

Research Article

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Pharmaceutical and Analytical Studies on *Guduchi Kwatha*Prepared by Varying Proportions of Water

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ABSTRACT

Background: Kwatha preparation is one among the Pnachvidha Kashaya Kalpana. The ratio of water and hardness of drug plays an important role during preparation of Kwatha. Disparities in proportions of liquid (water) to be mentioned can be observed in the classics, which are based on the quality (the hardness) and quantity of the raw material being used in the pharmaceutical procedure. Aims and objective: The objective of the present study was to evaluate the significance of proportion of water in the preparation of Kashaya and Ghana. The water proportion was fixed as 4 parts, 8 parts and 16 parts. Material and methods: Three batches of Kashaya and Ghana with each proportion were prepared considering the batch size as 1 kg and findings were systematically recorded. Organoleptic evaluation, Physico-chemical parameters of Kwatha and Ghana, HPTLC and HPLC were carried out. Result and discussion: Average time took for 1/4th reduction was found to be 339 min, 570 min and 960 min and in the preparation of Kashaya with 4 parts, 8 parts and 16 parts of water respectively while Average temperature maintained during the process was 95-100°C. An average of 6.23%, 6.24% and 6.32% Ghana was obtained from the Kashaya in 4,8,16 parts of water respectively. The change in organoleptic parameters, physico - chemical parameters, HPTLC & HPLC study was observed. The physico-chemical parameters of all the samples presented negligible differences. After spray, 7 spots were observed in the sample prepared with 4 parts of water and remaining samples provided only 5 spots. In HPLC study, the batch 4, 8 and 16 have almost similar concentrations. Conclusion: Though the percentage of Ghana is similar in all the batches; during the pharmaceutical procedure, it is advisable to add 8 parts of water. This facilitates proper soaking and boiling of the raw

Keywords: Guduchi, Kwatha, Proportion, Ghana, HPLC.

INTRODUCTION

Guduchi is widely used in *Ayurveda* and vertinary medicine as a general tonic, antiperiodic, antispasmodic, antiarthritic, antiallergic and antidiabetic properties. The Guduchi is mainely used as a "Rasayana" to improve the immune system and the body resistance against the infections. The aqueous fraction of *Tinospora codifolia* stem part is effective in ameliorating immunosuppressive effect and prevents pathogenic insults in immunocompromised stage^[1].

In modern pharmaceutical science, the method of preparation of extract is selected depending upon the physico-chemical properties of the crude drugs and main motto of the method adopted for a particular preparation of any drug has to extract the maximum pharmaceutically active ingredients of the crude drug. Therefore the nature of pharmaceutically active ingredient is an important factor in deciding the method of preparation. In case of herbal drugs the active ingredients may be in their soup or in fibrous portion or in water-soluble content at different temperature etc. These pharmaceutically active ingredients in extractive must be pharmacologically active, so that the extractives can be used for therapeutic purpose.

In *Ayurveda* different type of dosage forms are described for diverse therapeutic purpose in various classics and method of preparation of all the dosage form are different. *Panchavidha Kashaya Kalpana* (five primary dosage form) are the basis of *Ayurvedic* preparations, they have some drawbacks such as non-availability of crude drugs all the time, very short shelf-life, inconvenient taste and dose etc. To counteract these problems, *Upakalpanas* and secondary *Kalpanas* were developed by using the basic *Kalpanas*. Another motto behind the development of *Upakalpanas* seems to enhance the potency as well

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as the selectivity in the pharmacological actions. *Kwatha* is acknowledged as one of the most important dosage forms by all the seers of *Ayurveda*, who advocated it for diverse purposes. Apart from its use in therapeutics, *Kwathas* have been used in *Bhavana*, *Shodhana*, *Marana* of different metals and other *Visha Dravyas*. They were also useful as best *Anupaana Dravyas* and are main base in the preparation of different secondary preparations like *Avaleha*, *Sneha*, *Sandhana* etc [2, 3, 4]

Different Acharyas mentioned different opinion regarding the ratio of water and drug in different Samhitas. In SharangdharaSamhita, Acharya has mentioned different ratios of water and drug particularly for SnehaKalpana on the basis of hardness of drug^[5]. In the same chapter ratio of drug and water with relation to its quantity was given^[6]. On the other hand in general method of preparation of Kwatha forSnehaKalpna different proportion of Drug and water (1:4) was given^[7].

