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Standardization of *Bilwadi Gutika* through Physiochemical Analysis, Phytochemical Analysis and GC-MS

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ABSTRACT

Bilwadi Gutika is a commonly used *Agada* formulation in clinical practice. It is explained in *Ashtanga Hridaya* in *Sarpa Vishapradhishedha adhyaya*. Other indication of *Bilwadi Gutika* include poisonous bites of snake, spider, mice, scorpion, etc. *visuchika*, (simultaneous vomiting and diarrhoea), *Ajirna* (indigestion), *Gara visha* (Concocted poison) and *Jwara* (fever), makes the persons suffering from possession of evil spirits healthy again. Hence the formulation is subjected to physiological analysis, Phytochemical analysis, and Gas chromatography-mass spectrometry (GC-MS) analysis for its Standardization. *Bilwadi Gutika* was prepared by powdering thirteen ingredients and subjecting them to trituration using Goat's urine. Aqueous and hydro-alcoholic extracts were prepared. Then these extracts were subjected to Physiochemical, Phytochemical analysis and GC-MS to find out the phytoconstituents present in the sample extracts. The drug's physicochemical examination yielded results that fell within acceptable limits. Phytochemical analysis showed the presence of Alkaloids, Carbohydrates, Tannins, Glycoside, Steroid, Flavonoids and Mucilage. The GC-MS analysis recorded a total 40 peaks, among which Propanamide, 2-hydroxy was found to be in highest percentage of 33.4%.

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INTRODUCTION

Traditional medicine refers to the medicinal applications of traditional knowledge that have evolved over many generations within different communities' folk beliefs. According to the World Health Organization, more than 80% of people in developing countries depend on traditional Medicine for their primary health needs.[1] India, the birthplace of *Ayurveda* medicine, is home to over 45,000 different plant species. Ayurveda is a holistic science that emphasizes both disease prevention and disease treatment. It primarily uses plants for its medicinal properties. Due to their natural source and absence of adverse effects, ayurvedic medications are in high demand worldwide. Because genuine pharmaceuticals are expensive and hard to come by, the commercialization of traditional treatments has resulted in the widespread usage of adulterants and low-cost replacements. Therefore, to standardise and assess the quality of polyherbal formulations, it is now essential to testify in accordance with contemporary research guidelines.[2]

In traditional medicine, quality assurance plays a crucial role in ensuring the delivery of appropriate dosages of standard medications. Drugs of the highest caliber, free of adulteration, can be verified by physiochemical evaluation and comparison with reference values. A drug's phytochemical analysis can be used to identify its various chemical constituents. Gas chromatography-mass spectrometry (GC-MS) is the tool of choice for the analysis of volatile compounds in medicinal plants. It gives the percentage of components present in it.

The formulation *Bilwadi Gutika* is a commonly used *Agada* formulation in clinical practice. It is explained in *Ashtanga Hridaya* in *Sarpa Vishapradhishedha adhyaya*. Other indication of *Bilwadi Gutika* include poisonous bites of snake, spider, mice, scorpion, etc. *visuchika*, (simultaneous vomiting and diarrhoea), *Ajirna* (indigestion), *Gara visha* (Concocted poison) and *Jwara* (fever), makes the persons suffering from possession of evil spirits healthy again. It is an herbal formulation containing 13 ingredients in equal quantity and Goat's urine is used as *Bhavana dravya* (Liquid media for trituration).[3] Hence an attempt was made to confirm quality and purity with standardization of *Bilwadi Gutika.*

MATERIAL AND METHODS

Preparation of the formulation

All authenticated thirteen ingredients were checked for any impurities and cleaned. All thirteen ingredients were made into fine powder by using *Khalva yantra* separately. 60 gms of each of the fine powders of 13 ingredients were then mixed to form a homogeneous mixture. To this homogeneous mixture, *Bhavana* (Trituration) was given with Goat's Urine by using a wet grinder. After attaining *Subhavitha lakshana* the mixture was rolled into pills by using pill making machine. The pills were then dried under shade and stored in airtight container. Total 802 gms of *Bilwadi Gutika* was prepared.

