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PHYSICOCHEMICAL ANALYSIS OF *BILWADI AGADA* – AN AYURVEDIC POLY-HERBAL FORMULATION

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ABSTRACT

The quality control assessment of Poly- herbal formulations is of great significance in order to justify their acceptability in modern system of medicine though the drug may be therapeutically potent. Ayurveda and its rapidly increasing use by public have given rise to many newer issues and challenges. Ayurvedic formulations prepared by several manufactures are guaranteed to carry out the quality control test as per preliminary guidelines given by CCRAS (Central Council of Research in Ayurveda & Siddha).Though the standards are followed, still the variability in their results has been observed when compared between same formulations. *Bilwadi Agada* is one such Poly- herbal formulation consisting of 13 drugs & treated in *Garavisha* & *Jangama visha* etc. The condition which produces ailments like *Pandu, Krusha, Alpaghni,Kasa, swasa, Aridta* ,when triggering factor are congregate. An attempt is made here *Bilwadi Agada* prepared by GMP certified pharmacy within house preparation. Results revealed that the samples in their organoleptics, pH & Physico- chemical properties, Thin layered chromatographic study showed result was seen in disintegration time & hardness of sample i.e., hardness is 7.1 but none of them disintegrates in 35min. The physico-chemical data of this study assists in maintaining the standard limits of *Bilwadi Agada*.

Key words: *Bilwadi Agada* , Hepatoprotective, *Vruschika Visha, Garavisha,* TLC.

INTRODUCTION - Ayurveda the ancient spiritual science & Indian system of medicine in the present scenario has gained popularity & also increased inclination towards herbal formulations globally due to its effective & efficacious results witnessed in various diseases & syndromes in the recent epic. Herbal drugs are the core of this system of medicine as the drug possesses all the required quality to prevent & cure various ailments. The principles to standardize the drugs that were developed in ancient period were subjective & are based on the scientific background prevailing in those days .Now they are to be viewed & answered looking the advancement of science & technology in current days. Hence there is very much need to validate Ayurvedic formulations with the aid of sophisticated instrumental & analytical techniques. The need of quality control for Ayurvedic formulations is due to reduction in the procedure of preparing drugs by ancient method due to commercialization of Ayurvedic pharmacy in present era.[1] these manufactures though prepare similar formulation fails to meet the standard quality control parameters when compared. *Bilwadi Agada* is one such Poly- herbal formulation explained in the context of *Garavisha* in Ayurvedic classics which is a combination of 13 drugs & it has efficacy over a condition where in a *visha* (toxins) due to improper elimination from body or when low potent toxins by virtue of which are battered by

climatic conditions stays in body, later produces when *Pandu*, *Krusha*, *Alpaghni*, *Kasa*, *swasa*, *Aridta*, triggering factors are congregate [2].

Table No.1 INGREDIENTS OF *BILWADI AGADA* ARE AS FOLLOWS [3]

SI.NO	Drug Name	Latin name	Used part	Quantity
01	*Bilwa*	*Aegle marmalos* corr ex Roxb	*Mula* (Root)	1Part
02	*Surasa*	*Ocimum santum* Linn	*Puspha* (Inflorosence)	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

MATERIALS AND METHODS:

Collection and Identification of plant materials-

Bilwadi Agada consists of 13 ingredients of which 13 are herbal only. All the drugs of *Bilwadi Agada* 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.

Instruments and Equipment's

Weighing machine, Analytical balance, Pulverizer, Clean cotton cloth, Steel vessel, Mask, Cap, Apron, Sieve no 85 and 120, Gas and stove.

Chemicals - Solvents and chemicals of analytical grade were procured from E. Merck and S.D. fine chemicals, Mumbai for analysis of *Bilwadi Agada*.

Stationary Phase: Silica gel GF254 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.

Method of Preparation of *Bilwadi Agada*: [4]

Preparation of *Churna:* The *Churna* (powder) was prepared as per the procedure explained in Ayurvedic Formulary of India. All drugs were made into fine powder in a pulveriser. These *churna* are passed first through 85# mesh followed by 120 # sieve individually and then all are mixed together in equal proportions to get uniformly blended homogenous mixture.