The ratio of water and hardness of drug plays an important role during preparation of *Kwatha*. Disparities in proportions of liquid (water) to be mentioned can be observed in the classics, which are based on the quality (the hardness) and quantity of the raw material being used in the pharmaceutical procedure. At the same time, there are references, where different proportions of water have been mentioned in the *Kwatha* preparation for a same drug. Such references will lead to confusion over addition of water proportion to a drug, when the same is not mentioned (*Anuktam*). Possibilities of differences in the therapeutic efficacies can be anticipated with variations in the proportions of the extracting media (water). Considering this, the present study has been attempted on preparing *Guduchi Kwatha* and *Ghana* with varying proportions of water (4, 8, 16) and develop possible analytical profile.

MATERIAL AND METHODS

Collection of raw material and its authentication

Fresh *Guduchi* was collected from the botanical garden of Gujarat Ayurved University Jamnagar after obtaining due permission from the concerned authorities. The raw drug sample was collected and authenticated in the Pharmacognosy laboratory, IPGT & RA, Jamnagar. Later, it was undertaken for the Pharmaceutical study.

Preparation of Kwatha

The Fresh Guduchi was procured from the botanical garden of Gujarat Ayurveda University Jamnagarand authenthicated at the pharmacognosy laboratory, IPGT&RA, Gujarat Ayurved University, Jamnagar, cleaned with tap water and crushed thoroughly in grinder, added with 4 parts of water and subjected to mild heat with infrequent stirring without covering its mouth. Reduction was done until the quantity reduced to 1/4th of its original volume and contents were filtered throughdouble-folded clean cotton cloth in to a stainless steel vessel and the residue was discarded.

The similar procedure was followed for all the groups. Average data obtained during preparation of *Kwatha* was depicted in Table 1

Preparation of Ghana

Previously preparedGuduchi *Kwatha* has been used forthe preparation of Guduchi *Ghana*. The *Kwatha* was subjected to the process of reboiling with intermittent stirring over mild flame till the contents become semisolid. Then the contents were subjected to indirect heating onwater bath and constant stirring was done to avoid possibilities of burning. Heating in water bath was stopped when most of the water was evaporated. Ghana was collected in a SS plate and subjected to drying in a hot air oven maintaining the temperature around 50°C.

After complete drying the *Ghana* was collected in duly labelled glass bottle.

The similar procedure was followed for all the groups. Observations and results obtained during the pharmaceutical process of *Guduchi Ghana* was depicted in Table 2

Analytical Study

All the samples of *Guduchi Kwatha* and *Ghana* were subjected to evaluate organoleptic characters (Table-3 & 4), preliminary physicochemical analysis (Table 5 & 6), qualitative estimation of functional groups (Table-7), HPTLC & HPLC profile by following standard guidelines in order to develop possible analytical profile.

HPTLC Profile

In HPTLC study all tracks of Ghana (1:4, 1:8, 1:16) were scanned under 254 nm & 366 nm, maximum Rf values are mentioned in table. Chloroform: Methanol (1: 9) was used as a solvent system and vanilline sulphuric acid was used as a spray reagent in this study (Table-8).

HPLC Profile

The work has been carried out at Natural remedies private limited. The results are as shown in the table (Table-9).