Table No 1: Quantity of ingredients used in the formulation

SI.no	Drug	Amount of raw Drugs in grams	Amount of course Powder in grams
1	*Bilwa*	90 gms	60 gms
2	*Surasa*	60 gms	60 gms
3	*Karanja*	264 gms	60 gms
4	*Natam*	103 gms	60 gms
5	*Surahwam*	94 gms	60 gms
6	*Haritaki*	96 gms	60 gms
7	*Bibhitaki*	81 gms	60 gms
8	*Amalaki*	85 gms	60 gms
9	*Shunti*	89 gms	60 gms
10	*Maricha*	102 gms	60 gms
11	*Pippali*	92 gms	60 gms
12	*Haridra*	124 gms	60 gms
13	*Daruharidra*	85 gms	60 gms
14	*Basta mutra* (Goat's urine)	-	-

Preparation of extract:[4, 5]

Aqueous extract and hydro-alcoholic extract of the formulation *Bilwadi Gutika* prepared based on API (Ayurveda Pharmacopoeia of India) standards. Extraction was done using the Soxhlet apparatus.

Physicochemical analysis of *Bilwadi Gutika*

The Physicochemical parameters and organoleptic characteristics of *Bilwadi Gutika* includes colour, odour, taste, shape, consistency, pH, loss on drying, friability, hardness, and

disintegration time and total Ash value. Which were conducted according to Laboratory guide for the analysis of Ayurveda and Siddha formulation[6].

Phytochemical analysis of *Bilwadi Gutika* [7]

Phytochemical analysis of *Bilwadi Gutika* includes tests for the presence of Alkaloids, Carbohydrates, Tannins, Glycoside, Steroids, Saponin, Flavonoids and Mucilage. Tests were conducted according to standard guidelines.

GC-MS of *Bilwadi Gulika*[8]

GC-MS information

Make: Perkin Elmer

GC model: clarus 680

Mass Spectrometer: Clarus SQ 8C

Software: Turbo Mass ver 6.1.1

Library ver: NIST-2014

Acquisition parameters

Oven: Initial temp 50°C for 2 min, ramp 15°C/min to 150°C, hold 1 min, 25°C/min to 235°C, hold 12 min

Total Run Time: 25.07 min

InjA auto=250°C, Volume=1 µL, Split=1:10,

Flow Rate: 2 mL/min

Carrier Gas=He,

Column=Elite-5MS (30.0m, 0.25mmID, 0.25µm)

Mass condition

Solvent Delay=3.00 min,

Inlet Line Temp=250°C,

Source Temp=230°C,

Scan: 40 to 600Da,

GC-MS Procedure

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 2 ml/min. The injector temperature was set at 250°C during the chromatographic run. The 1µL of extract sample was injected into the instrument the oven temperature was as follows: 50 °C (2 min); followed by 150 °C at the rate of 15 °C min−1; and 150 °C, where it was held for 1min and then followed by 235°C at the rate of 25°C min−1; it was held for 10 min. The mass detector conditions were: Inlet line temperature of 250 °C; ion source temperature of 230 °C; and ionization mode electron impact at 70 eV, a scan time of 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2014) library.

RESULTS

Table No. 2– Results of Physiochemical analysis of *Bilwadi Gutika*

Sr. No	Parameter	Result
1.	Color	Blackish- brown
2.	Oduor	*Ajamutra Gandhi*
3.	Taste	*Tikta, Katu*
4.	Touch	Rough
5.	Appearance	Solid
6.	pH	1.64
7.	Loss on drying	0.068%
8.	Total Ash value	8.1 %
9.	Friability	0.1%
10.	Hardness	4.3
11.	Disintegration time	27 mins

Table No.3- Results of extraction of *Bilwadi Gutika*

Details	Extract value
Aqueous extract of *Bilwadi Gutika*	15.08%
Hydroalcoholic extract of *Bilwadi Gutika*	22.10%

