**16**	**62%**
4	Glycosides/Sugar	-		
5	Tocopherols	+	**11**	**20%**
6	Phytosterols/ Steroids	+	**2**	**2.5% (including Glycosides)**
7	Miscellaneous Category	-		

Discussion

In Samhita *Dooshivishari Agad* is basically mentioned for the latent poisoning. Now a day this formulation is widely used by all clinician for treating all the skin disease & chronic diseases condition. In the current study DA was analysed for physicochemical and phytochemical analysis. Organoleptic analysis, which serves in the initial quality assessment, is the evaluation of a product as it is perceived by sense organs. Thus DA was subjected to an organo- leptic analysis as a preliminary quality check, which proved that the brown colour and bitter taste were distinctive features of DA. Loss on drying at 105^{o}C was 2.5% which suggest quality and stability of formulation as due to the less moisture it is not likely to get contaminated by fungal growth (9,10).

Less Ash value indicates less inorganic matter and silica were detected (11). pH of DA was 5.6 which indicate that it is basic in nature and does not cause any

adverse effect on the gastric mucosa. The extractive value is a valuable tool to assess and determine the chemical ingredients contained in the crude medication as well as useful in determining the chemical proportions soluble in a certain solvent system. The extractive value of DA in water & ethanol is 16.89% and 23.94% respectively .High alcohol-soluble extractive value indicates the presence of polar ingredients such as steroids, phenolics, flavonoids, and glycosides, while water-soluble extractive value shows the presence of acids, sugars, and inorganic compounds. (12).

Phytochemical analysis by HPLC indicates that DA is a rich source of phytoconstituents Polyphenols , and Terpenoids. Animal and human researches have provided evidence that polyphenols' is having anti-inflammatory and antioxidant capabilities. (13)

Phenolic compounds may prevent systemic and local inflammation by restoring the redox equilibrium to reduce oxidative stress and by modifying inflammatory responses by mitigating cytokine pathways(14) Flavanoids such anthocyanins, catechins, flavanols, flavones, flavanones, and isoflavones may combat free radicals and lower the risk of cancer by stopping cellular growth in tumours(15,16). These phytochemical both flavonoids & phenolic components have been also reported for immune system promoting, skin protection from UV radiation (17, 18). Terpenoids have been found to be useful in the prevention and management of many diseases like cancer. It also possesses antimicrobial, antifungal, anti-parasitic, antiviral, anti-allergic, anti- inflammatory and immune -modulator properties. (19, 20).

Conclusion

Physicochemical characters are of great significance for the purity, authenticity and action of drugs. Analytical findings of the current study can be considered as reference standard for DA. The scope of DA in medical practice is broadening as a result of phytochemical investigation revealing that the polyphenol/flavonoids are the active component of DA.

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