RESULT & DISCUSSION

In *Ayurveda* there is an explanation of different dosage forms or *kalpanas*. It is mainly classified in to two types; *Aushadhakalpana* and *Aharakalpana*. *Aushadhakalpana* can be again classified into two; Primary *kalpanas* and Secondary *kalpanas*. *Panchavidha kashaya kalpanas* are considered to be fundamental preparation in *Ayurvedic* pharmaceutics which is having good therapeutic action, prepared for instant use and the most widely used formulations as a starting dosage form as well as a base for many different dosage forms. *Kwatha* (decoction) is one among most popular and effective dossage form. Literally, the word *Kashaya* means that which brings about normalcy to the body by maintaining equilibrium of physiological factors by removing pathology^[8].

Ayurvedic pharmaceutical industry was increasing at a rapid pace over the years and in the industry, Kwatha is the basic material for the preparation of other dosage form like sneha, Vati, Asav- arista, as a Bhavnadravya (levigation media) in various Rashashatriya preparations and if it is not prepared properly, ultimately it affects the quality of final product. Another aspect is that people are not aware about the proportion of water and randomly added the water for the preparation of decoction without thinking the nature of raw material (hard, moderately hard or soft). For example for the preparation of good quality of Bhasma, Bhavnadravya should be sufficient in quantity for proper trituration of the material but as we concern about quantity of liquid media, we have to concern about the concentration of that particular liquid media so that final prepared Bhasma fulfills all classical quality control parameter and it is possible when the exact proportion of water was used during preparation of decoction. If we follow the classical guidelines properly given by our ancient sages then only good quality of final product can be achieved.

Different Acharya has different opinions regarding the types, addition of water etc

Acharya *Charaka* was first to mention about *Panchavidha kashaya kalpana*, but he has given the name *Shrita* for *kashaya*, wherein he describes the boiling of dravya in the *mandaagni* is *Shrita*^[9]. *Acharya Sushruta* considers six types of *kashaya kalpanas Ksheera* (milk),

Swarasa Kalpana (formulations), Shrita, Sheeta (cold) and Churna (powder)^[10]. In Sharangadhara samhita method of preparation of kashaya (decoction) is mentioned and includes kashaya under Panchavidha kashayas^[11]. Acharya Kashyapa has also mentioned it under 7 types of kashaya (decoction). Churna (powder), Sheeta kashaya, Swarasa, Abhishava (fermented preparation), Phanta, kalka (paste) and Kwatha (decoction). Acharya Harita has classified kashayas into 7 types depending on the quantum of reduction and the action exhibited by that particular kwatha^[12].

The criteria of boiling observed by *Agnivesa* is "*Gatarasheshu Aushadheshu*" means the active principle of the drug should completely enter in to the water and no active principle should remain present in the residual drug^[13].

During preparation of decoction of Guduchi, initially Colour of liquid (Kwatha) was Green with slightly bitter taste and presence of froth on surface. At 60°C drug started settling at the bottom and froth seen on surface was increases. Evaporation was started between 80-90°C which aggravated on stirring and colour of liquid darkens and light brown in colour. Average 5.5, 9.5, 16 hrs. are required for the preparation of Guduchi Kwatha by addition of 4, 8 and 16 parts of water respectively. Average temperature maintained during the process was 95-100°C. Average yield of Kwatha was 1.01, 2.04 and 4.04 lit respectively.

Raw drug should be crushed and made in to pieces (*Anusho bhedayitva*) before initiating the actual process of *Kwatha*, by which maximum extraction of the desired active principles can be expected. At the same time, reduction of material to finer levels (*Anusho bhedayitva*) will pose to other problems at different levels, considering which the particle size has been finalized as '*Yavakuta*' by the Ayurvedic Pharmacopoeial Committee for the preparation of *Kwatha*.

The proportion of water to be used during the *Kwatha* preparation depends upon the quantity and quality (soft, hard etc.) of the raw material. In general, if the quantity is less, excess proportion (16 times) of water and vice versa has been advocated in classics. In addition, the proportion of water also depends on the nature of the raw material ^[5]. The variation of the quantity is to facilitate the optimum extraction of the needful principles in to the end product i.e. *Kwatha*, which has been mentioned by *Charaka* as *Gata Rasata*.

