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# ROLE OF *AJAMOOTRA BHAVANA* ON PHYSICO-CHEMICAL ASPECTS OF *BILWADI AGADA* WITH SPECIAL REFERENCE TO *KALA* (DURATION) – AN ANALYTICAL STUDY

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## ABSTRACT

**Background:** *Bilwadi Agada* is indicated for the management of *sarpa visha, lootavisha, Garavisha* etc. It needs to be potentiated in order to increase its *veerya*. *Bhavana* samskara, is a wet trituration process and also considered as a size reduction technology, is an essential processing practice in preparation of *Bilwadi Agada.* Ambiguity in Protocol of *bhavana samskara* still prevails among Ayurveda fraternity as no specific time duration is indicated and hence need to be standardized with respect to safety and efficacy. Hence preclinical evaluation of *Bilwadi agada* is undertaken to standardize time duration of *bhavana samskara* by keeping particle size as analytical parameter. **Aims and Objective:** To evaluate the impact of *Ajamootra bhavana* on Physico-chemical and Phyto-chemical parameters of *Bilwadi Agada* and To evaluate variation in particle size of *Bilwadi Agada* depending upon different time interval of *Bhavana samskara* **Methodology:** *Bilwadi Agada* was prepared using *Ajamootra bhavana* in different time intervals. Three samples were analysed for physico and phytochemical parameters by keeping particle size as one of the investigating tool. **Observation and Results:** Qualitative analysis revealed presence of Carbohydrates, reducing sugar, alkaloids, proteins, amino acids, fats and oils, steroids, flavonoids, saponins, Carbonate, Iron, Chloride and Nitrate in all samples. Increase in particle size observed on time dependent manner with reference to bhavana samskara. **Conclusion:** particle size varies in relation with time duration, demands further studies.

**Keywords** *Bilwadi Agada*, *Bhavana samskara* and Particle size

**INTRODUCTION:** Pharmaceutics of Ancient traditional science is well developed and established which is called as *Bhaishajya kalpana* conglomerating many principles of *Ayurveda* while formulating a medicine without violating the fundamentals. Among such concepts, *Samskaras* (The procedures intended to alter qualities of a drug for better efficacy) is one of the techniques developed at that time.

The word *Samskara* is an significant concept headed by ancient Ayurveda researchers and is defined as transformation (*Samskaro hi Gunantaradhanam uchyate*) of the inherent qualities (*Swabhavika Guna*) of a substance which leads to the addition of new properties.[1,2,3] Various modes of *Samskara* are mentioned in *Ayurvedic* pharmaceutics such as *Swedana* (boiling), *Mardana* (grinding), *Manthana* (churning), *Bhavana* (impregnation) etc. [4]

Amongst them, *Bhavana* is an important *Samskara* with the help of which, not only the potency of a drug can be altered; but is also capable to bring about changes in characteristics of drug viz. regulation, addition of new or removal of undesirable characteristics.[4] Bhavana is a unique pharmaceutical procedure in which a drug or mixture of drugs in powdered form is triturated with sufficient quantity of liquid media [viz. plant extractives (expressed juice, decoction etc) or animal products (urine, milk etc)] till liquid portion gets absorbed completely.[5,6]

*Bilwadi Agada* is unique herbal formulation which is indicated in *Sarpavisha, Lootavisha, Vruschika visha Vishuchika, Ajeerna, Garavisha and Jwara.*[7] It needs to be potentiated in order to increase its medicinal value and *bhavana samskara* can be adopted by using *Ajamutra* (Goat urine) and triturated till it becomes dry.[8] The present study intended to evaluate the changes which occur in *Bilwadi Agada* before and after *bhavana* with *Ajamutra* with respect to the particle size of the finished product along with phyto-chemical changes.

**Material and Methods**

**Procurement and Authentication of drugs**

Raw drugs were procured from KLE's Ayurveda Pharmacy, Belagavi and authentication was carried out at AYUSH approved Drug Testing Laboratory, BMK Ayurveda College, Belagavi (Voucher No CRF 14/603-614).