Preparation of *Bilwadi Agada:-*

Authenticated drugs were pulverised to powder & then sieved through 120 sieve mesh. Daily fresh *Ajamutra* was collected & subjected to urine routine examination to confirm that, its free from pus cells, Micro-organisms, sugar, proteins etc contaminations at KLEU'S Shri

B.M.K.Ayurveda Mahavidyalaya & Research center Laboratory Shahapur, Belagavi, Karnataka. All the individual *choornas* (50gms each) were mixed with *Ajamutra* (Q.S) and *bhavana* was given. *Bhavana dravya* was mentioned in *Bilwadi Agada.* Daily fresh *bhavana dravya* was taken in require quantity & *Mardana* was done till proper consistency was obtained (8hrs). *8hrs Bhavana* was done daily for 22days at KLEU'S Shri.B.M.K. Ayurveda Mahavidyalaya& Research centre RS&BK Laboratory. During the course of preparation of *Bilwadi Agada*- daily temperature & humidity were noted down. & *Mardana* time, colour, consistency, odour, taste of *Bilwadi Agada* were recorded in chart. This procedure was followed for 22days (for more than 170hrs). On 22nd day the vati were prepared & shade dried in stainless steel plate. Then i.e stored in dried air tight container. Good manufacturing procedure followed throughout the preparation of *Bilwadi Agada.*

Physicochemical Evaluation of *Bilwadi Agada* [5]

Analytical study was carried out in AYUSH approved Central Research Laboratory of Shri B.M.K. Ayurveda Mahavidyalaya Belgaum. Microbial Limit Test was carried out in Microbiology Laboratory of KLE University's Shri B.M. Kankanwadi Ayurveda Mahavidhyalaya Belgaum, Karnataka, India.

BA was subjected to various analytical parameters as follows – Organoleptic parameters: *Rupa* (colour), *Rasa* (Taste), *Gandha* (odour), *Sparsha* (Touch).[6] Physico-chemical Parameters: pH% w/v of aqueous solution. [7] Loss on drying at 110oC. [8] Ash value.[9] Acid insoluble ash.[10] Water soluble extractive.[11] Hydro alcoholic soluble extractive, alchohol soluble extractive. [12] Quantitative tests for *Gullika*: Weight variation test. [13] Tablet hardness test. [14] Tablet disintegration time. [15]Friability.[15]

Qualitative test for various functional groups – [16, 17] Microbial limit Test was carried out for Fungal and Bacterial study. [18] Fluorescence analysis: The powdered sample of BA was exposed to UV light at wavelength of 254nm and 366nm. Results were recorded. [19]

Results & discussion:-

Physicochemical analysis of *Bilwadi Agada* by using in house prepared sample has been carried out and the results are shown in tables. Ayurvedic formulations claimed to be made according to CCRAS guidelines are effective but it is very difficult to maintain uniformity in formulations which is may be due to natural heterogeneity, the quality of herbal starting material obtained from wild collection shows more and more fluctuations which can be depicted from present experimental data.[20]

Analytical study was conducted at final product of *Bilwadi Agada*. Physicochemical analysis & preliminary phytochemical analysis of final product of *Bilwadi Agada* was done.

Organoleptic Study -

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

Table No.2: Showing the Organoleptic Characters of *Bilwadi Agada*

Sr. No.	Parameters	*BA*
1	Colour	Brownish black
2	Odour	Ajamutra Gandhi
3	Taste	Pungent, Bitter
4	Consistency	Hard

Physico chemical analysis – pH value represents alkalinity and acidic nature of formulation, pH of DA was bit alkalinity. Alkalinity nature of BA was due to *Bhavana dravya* (Triturating media) given for more duration. Loss of drying indicates the moisture content, in BA it was 12%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 20.7%w/w. Various components have different solubility media, present formulation solubility was seen in water and Alchohol , Water and methanol soluble extractive value of BA was 15.4%, 7% respectively which shows that BA having more bioavailability in water media than alcohol.[Table 3]

Table No 3: Showing the Physicochemical Properties of *Bilwadi Agada*

Sr. No.	Parameters	*Bilwadi Agada(a)*
1	pH at 5% aqueous solution	8.4
2	Loss on Drying at 110^0C (% w/w)	12%
3	Total Ash (% w/w)	20.7%
4	Acid Insoluble Ash (% w/w)	2.415%
5	Water Soluble Ash (% w/w)	4.16%
6	Water Soluble Extractive (%w/w)	15.4%
7	Alcohol Soluble Extractive (%w/w)	7%

Average Weight, Disintegration time, Hardness of BA were given in [Table 4], the weight variation is +/- 2%, by this proper fixation of therapeutic dose can be achieved. Hardness and Disintegration of BA is more due to more *Mardana* duration.