Table No.4 – Results of Phytochemical Screening of aqueous and Alcoholic extract of *Bilwadi Gutika*

Test	Aqueous	Hydro-alcoholic
Alkaloid		
Dragendroff's test	Positive	Negative
Mayers's test	Positive	Negative
Wagner's test	Positive	Negative
Carbohydrates		
Fehling's test	Positive	Positive
Tannin		
Bromine	Negative	Negative
Lead acetate solution	Negative	Positive
Glycoside		
Liebermann's – Burchard reaction	Negative	Negative
Borntrager's test	Positive	Positive
Steroid		
Salkowski reaction and Liebermann's	Negative	Positive
Saponin		
Foam test	Negative	Negative
Flavonoids	Negative	Positive
Mucilage	Positive	Positive

The Phytochemical investigation of the Aqueous and Hydro-alcoholic extract of *Bilwadi Gutika* revealed the presence of Alkaloids, Carbohydrates, Tannins, Glycoside, steroids, Flavonoids and Mucilage.

Results of GC-MS of *Bilwadi Gutika*

Table No.5- At Retention time 10.5 min

Peak number	**Name of the component**	**Formula**	**Probable %**
1	Propanamide, 2-hydroxy-	$C_3H_7NO_2$	33.4%
2	2,3-Epoxybutane	C_4H_8O	12.2%
3	Silane, methyl-	CH_6Si	11.7%
4	2,3-Epoxybutane	C_4H_8O	12.2%
5	Ethanol, 2-nitro-	$C_2H_5NO_3$	4.98%
6	2,3-Epoxybutane	C_4H_8O	12.2%
7	Hydrazine, methyl-	CH_6N_2	4.01%
8	Propanamide, 2-hydroxy-	$C_3H_7NO_2$	33.4%;
9	Oxirane, 2,3-dimethyl-, trans-	C_4H_8O	4.01%
10	Butanal, 3-hydroxy-	$C_4H_8O_2$	3.85%

Table No.6 - At Retention time 12.4 min

Peak number	**Name of the component**	**Formula**	**Probable %**
1	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	27.4%
2	Benzeneethanamine, 2,5-difluoro- β,3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	23.2%
3	l-Gala-l-ido-octose	$C_8H_{16}O_8$	5.02%
4	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	$C_{11}H_{17}NO_2$	4.43%
5	Benzeneethanamine, 2-fluoro-β,3-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	4.09%
6	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	3.45%
7	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	2.58%
8	Pyrazole[4,5-b] imidazole, 1-formyl-3-ethyl-6-β-d-ribofuranosyl-	$C_{12}H_{16}N_4O_5$	1.92%
9	7-[β-d-Ribofuranosyl]imidazo[4,5-d][1,2,3]-triazin-4-one (2-azainosine)	$C_9H_{11}N_5O_5$	1.43%
10	2-Amino-4-dimethylaminomethylenepentanedinitrile	$C_8H_{12}N_4$	1.38%;

Table No.7 - At Retention time 13.2 min

Peak number	**Name of the component**	**Formula**	**Probable %**
1	1,1-Dichloro-2-methyl-3-(4,4-formyl-1,3-butadien-1-yl) cyclopropane	$C_{10}H_{10}Cl_2O_2$	14.7%
2	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	9.79%
3	Methyl-3-phenoxybenzoate	$C_{14}H_{12}O_3$	8.27%
4	Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-	$C_{12}H_{19}FN_2O$	2.43%
5	α-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methyl boronate	$C_{13}H_{26}BNO_6Si$	2.43%
6	2-(Hydroxyphenylmethyl)[1,4]benzoquinone	$C_{13}H_{10}O_3$	2.34%
7	Benzeneethanamine, 2,5-difluoro-β,3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	2.24%
8	Morphinan-3,14-diol, 4,5-epoxy-, (5α)-	$C_{16}H_{19}NO_3$	2.16%
9	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	2.07%
10	1-Chloromethyl-1-cyclohexyl oxy-1-silacyclohexane	$C_{12}H_{23}ClOSi$	1.99%

