Quality control evaluation of *Guduchi Ghana* [dried aqueous extract of *Tinospora cordifolia* (Willd.) Miers] – an herbal formulation

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Abstract:
Ghana (solidified aqueous extract) is mentioned in Ayurvedic pharmaceutics as a secondary derivative of *Kwatha Kalpana* (decoction). *Guduchi Ghana* is popularly known in ayurvedic fraternity for its huge therapeutic credentials. However, published information on physicochemical profile of *Guduchi Ghana* is very minimal. Aims and Objectives of this study are to develop Standard Manufacturing Procedure (SMP) and quality control parameters for *Guduchi Ghana*. Fifteen batches of *Guduchi Ghana* were prepared and findings were systematically recorded. Physicochemical parameters and qualitative tests for various functional groups, quantitative estimation of total alkaloids, starch content, fluorescence analysis, HPTLC profile, heavy metal analysis, and microbial load of *Guduchi Ghana* were carried out. An average 5 % *Ghana* was obtained. The pH, Loss on drying, Ash value and Water soluble extracts values of *Guduchi Ghana* were 5.90, 7.69%w/w 16.60% w/w and 48.57 % w/w respectively. Among functional groups Glycosides, Alkaloids, Tannin, Phenols, Carbohydrates, Starch and Sterol/Steroid were found present. Number of peaks obtained in HPTLC also corresponds to this finding. Percentage of total alkaloid content was 0.58 %. No heavy metal and microbial load were detected in *Ghana* sample.

Introduction

Quality control and standardization of herbal medicines involve several steps like source and quality of raw materials, good agricultural practices, good manufacturing practices, developing standard analytical profiles etc. These practices play a pivotal role in guaranteeing the quality and stability of herbal preparations [1]. It is a herculean task to standardize herbal products due to a number of factors. Environmental conditions (soil, altitude, seasonal variations, humidity, rainfall pattern, shade, dew, frost etc) can create substantial variability in product quality and its level of components within the batches. Herbal extracts contain intricate mixture of numerous organic chemicals such as secondary metabolites in complex matrices. Moreover non availability of reference standards also snags the study. It creates a challenging situation. In spite of that, the present task is undertaken to develop physicochemical standards of *Guduchi Ghana* to compare the formulation with the available modern physicochemical parameters.

Guduchi (*Tinospora cordifolia* (Willd.) Miers) is incredibly versatile vine in Ayurvedic system of medicine since ancient times and is indicated for potential use in wide range of diseases. The term *Ghanavati* is available in *Siddha Yoga Samgraha* by the name of *Samsamani Vati* [2]. *Ghana* is prepared by boiling decoction to semisolid form and at the end they dried to solid form [3]. Though, *Guduchi Ghana* is widely in practice, information on its physicochemical profile is not available. Considering this, *Guduchi Ghana* is prepared to develop analytical profile.

Materials and Methods

Collection of raw materials

Fresh *Guduchi* stem spreading over *Nimba* (*Azadirachta indica*) tree was collected from the campus of Gujarat Ayurved University, Jamnagar, Gujarat, India, and authenticated at the Pharmacognosy laboratory from same institute, in between date 5/08/2011 to 26/08/2011.

Fresh *Guduchi* stem was collected as per classical guidelines [4]. *Guduchi* creeping over *Nimba* is said to be the best as the synergy between these plants enhance its efficacy [5]. Matured stem was separated from other parts of the plant like roots, leaves, flowers, fruits and other physical impurities and washed thoroughly with potable water for three times.

Preparation of the Ghana

To develop SMP, 15 batches of *Guduchi Ghana* were prepared. The whole process was divided into two parts i.e. preparation of *Guduchi Kwatha* and *Ghana*. Firstly *Guduchi Kwatha*[6] and later its Ghana[7] was prepared by

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following classical methods. Initially 5 kg raw drug was cut into pieces of 1.6-2.1 cm and pounded thoroughly. Pounded drug was mixed with 4 times potable water (20 L) in a stainless steel vessel and kept aside overnight for soaking (12 hrs). The next day, contents were subjected to mild heat (90°-95°C) to facilitate the evaporation with intermediate stirring till reduction to pada-shesha i.e. 1/4th of the initial quantity, which took 8 hrs. Then, the contents were filtered through four folded cotton cloth and collected into a separate vessel to obtain *Kwatha*. The residue remained over the cloth was discarded.