The quantum of heat applied for the preparation of Kwatha should always be Mridu (around 100°C), which is needed for Samyak Virya Utkrishtata (proper extraction of the needful principles)^[6]. Most of the alkaloids and other constituents of the herbals will get destroyed at high levels of temperatures and probably, because of this, the seers preferred mridu agni in the pharmaceutical preparation. Quantum of heat and the duration of heating are of prime concern for Kwatha nirmana. The purpose of boiling is to drive therapeutically active principles from the source drug up to maximum possible extent without damaging any of the useful constituents in the process. If a drug doesn't contain any of the heat labile substance, then, it could be subjected to repeated-boiling for better extraction of the therapeutically active principles from the source drug^[14]. All the seers of Ayurveda emphasized on continuous agitation (Darvyavaghattayan) of the contents during the process of boiling. This will hasten the extraction and also avoid the drugs to settle down to the bottom of the container, avoiding possibilities of charring.

Agitation will help to increase a dissolution pathway and by bringing fresh solvent into contact with the boundary layer so producing a high value for concentration gradient. The rate of dissolution may therefore be markedly affected by agitation or stirring. A drug in the dry state is porous due to shrinkage, and the porescontain air that must be displaced as the solvent enters into the pores and penetrates into the cells. The process will depend on the character of the drug. The cell

walls consist basically of cellulose molecules, known as micelle and in fresh material are surrounded by a film of water. When the drug is dried, this film is lost and the micelle move together to form a continuous membrane. When the dry drug is moistened the reverse occurs & the micelle takes up a liquid film and tissue swell. The amount of swelling is variable being greatest with liquids when hydroxyl groups form a great part of the molecule. Thus water causes considerable swelling. So it is very much necessary that thedry drug should be immersed in water for considerable amount of period before initiating the process of boiling. The immersion is mandate, when the raw material falls under the categories of Kathina (hard) or Atyanta Kathina (too hard). The chief desired characteristic of Kwatha is mentioned as Gata rasatwam by Charaka. It indicates that, the Kwatha Dravya (raw material) should attain the state of Gata rasatva (devoid of taste). This state can be interpreted as that, the raw material became tasteless i.e. all the therapeutically viable principles have been entered in to the liquid (Kwatha). After confirming the 'Gatarasa' state i.e. exhaustion of the drug of its therapeutic active principles, straining should be done and the filtrate is to be mustered [7].

The assessment of *Gatarasata* can be beneficially used in establishing duration of boiling, quantum of the water to be taken while preparing the *Kwatha* etc.

The *Guduchi Kwatha* (of three batches) were processed further to prepare *Ghana*. After 6 hours of boiling mild sticky nature was observed during rubbed between two fingers. After 8 hours of heating stickiness of the liquid and adhesiveness to the vessels was increased. Thickness of liquid and adhesiveness of vessel was gradually increased. After dried in the oven brownish green colored and semi-solid material was converted into dark brown colored solid material. During processing, the application of mild heat is required with occasional stirring to avoid destruction of the components sensitive to higher temperature.

Respectively, an average of 6.23%, 6.24% and 6.32% *Ghana* was obtained from the *Kashayas* and average 9.43, 10.14, 12.23 hrs. are required for the preparation of *GuduchiGhana* (by addition of 4, 8 and 16 parts of water) respectively. It is observed from the practical that the difference in between the final quantity of obtained *Ghana* is insignificant.

Three batches of *Kwatha* and *Ghana* were prepared to maintain the Standard manufacturing Procedure.

Analytical study was carried out with a view to know particular chemical configuration of the final product and to point out the physico-chemical changes and effect of different *Samaskaras* (*Agni Sannikarsha*) during the pharmaceutical processing.

In the qualitative analysis Saponin, Glycosides, Protein and Carbohydrate (starch) were found to be present in all the samples, while they are free from Sterols and Quinines.

The physico-chemical parameters of all the samples presented negligible differences.