**Table .1 Ingredients of Bilwadi Agada: [9]**

| SI.NO | Drug Name | Latin name | Useful part | Proportion |
|---|---|---|---|---|
| 01 | *Bilwa* | *Aegle marmalos* corr ex Roxb | *Mula* (Root) | 1part |
| 02 | *Surasa* | *Ocimum sanctum* Linn | *Puspha* (Inflorescence) | 1part |
| 03 | *Karanja* | *Pongamia pinnata* Linn | *Beeja* (Seeds) | 1part |
| 04 | *Nata* | *Valerinia wallichi* DC | *Kanda*(Rhizome) | 1part |
| 05 | *Devadaru* | *Cedrus deodaru* Roxb | *Saara*(Heartwood) | 1part |
| 06 | *Haritaki* | *Terminalia chebula* Retz | *Phala*(Fruit) | 1part |
| 07 | *Bibhitaki* | *Terminalia belerica* Roxb | *Phala*(Fruit) | 1part |
| 08 | *Amalaki* | *Emblica officinalis* Gaertn | *Phala*(Fruit) | 1part |
| 09 | *Shunti* | *Zingibera officinale* Rose | *Kanda* (Rhizome) | 1part |
| 10 | *Maricha* | *Piper nigrum* Linn | *Phala*(Fruit) | 1part |
| 11 | *Pippali* | *Piper longum* Linn | *Phala*(Fruit) | 1part |
| 12 | *Haridra* | *Curcuma longa* Linn | *Kanda*(Rhizome) | 1part |
| 13 | *Daruharidra* | *Berberis aristata* DC | *Twak*( Stem bark) | 1part |
| 14 | *Ajamutra* | Goat urine | Urine | Q.S |

**Method of Preparation:** The individual ingredients were powdered using pulveriser and sieved through 120 mesh to obtain a *sukshma churna* and taken to a clean dry vessel in equal proportions (50gms each) and mixed well to obtain a homogeneous mixture. *Bhavana* was given with *Ajamutra* by adding sufficient amount (required amount) to soak and was triturated in a clean *khalwa yantra* till

proper consistency was attained. The formulation was triturated for 56 hours (7days) (Sample B.A 1). The formulation triturated for 112 hours (14days) (SampleB.A2) and the formulation triturated for 168hours (21days) (Sample B.A 3) and were stored in air tight containers.

**Quantity of Ajamutra added during Bhavana:**

*Bhavana* was conducted for total 21days with an average of 6-8hrs per day. On day 1st 1liter of *Ajamutra* was added to *Bilwadi Agada churna* (650g) and 400ml-600ml *Ajamutra* was added on 2nd, 3rd, 4th, 5th, 6th, 7th day respectively. 350ml-400ml *Ajamutra* was added on 8th, 9th10th, 11th, 12th, and 13th, 14th. 180ml- 300ml *of Ajamutra* was added on 15th, 16th, 17th, 18th, 19th, and 20th, 21th respectively.

**Physico-chemical Evaluation:**

Organoleptic characters, Loss on Drying, Ash value, Water soluble extract, Alcohol soluble extract and pH in 5% aqueous suspension were assessed. Qualitative assessments of functional groups were also carried out. [9.10]. Qualitative analysis of both Phyto-chemicals and functional groups along with inorganic components were carried out. Thin Layer Chromatography was carried out to ascertain separation of bands and band pattern. Ethanolic extract was used for TLC analysis. Toluene and Ethyl acetate (7:3) were used as solvent system.

**Particle size Evaluations -** Particle size was evaluated by using a Carl zeiss microscope and counting method was adopted for the measurement. Particle size was evaluated on 7th, 14th and 21st day; it was estimated to see the effect of continuous trituration in a liquid media under pressure. [10]

**Observations and Results:**

*Abhavita churna* was Light yellow in colour with amorphous consistency and having slightly *tikta pradhana rasa*. After *Bhavana samskara, Bilwadi Agada* was brownish black in colour with significant odour of the *Ajamutra* and increased taste of *Tikta* and also of *Kashaya rasa.* Presence of organic functional groups and inorganic elements has been enumerated in Table 1 and 2 respectively. Changes observed with respect to physico-chemical parameters are enumerated vide Table No 3. Before *bhavana samskara*, particle size was measuring with a range of 248.07 microns. Later on 14th day there was increase in the particle size (368.32 microns). Half of the particles were increased to more than 461.4 microns on 21st day. TLC analysis revealed the presence of bands with the Rf values 0.05, 0.98, 0.22, 0.27, 0.37, 0.45, 0.57, 0.66, 0.71 on exposure to 254 nm of UV and 0.10, 0.14, 0.20, 0.24, 0.28, 0.32, 0.37, 0.41, 0.55, 0.59, 0.70 on exposure to UV 366 nm (Table No 4).

*Table 2(Data Showing Assessment of Functional groups in three samples)*

| Organic Constituents of Bilwadi Agada | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Sl.No* | ***Phyto chemical*** | *BA1* | *BA2* | *BA3* | *Sl.No* | ***Phyto chemical*** | *BA1* | *BA2* | *BA3* |
| *1* | ***Carbohydrates*** | + | + | + | *7* | ***Non Reducing Poly saccharides (Starch)*** | - | - | - |
| *2* | ***Proteins*** | + | + | + | *8* | ***Glycosides*** | - | - | - |
| *3* | ***Reducing*** | + | + | + | *9* | ***Alkaloids*** | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***sugars*** | | | | | | | | |
| *4* | ***Mono saccharides*** | - | - | - | *10* | ***Saponins*** | - | - | - |
| *5* | ***Pentose sugars*** | - | - | - | *11* | ***Flavonoids*** | - | - | - |
| *6* | ***Fats and oils*** | + | + | + | *12* | ***Steroids*** | + | + | + |