Table 4: Quantitative parameters of BA

Sr.No	Parameters	BA
1	Wt. Variation Test	+/-2
2	Tab. Disintegration Time (min)	35min
3	Hardness (Kg/cm2)	7.1 kg/cm3
4	Friablity	0.784%

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 No.5: Showing Qualitative Parameters of *Bilwadi Agada*- Organic Test

Sr. No.	Parameters	Test	*Bilwadi Agada* (a)Ext.	
			Aq	A/L
1	Carbohydrates	Molish	+	+

2	Reducing Sugar	Benedict's	+	+
3	Monosaccharides	Barfoed's	-	-
4	Non reducing sugar	Benedict's	-	-
5	Pentox sugar	Bial's	-	-
6	Hexose sugar	Selwinoff's	+	+
7	Proteins	Million's	+	+
8	Amino Acids	Cysteine	+	+
		Tyrosine	+	-
9	Steroids		+	+
10	Glycosides	Cardiac Glycosides	-	-
11	Flavonoids		+	-
12	Alkaloids	Dragandroff's	+	+
13	Saponin		+	-
14	Fats & Oil	-	+	+

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

Table No.6: Showing Qualitative Parameters of *Bilwadi Agada*- Inorganic Test

Sr. No.	Parameters	*Bilwadi Agada*
1	Calcium	Present
2	Magnesium	Present
3	Sodium	Present
4	Potassium	Absent
5	Iron	Present
6	Sulphate	Present
7	Chloride	Present
8	Carbonate	Absent
9	Nitrate	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 No 7: Physical Characteristics of *Bilwadi Agada*

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

Fluroscence Analysis of *Bilwadi Agada*: The powder of the BA was made and subjected to various reagents. It was then observed under Normal light, 254nm and 366nm.The results are mentioned below in table no.8.

Table No .8 Showing Fluroscence Analysis of *Bilwadi Agada*

Sr.No	Materials	*Bilwadi Agada*		
		DL	**UV 254nm**	**UV366nm**
1	Powder As such	DB	DB	DB
2	P + N. NaOH	DY	G	B
3	P + Picric Acid	B	LG	B
4	P + Acetic Acid	B	G	B
5	P + 1N. HCL	B	G	DB
6	P + 1N. HNO3	B	G	DB
7	P + Iodine 5%	B	DG	DBL
8	P + 5% FeCL3	B	G	BL
9	P + 50% HNO3	B	G	BL
10	P + Methanol	B	B	BL
11	P + Methanol + NaOH	B	G	BL

P-Powder, DL- Day light, DB-Dark Brown, DY-Dark Yellow, B-Brown, G - Green, LG-LightGreen, DG – Dark Green, DBL – Dark Black, BL – Black, LB – Light Brown, YB –Yellowish Brown

Microbial limit test has been carried out for *Bilwadi Agada* samples and study reveals BA sample was within the limits as per Indian Pharmacopeia Standard.

Table No.9: Showing the Results of Microbial Limit Test of *Bilwadi Agada*

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

Sr. No	Description macroscopic	Limits (As per IP)	Results
1.	Total bacterial count	30-300cfu/ml	37cfu/ml
2.	Total fungal count	10-100cfu/ml	22cfu/ml

TLC study is carried out on 60F 254 pre-coated TLC plate under the solvent system Toulene and Ethyle acetate in the ratio 7:3 after various trial and errors. Ethanol extracts of all the samples have been taken and visualized under UV light chamber at the range of 255nm and 365nm. This parameter gives idea about qualitative estimation presence of various components of drugs. Results of TLC are shown in table no.10. [20]

Table 10: Showing TLC Analysis- RI values of Alcoholic Extract of *Bilwadi Agada* with solvent system Toluene: Ethyl Acetate (7:3).

Sl. No.	Condition	Rf values of ***Bilwadi Agada***
1.	Short wave (Spots at UV 254nm)	0.05, 0.98, 0.22,0.27,0.37, 0.45, 0.57, 0.66, 0.71

2.	Long wave (Spots at UV 366nm)	0.10,0.14,0.20,0.24,0.28,0.32, 0.37,0.41,0.55,0.59,0.70

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

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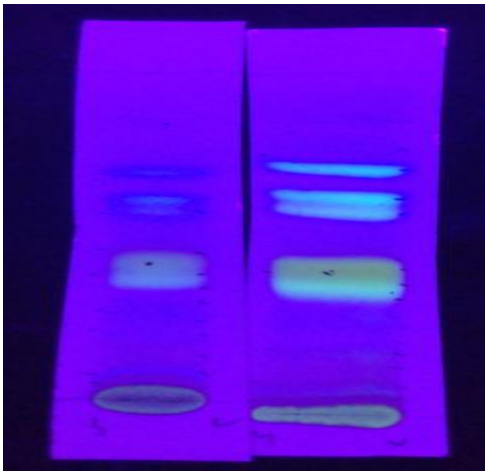

TLC of Inhouse prepared *Bilwadi Agada*