Chromatographic study (HPTLC) was carried out to establish the fingerprinting profile for the different batches of *Ghana*, reveal the possibly active phyto-constituent and to compare them. At 254 nm visualization. HPTLC provided maximum number of spots 6 with 8 parts of water, 5 with 4 parts of water and least (4) in 16 parts of water. After spray, 7 spots were observed in the sample prepared with 4 parts of water and remaining samples provided only 5 spots. Thus, it implies that although the raw material is same, change in the procedure (proportion of water and duration of heating) induces changes in product formation.

HPLC revealed that batch 4, 8 and 16 have similar concentrations. Peak at 11 min is maximum in Batch 16 which is minimum in all other batches. The peaks observed in between the 1-10 min. were found to be more in Batch 4 (13), followed by 11 in Batch 8, 10 in Batch 16. More or less the peaks are nearby with changes in the height of the peaks.

CONCLUSION

In *Panchavidha Kashaya Kalpana, Kwatha* is having vital importance as a initiation media in the preparation of any classical dosage form.

Water is an universal and most acceptablesolvent for the extraction of different chemical moieties. At an average 6.23% of *Ghana* can be expected from fresh *'Guduchi'*. Fresh Guduchi is considered as *Madhyam* type of *dravya* depending upon its hardness. Though the percentage of *Ghana* is almost similar in all the batches; during the pharmaceutical procedure, it is advisable to add 8 parts of water. This facilitates proper soaking and boiling of the raw material. The analytical parameters in the samples prepared with varying liquid portions were found to be in a narrow range.

Table 1: Average data of Kwatha prepared with varying proportion of water

Batch	Quantity of		Proportion	% of reduction	Kwatha obtained	Temp. Range	Duration	
Batch	Drug	Water	Proportion	% of reduction	Kwatna obtained	remp. Kange	(min.)	
1	1000 g	41	1:4	1/4	1016.67	95-100°C	330	
2	1000 g	81	1:8	1/4	2046.66	95-100°C	570	
3	1000 g	16 l	1:16	1/4	4045.00	95-100 ⁰ C	960	

Table 2: Observations and results obtained during the pharmaceutical process of Guduchi Ghana.

Batch	Quantity of		Proportion	Ghana obtained	Yield in (%)	Duration (hrs)
	Drug	Water				
1	1000 g	41	1:4	62.33	6.23	09.43
2	1000 g	81	1:8	62.43	6.24	10.14
3	1000 g	16	1:16	63.25	6.32	12.23

Table 3: Organoleptic characters of Guduchi Kwatha.

	Organoleptic characters	Batch				
	Organoleptic characters	1:4	1:8	1:16		
1	Colour	Brown	Brown	Brown		
2	Odour	Characteristic	Characteristic	Characteristic		
3	Appearance	Dark	Dark	Dark		
4	Taste	Bitter	Bitter	Bitter		

Table 4: Organoleptic characters of Guduchi Ghana.

S. No.	Organoleptic characters	Batch				
3. INO.	Organoleptic characters	1:4	1:8	1:16		
1.	Colour	Brown	Brown	Brown		
2.	Odour	Characteristic	Characteristic	Characteristic		
3.	Appearance	Dark	Dark	Dark		
4.	Taste	Bitter	Bitter	Bitter		

 $\textbf{Table 5:} \ \textbf{Results of physicochemical parameters of } \textit{GuduchiKwatha}.$

S. No.	Parameters	Batch			
		1:4	1:8	1:16	
1.	Total Solid Content (% w/v)	2.66	1.645	1.365	
2.	рН	6	6	6	
3.	Specific Gravity	1.005	1.002	1.003	
4.	Viscosity (mill poise)	1.229	1.326	1.836	
5.	Refractive Index	1.347	1.346	1.344	

Table 6: Results of physicochemical parameters of *GuduchiGhana*.