**Table 3 (Qualitative analysis of inorganic compounds)**

| **Inorganic Constituents of Bilwadi Agada** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Sl.No* | *Inorganic Elements* | *BA1* | *BA2* | *BA3* | *Sl.No* | *Inorganic Elements* | *BA1* | *BA2* | *BA3* |
| *1* | ***Calcium*** | + | + | + | *6* | ***Sulphate*** | + | + | + |
| *2* | ***Magnesium*** | + | + | + | *7* | ***Phosphates*** | - | - | - |
| *3* | ***Sodium*** | + | + | + | *8* | ***Chloride*** | + | + | + |
| *4* | ***Potassium*** | - | - | - | *9* | ***Carbonate*** | - | - | - |
| *5* | ***Iron*** | + | + | + | *10* | ***Nitrate*** | - | - | - |

**Table No.4 (Physicochemical Properties of Bilwadi Agada)**

| **SL.NO** | **PARAMETERS** | **BA1** | **BA2** | **BA3** |
|---|---|---|---|---|
| 1 | pH at 5% aqueous solution | 7.80 | 7.4 | 8.4 |
| 2 | Loss on Drying at $110^0$c (%w/w) | 10.243% | 12.195% | 12% |
| 3 | Total Ash(%w/w) | 14.099% | 17.475% | 20.7% |
| 4 | Acid insoluble value(%w/w) | 0.966% | 1.941% | 2.415% |
| 5 | Water soluble Extract(%w/w) | 13.40% | 15.2% | 15.4% |
| 6 | Alcohol Soluble Extract (%w/w) | 7.803% | 10.44% | 7% |

**Table No. 5 (TLC Report)**

| **Extract** | **Solvent System** | **Spots at UV 254 nm** | **Spots at UV 366 nm** |
|---|---|---|---|
| Ethanol | Toluene : Ethyl Acetate (7:3) | 0.05,0.98,0.22,0.27,0.37, 0.45,0.57,0.66,0.71 | 0.10,0.14,0.20,0.24,0.28,0.32, 0.37,0.41,0.55,0.59,0.70 |

**DISCUSSION:** Ambiguity related to duration of *bhavana* remains persistent till standardization process and present study is an effort in studying different *bhavana kala* and its effect on the physico-chemical qualities of *Bilvadi agada.* Conventionally followed duration for *bhavana samskara* such as 7, 14 and 21 days is adopted in this study to evaluate particle size and other functional group analysis. Significant increase in *Tikta-Kashaya Rasa* and Tingling sensation attributed of the *Bilwadi Agada* can be related to the amount of *Ajamootr*a used for *Bhavana samskara*. pH of goats urine (*Ajamootra*) is generally alkaline (7.5 – 8.5), hence increased in the *Bilwadi Agada* with respect to *bhavana kala.* Slight decline in pH of *Bilwadi agada* on $14^{th}$ day may be due to buffering activity of compounds present in organic media such as citrates and oxalates but on continuous usage of *Ajamootra* had resulted in elevation of pH on $21^{st}$ day. Salts present in the urine are of hygroscopic in nature hence hold more moisture even if attempt is made to dry them under shade, which might have played vital role in increased loss on

drying of sample 3 (21 days) as it composed maximum salt content due to usage of *ajamootra* for 21 days and the similar phenomena may also be responsible for ash and extractive values. Both plants and animal products contain traces of carbohydrates, proteins, lipids and steroids/sterols. Accumulation of traces of such compounds over a period of time during *bhavana samskara* might have caused their detection during qualitative analysis. Functional groups such as alkaloids, saponins and glycosides were not detected during qualitative analysis, which may be due to predominance of compounds present in *ajamootra*. Concentrations of inorganic compounds are related with quality of food intake and hence responsible for varied detection during qualitative analysis. Though potassium and phosphates are excreted by kidneys, absence of such compounds in *Bilwadi agada* may be attributed to diet of goats from which urine is collected. Chromatography is an important qualitative analysis and generally conducted in presence of reference standards, which is difficult to derive for poly herbal compounds, whereas banding pattern may be studied with suitable solvent system. TLC analysis in present study is a symbolic and an effort to develop suitable solvent system for future analytical studies.

**CONCLUSION:** Influence of *bhavana dravya* on the qualities of final product is evident as it brings about marked changes in many aspects of physico and phyto-chemical parameters of *Bilwadi agada*. *Ajamootra* over a period of 21 days brings about increase in particle size along with elevation in organoleptic parameters. Efficacy of *bhavana samskara* may be further assessed with experimental and clinical studies.

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