Further, *Kwatha* was subjected to heat maintaining temperature in between 70°-75°C till a semisolid consistency was obtained. The material was shifted into glass tray and placed in oven for drying at 45°-50°C till complete drying. After complete drying, it was grinded to fine powder, filtered through 80 no. sieve and packed in air tight containers. By following similar process, 14 more batches were prepared to ensure standard manufacturing procedure.

Analytical study

Green *Guduchi* stem, *Guduchi kwatha* and *Guduchi Ghana* were analyzed by employing various analytical parameters. Out of 15 batches, physicochemical analysis was carried out on five randomly selected batches. Organoleptic characteristics (color, odor, taste, touch) and physicochemical analysis like loss on drying at 110°C [8], ash value [9], acid insoluble ash [10], pH value [11], specific gravity at 40°C [12], total solid content [13], and various extractive values (water soluble [14], methanol soluble [15], chloroform soluble, benzene soluble, diethyl ether soluble [16]) were carried out. *Guduchi Ghana* was further subjected to qualitative tests for various functional groups [17,18], quantitative estimations of total alkaloids [19] and percentage of starch contents. Tests for presence of certain heavy metals [20] (mercury, arsenic, lead, cadmium, tin, iron, copper and zinc) and microbial contamination [21] were carried out. Fluorescence analysis was also done for test sample observation under ordinary light, short wave (254 nm) and long wave (366 nm). *Guduchi Ghana* obtained from batch 1 to 15 was mixed thoroughly and this sample was subjected to heavy metal analysis, microbial contamination, fluorescence analysis and HPTLC study.

HPTLC profile

Initially sample solutions were prepared. Accurately weighed 500 mg *Guduchi Ghana* was taken in methanol and was filtered through Whatman I filter paper (Nair Industries, Mumbai). The filtrate was further subjected to chromatographic separation. The Solvent system used was Chloroform: Methanol (9:1 % v/v). 5 µl of sample solution was applied on pre-coated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and the plate was developed in the solvent system up to a distance of 8 cm. The plate was visualized under short and long UV radiation and density of the separated spots was recorded using scanner III. The plate was sprayed with Vaniline-sulphuric acid reagent and observed in visible light. The Rf values were recorded. (Figure 1) Peak display densitogram of *Guduchi Ghana* at 254 nm and 366 nm is placed in Figure 2.

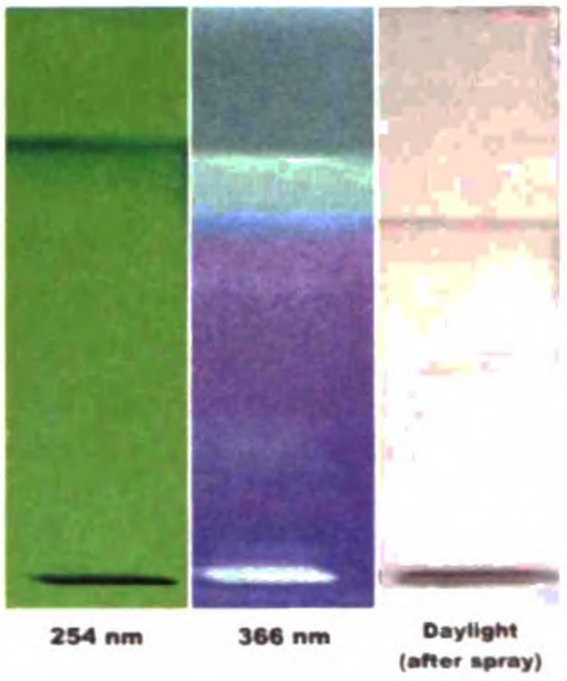

Figure 1: Visualization of *Guduchi Ghana* at 254 nm, 366 nm and in visible light