Table No.8 - At Retention time 14.0 min

Peak number	**Name of the component**	**Formula**	**Probable %**
1	Trifluoroacetamide,-(4-bromobenzyl)	$C_9H_7BrF_3NO$	12.8%
2	2-Iodo-5,6-dimethylpyridine-3,4-carbonitride	$C_9H_6IN_3$	7.76%
3	Succinic acid, 2-bromo-4-fluorophenyl 2,2,3,3,4,4,4-heptafluorobutyl ester	$C_{14}H_9BrF_8O_4$	7.15%
4	Succinic acid, 5-fluoro-2-nitrophenyl 2,2,3,3,4,4,4-heptafluorobutyl ester	$C_{14}H_9F_8NO_6$	6.60%
5	9,10-Anthracenedione, 1,4-diamino-5-nitro-	$C_{14}H_9N_3O_4$	5.83%
6	Succinic acid, 3,5-fluorophenyl 2,2,3,3,4,4,4-heptafluorobutyl ester	$C_{14}H_9F_9O_4$	4.58%
7	Quebrachamine	$C_{19}H_{26}N_2$	4.05%
8	Succinic acid, 2-chlorophenyl 2,2,3,3,4,4,4-heptafluorobutyl ester	$C_{14}H_{10}ClF_7O_4$	3.89%
9	Papaveroline	$C_{16}H_{13}NO_4$	3.59%
10	Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester	$C_{15}H_{21}F_7O_4$	2.82%