S.No.	Parameter	Result
1.	Loss on drying at 110°C (%w/w)	7.53
2.	Ash value (%w/w)	10.15
3.	Acid insoluble ash (%w/w)	0.50
5.	Water soluble extractive (%w/w)	39.12
6.	Alcohol soluble extractive (%w/w)	2.23
7.	pH value	5.59

Table 7: Qualitative tests of GuduchiKwatha

	Functional Group	Result
1	Alkaloid	-ve
2	Tannin	-ve
3	Saponin	+ve
4	Glycosides	+ve
5	Flavanoids	-ve
6	Phenols	-ve
7	Protein	+ve
8	Sterols	-ve
9	Quinines	-ve
10	Carbohydrate(starch)	+ve
11	Triterpenoids	-ve

Table 8: Rf values of (All the samples of Guduchi): After Spray

Track	No of spots	Rf Value
Track 1(1:4)	5	0.04,0.15,0.20,0.81,0.07
Track 2(1:8)	7	0.05,0.14,0.18,0.28,0.41,0.55,0.62
Track3(1:16)	5	0.05,0.16,0.22,0.24,0.83

Table 9: Peaks of HPLC of different samples of *Guduchi Ghana*& extracts;

Ret. Time	Batch						
Ret. Tille	4	8	16	w	E		
2.65	70126	57781	37318	83686	127546		
2.78	-	-	-	82230	-		
2.82	211843	227796	182561	-	237350		
3.16	74106	-	97990	-	-		
3.52	-	-	-	6822	-		
3.64	47990	93870	39183		66122		
4.12	51072	36947	52481	62688	93696		
4.5	-	24876	-		138006		
4.63	-	-	15047	1458	-		
5.35	28088	12414	10363	26835	-		
5.41	-	-	-	-	100202		
5.77	-	-	-	14632	-		
5.98	-	2939	-	-	52473		
6.25	7448		15653	26431	-		
6.46	59210		8065	23562	-		
7.1	-	51883	49273	35393	163666		
7.42	27897	47207	61287	104725	127020		
7.96	75643	-	134851	179065	640134		
8.04	-	77926	-	-	-		
8.63	-	-	15609	-	-		
9.19	-	100193	31596	-	1045382		

9.36	34789	4905	- 1	-	-
9.42	-	-	11963	15197	68273
9.59	44007	7368	-	26540	66183
9.71	-		17127	-	-
10.11	-	12306		27046	-
10.46	-	4050			-
10.9	78233	250248	142767	22676	134719
11.41	37280	42538	46855	42350	62288
11.68	-	12842	5586	5331	-
11.76	-	-	-	-	47699
11.96	80241	37825	24595	39500	106348
12.4		50858	42294	39649	121233
12.59	73865	-	-	-	
13.13	17447	-	7376	-	25615
13.34		5216		-	-
13.44	15995	-	5354	16490	-
13.86		-	-	1595	-
14.06	10931	-	-	4971	-
14.34	-	-	-		43473
14.45	-	-	-	3206	
14.79	11999	4448	5863		34755
14.9	-			31850	
15.25	4019	19515	13158	8771	
15.35	-	4867	-		63678
15.77	82972	4881	-	4590	
16.1	-	3244	5160	3666	48997
17.16	2375	-	1692	-	28051
17.6	-	5076	-	-	-
17.98	-444	-	-	-	-
18.14	-	-	-	1377	11119
18.5	-	4824	3075		12908
18.61	1233	-	-	-	
18.91	2505	1903	2871	-	10293
19.45	1353	829	1383	2919	16550
19.95	7761	2368	6962	3545	-
20.4	-	-	-	1359	
21.06	67307	3168	5695	4189	-
21.1	-	-	-	-	7972
21.54	-	-	6580	2073	-
21.98	-	-	-	1668	-
23.47	4965	4267	4817	4647	4603
24.4	-	-	4473	4508	-
25.56	-	-	-	3371	-
25.94	3303	2873	2947	3146	3324
26.54	1235	1387	1603	1427	1471
28.06	-	2629	2819	2450	-
28.4	-	-	1201	-	-
28.83	-	5900	-	-	-
28.96	11672	5853	8570	11698	10441
30.47	-	-	-	916	-

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