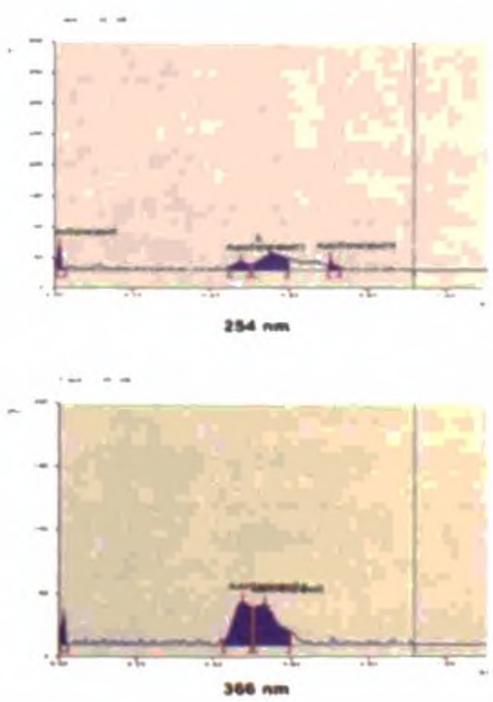

Figure 2: Peak display densitogram of *Guduchi Ghana* at 254 nm and 366 nm

Results and Discussion

In earlier studies, few scholars attempted to prepare *Guduchi Ghana* by using 4 times and 8 times water and the average percentage of *Ghana* obtained was 3.2–3.68%, 4.04–5.56%, 5.0–5.50% and 8.42% respectively [22,23]. Validation of Standard Manufacturing Procedure of *Guduchi Ghana* is also established in a foregoing study [24]. Moreover, its pharmacological [25] and clinical profile [26] is also established on ailments viz. diabetes in recent works. However, published data on physicochemical and phyto-chemical profile of *Guduchi Ghana* is very minimal, considering which an effort has been made by this study to throw some more light toward development and evaluation of analytical profile of the same.

Freshly collected raw drug was authenticated and analyzed before processing because good quality products mainly depend on genuine raw materials. Before preparation of *Kwatha*, separation of physical impurities and outer skin was done for quality maintenance, specific size reduction up to a certain extent to facilitate proper extraction of water-soluble constituents. *Acharya Yadavji Trikamji* has mentioned to use '*Angustha pramana*' (thumb size) of *Guduchi* stem [27]. Accordingly thumb sized or medium size stem diameter (1.6-2.1 cm) was selected for study [28]. Mild heating with peak temperature 70°-75°C was maintained along with continuous stirring during preparation of *Ghana* from *Kwatha*. It was applied for proper extraction and reducing the chances of burning of heat labile constituents in higher temperature.

The Organoleptic characters which correspond to the *panchagnanendriya pariksha* (perception by five sense organs) of *Ayurveda*, were performed at three stages of preparation (for fresh *Guduchi* stem, its *Kwatha* and *Ghana*) because these parameters can change at different stages [Table 1]. The taste of finally prepared *Ghana* was very bitter (+++) due to extraction of bitter principle from the material.

Observations of physicochemical data are tabulated at Tables 2-4. The pH conventionally represent the acidity and alkalinity; pH of Kwatha and different batches of Guduchi Ghana did not revealed much difference and showed to be weak acidic in nature.

Table 1: Organoleptic characters of raw (Fresh *Guduchi* Stem), intermediate product (*Kwatha* and wet *Ghana*) and finished product

Sr. No.	Parameter	Raw (Fresh *Guduchi* stem)	Intermediate product		Finished product
			Kwatha	Wet *Ghana*	Powdered *Ghana*
1	Rupa (Color)	Creamish brown	Brownish green	Greenish brown	Dark brown
2	Rasa (Taste)	Bitter (+)	Bitter (++)	Bitter (+++)	Bitter (+++)
3	Gandha (Odour)	Not specific (bitter smell after removing outer loose skin)	Characteristic	Not specific	Aromatic (chocolaty)
4	Sparsha (Touch)	Soft, Slimy	Liquid, Sticky	Soft, Slimy	Smooth