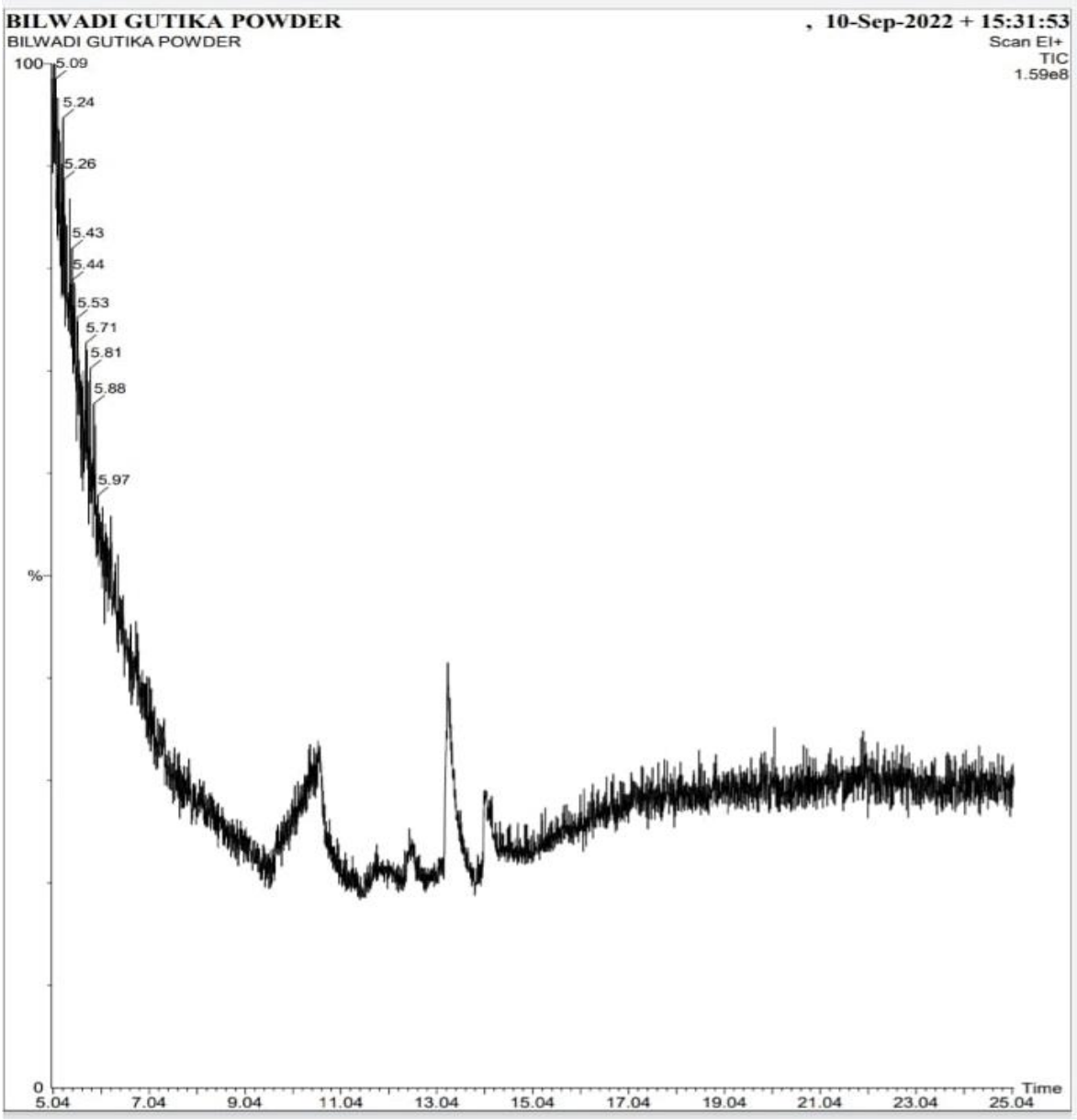

Fig No.1: Graphical representation of GC-MS analysis of *Bilwadi Gutika*

DISCUSSION

The pH values determine the acidic nature of the formulation. The pH of stomach acid comes in between 1.5 and 3.5. pH values of *Bilwadi Gutika were* found to be 1.64, indicates *Bilwadi Gutika* is slightly acidic in nature and comes close to the pH of the stomach. The Friability of tablet indicates good mechanical resistance for the tablets while handling or shipping. Hardness also measures the strength of the tablet. The hardness and Friability of tablet were found to be 0.1% and 4.3 indicates good strength and mechanical resistance of *Bilwadi Gutika.* The loss of drying value indicates the presence or absence of moisture. Loss on

drying value of *Bilwadi Gutika.* Found to be 0.068%, which lies between the standard limits. The residue remaining after incineration is the total ash content of the drug. The total Ash value of *Bilwadi Gutika* was 8.1 %, indicates less number of inorganic elements. Tablet Disintegration time, indicates how rapidly the solid de-aggregates to physiological solution and the drug get absorbed. *Bilwadi Gutika.* Disintegrated by 27 min, indicates good de-aggregation.

The nature of the chemical components that are present in a crude medication is measured by its extractive value. It is crucial for the estimation of specific chemical components that are soluble in the extraction solvent in question[9].

The present study used aqueous extract and hydro-alcoholic extract bioactive compounds from *Bilwadi Gutika*. Results showed that both solvents resulted in various extraction yields, which could cause variations in the level of bioactive compounds in the extract. The results of aqueous extract and hydro-alcoholic extract values of *Bilwadi Gutika* showed 15.08% and 22.1% respectively, indicate more solubility of extract bioactive compounds in hydro-alcoholic extract compare to aqueous extract of *Bilwadi Gutika*.

The Phytochemical investigation of the aqueous and hydro-alcoholic extract of *Bilwadi Gutika* revealed the presence of Alkaloids, Carbohydrates, Tannins, Glycoside, Steroid, Flavonoids and Mucilage.

In GC-MS analysis total 40 peaks were recorded, among which Propanamide, 2-hydroxy was found to be in highest percentage of 33.4%.

CONCLUSION

The results of Physicochemical analysis of drug was found to be within standard limits. Phytochemical analysis showed presence of Alkaloids, Carbohydrates, Tannins, Glycoside, Steroid, Flavonoids and Mucilage. The GC-MS analysis recorded total 40 peaks, among which Propanamide, 2-hydroxy was found to be in the highest percentage of 33.4%.

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