Table 2: Physicochemical data of Fresh *Guduchi* stem

Parameter	Batches					
	B - 1	B - 2	B - 3	B - 4	B - 5	Mean
Loss on drying (% w/w)	73.7	73.00	73.2	73.9	73.6	73.48
Ash value (% w/w)	1.69	1.73	1.67	1.70	1.69	1.69
Water soluble extractive (% w/w)	7.00	7.12	7.31	6.96	6.93	7.06
Alcohol soluble extractive (% w/w)	14.00	14.30	13.94	14.10	13.91	14.05

Table 3: Physicochemical data of *Guduchi Kwatha*

Parameter	Batches					
	B - 1	B - 2	B - 3	B - 4	B - 5	Mean
pH	5.53	5.64	5.56	5.62	5.64	5.60
Sp. Gravity	1.016	1.016	1.0212	1.013	1.017	1.0172
Total solid content (% w/w)	5.12	6.43	4.56	5.89	5.44	5.48

Table 4: Physicochemical data of *Guduchi Ghana*

Parameter	Batches					
	B - 1	B - 2	B - 3	B - 4	B - 5	Mean
pH value	5.90	5.93	5.90	5.91	5.90	5.90
Loss on drying (% w/w)	7.8	7.71	7.46	7.53	7.96	7.69
Ash value (% w/w)	16.87	16.50	16.35	16.52	16.78	16.60
Acid insoluble ash (% w/w)	0.48	0.51	0.49	0.48	0.50	0.49
Water soluble extract (% w/w)	48.65	48.79	48.32	48.44	48.66	48.57
Alcohol soluble extract (%w/w)	17.1	16.9	17.2	17.1	17.0	17.06
Chloroform soluble extract (%w/w)	0.88	0.85	0.83	0.86	0.87	0.86
Benzene soluble extract (% w/w)	0.08	0.07	0.07	0.09	0.08	0.08
Diethyl either soluble extract (% w/w)	0.05	0.04	0.06	0.05	0.06	0.05

Loss on drying indicates the moisture content; in the present sample, it was 7.69% w/w. Material absorbs moisture during the storage. In conjunction with a suitable temperature moisture will lead to activate the enzymes and suitable condition for prodiferation of living oraganisms is given. Hence, moisture contents may affect the quality of the drug. Although the weight loss in the samples is principally due to water, small amount of other volatile materials will also contribute to the weight loss. High loss on drying value in *Guduchi Ghana* indicates more hygroscopic nature of it.

Ash value depends upon the inorganic substances present in the particular drug. The higher the inorganic substances present in drugs, more will be the ash value. Here, the ash value was 16.60 % w/w; which may be due to the extensive heating proccss involved in preparation of *Ghana*.

Various components have their solubility in particular media. In this study, soluble principles of the samples were more in water and methanol; in water, it was 48.57% and in methanol, it was 17.06%. These two extractive values in *Ghana* were higher than similar extractive values of green *Guduchi* stem, which were 7.06% and 14.05% respectively. These findings are indicative of role of *Jala* and *Agni Samsakara* (soaking in water and subsequent maintained heating) in more extraction of these moieties in *Ghana*. In the solubility test, increase in water soluble extractive was found, which depicts its more bioavailability in water medium. Other obtained extracts (chloroform, benzene and diethyl ether) were fewer quantitatively, which signify that they might be playing minor role in expression of biological activities of *Guduchi Ghana*.

Qualitative tests were done to detect the functional groups. (Table 5) Presence of major active constituents of raw drugs into the finished product suggests the extraction of these moieties in the formulation. The study reveals the presence of glycosides, alkaloids, tannins, phenols, carbohydrates/starch and sterols in all batches of formulation whereas an absence of saponins, flavanoids and proteins was observed.

Table 5: Qualitative test for various functional groups

Functional groups tested	Batches					
	B - 1	B - 2	B - 3	B - 4	B - 5	Mean
Glycosided	+ve	+ve	+ve	+ve	+ve	
Alkaloids	+ve	+ve	+ve	+ve	+ve	
Tannin	+ve	+ve	+ve	+ve	+ve	
Saponin	-ve	-ve	-ve	-ve	-ve	
Flavanoids	-ve	-ve	-ve	-ve	-ve	
Phenols	+ve	+ve	+ve	+ve	+ve	
Proteins	-ve	-ve	-ve	-ve	-ve	
Carbhydrates	+ve	+ve	+ve	+ve	+ve	
Starch	+ve	+ve	+ve	+ve	+ve	
Sterol/Steroid	+ve	+ve	+ve	+ve	+ve	

Total alkaloid content and starch content was found to be 0.58% and 0.315% mg/ml respectively. Presence of starch in sample of *Ghana* suggests passage of suspended particles of starch through filter cloth. Observations on heavy metal analysis were found to be within permissible limits, thus showing the purity of raw drug and also the finished product. Inductive Coupled plasma-Optical Emission Spectrometer (ICP-OES) technique adopted for heavy metal detection (Instrument Make: Perkin Elmer, Model: Optima 3300 RL). Instrument calibrated with reference standard 100 ppm. No heavy metal viz. Mercury (Hg), Arsenic (As), Lead (Pb), Cadmium (Cd), Tin (Sn), Iron (Fe), Copper (Cu) and Zinc (Zn) were detected.

Medicinal plant matters normally carry bacteria and moulds in high numbers often originating in soil or after final drug preparation in product. In *Ghana*, the microbial count was within permissible limits. (Table 6) This indicates the proper hygiene norms followed during the preparation of formulation and packing which are essential to establish the quality standard at finished product level.

Table 6 : Pathogen and total microbial count of *Guduchi Ghana*

Sample	Test	Result (CFU/ml)	Speeification
Guduchi Ghana	Total Bacterial count	Absent	10^5 CFU/ g
	Yeast &Mould count	Absent	10^3 CFU/ g
	E. coli	Absent	Absent
	Salmonella	Absent	Absent
	Psuedomonas aeruginosa	Absent	Absent
	Staphylococcus aureus	Absent	Absent

Fluorescence analysis and HPTLC profile are placed in Table 7 and 8.

Table 7: Fluorescence analysis of *Guduchi Ghana*

Sample	Observation under		
	Ordinary light	Short Wave (254 nm)	Long Wave (366 nm)
Guduchi Ghana	Dark brown	Dark brown	Light brown

Table 8: HPTLC profile of *Guduchi Ghana*

Guduchi Ghana			
Under 254 nm		Under 366 nm	
No. of peaks (spots)	R_f values	No. of peaks	R_f values
4	0.04 0.14 0.26 0.46	2	0.04 0.46

Chromatographic study (HPTLC) was carried out under 254 nm and 366 nm UV to establish the fingerprinting profile and to reveal the possibly active phyto-constituents. It showed phyto-components with Rf values 0.04, 0.14, 0.26 and 0.46 in *Ghana* sample, which may be responsible for expression of its pharmacological and clinical actions.

Conclusion

Oraganoleptic parameters, physicochemical and phyto-chemical analysis as well as HPTLC, heavy metal profile, and microbial overload are essential parameters for quality assurance; thus were carried out as per the norms of WHO guidelines. The average percentage of dried *Ghana* obtained was 3.8 %. The pH, loss on drying, ash value and water soluble extracts values of *Ghana* were 5.90, 7.69, 16.60 w/w and 48.57 % w/w respectively. No heavy metal and microbial load were found; thus complying the official standards. Glycosides, Alkaloids, Tannin, Phenols, Carbohydrates, Starch and Sterol/Steroid were detected. Number of peaks obtained in HPTLC also corresponds to this finding. The data obtained by present study may prove a torch bearer for future studies.

References:

1. WHO. Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. World Health Organization, 2004; Geneva.
2. Acharya YT. Siddha Yoga Sangraha. Jwaradhikara, 13th ed. Nagpur: Baidyanath Ayurveda Bhavan Ltd; 2008; 4.
3. Shastri P, editor. Sharangadhara Samhita of Sharangadhara (Madhyama Khanda). 6th ed. Varanasi: Chaukhamba Orientalia; 2005; 8/1: 206.
4. Shastri P, editor. Sharangadhara Samhita of Sharangadhara (Prathamakhanda). 6th ed. Varanasi: Chaukhamba Orientalia; 2005; 1/45 : 11.
5. Anonymous. Quality Standards of Indian Medicinal Plants. Vol. 1, New Delhi: ICMR; 2003; 212.
6. Shastri P, editor. Sharangadhara Samhita of Sharangadhara (Madhyama Khanda). 6th ed. Varanasi: Chaukhamba Orientalia; 2005; 9/3 :212.
7. Acharya YT. Siddha Yoga Sangraha. Jwaradhikara, 13th ed. Nagpur: Baidyanath Ayurveda Bhavan Ltd; 2008; 4.
8. Anonymus, The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family Welfare, Govt. of India, part-1, vol-1, 1st ed, 1999 ; 214 (2.2.9).
9. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 213 (2.2.3).
10. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 213 (2.2.4).
11. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 230 (3.3).
12. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 236 (3.7).
13. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 214 (2.2.9).
14. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 214 (2.2.7).
15. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 214 (2.2.6).
16. Ibid, The Ayurvedic Pharmacopoeia of India, part-1, vol-1, Appendix-2 : 214 (2.2.8).
17. Baxi AJ, Shukla VJ, Bhatt UB. Methods of qualitative testing of some Ayurvedic formulations. 1st ed. Jamnagar: Gujarat Ayurved University; 2001; 5-12.
18. Khandelwal KR. Practical pharmacognosy techniques and experiments. 16th ed. Pune: Nirali Prakashan; 2006 ; 149-56.
19. Stephen K. Medicinal plant alkaloids: an introduction for pharmacy students. 2nd ed. University of Toronto press; 1965 ; 44.
20. Anonymous. Quality control methods for medicinal plant materials. W.H.O., Pharm; 1992; 559, Rev. 1 : 46-48.
21. Anonymous. Quality control methods for medicinal plant materials. W.H.O., New Delhi: A.I.T.B.S. Publishers; 2002; 48-55.
22. Umeratiya B. A Comparative pharmaceutical study of *Guduchi Ghana* prepared by *Kwatha* and aqueous extract method, *Ayu journal*, vol.30, No. 1 (Jan-Mar) 2009; 77-82.
23. Khedekar S. A Pharmaceutico-Pharmaco-clinical Standardisation of Makaradhwaja prepared by Swarna Patra, Swarna Varkha and Swarna Bhasma w.s.r. to Madhumeha (Diabetes Mellitus), M.D. Dissertation. Jamnagar: Dept. R.S. and B.K., I.P.G.T. and R.A; 2009; Pharmaceutical study : 90.
24. Parmar D. The effect of Puta in the preparation of Vanga Bhasma w.s.r. to Madhumeha (Diabetes mellitus), M.D. Dissertation. Jamnagar: Dept. R.S. and B.K., I.P.G.T. and R.A; 2009; 84.
25. Dhundi S. Pharmaceutical standardization of *Guduchi Ghana* (solidified aqueous extract of *Tinospora cordifolia* miers.), *International Research Journal of Pharmacy*, 2011;2 (11) : 102-104.
26. Sharma R, Galib, Prajapati PK. Validation of Standard Manufacturing Procedure of Guduchi Ghana [dried aqueous extract of *Tinospora cordifolia* (Willd.) Miers] and its tablets. *Ayurpharm Int J Ayur Alli Sci*. 2012;2 (7): 224-232.
27. Sharma R, Kumar V, Ashok BK, Galib R, Prajapati PK, Ravishankar B. Evaluation of hypoglycaemic and anti-hyperglycaemic activities of *Guduchi Ghana* in Swiss albino mice. *Int J Green Pharm* 2012; 7: 145-8.
28. Sharma R. The effect of two different dosage forms of *Guduchi* i.e. *Satva* and *Ghana* W.S.R. Antihyperglycemic effect on *Madhumeha* (NIDDM). M.D. Dissertation. Jamnagar: Dept. R.S. and B.K., I.P.G.T. and R.A; 2012; 132-33.