Physico-phytochemical Evaluation of Guduchi Ghana Prepared by Different Methods

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Abstract

Guduchi (Tinospora cordifolia) is described by the synonym of 'Amrita', which means it has the capacity of life restoring. So, it is the important Ayurvedic Rasayana drug, or rejuvenative tonics. Guduchi Ghana is rehabilitated form of Kwatha. Guduchi Ghana has mentioned in Sidhdha Yoga Sangraha by the name of 'Samshamni vati'

Guduchi Ghana was prepared by Kwatha and Soxhlet method as per above reference and modern technique respectively in the department of Rasashastra and Bhaishajya Kalpana, Jamnagar. The third sample was procured from the market for analytical comparison. All the samples were analyzed physico-chemically i.e. pH, Loss on drying, Acid insoluble ash, Water and Methanol soluble extractives, qualitative Tests , quantitatively Estimation of alkaloids content and starch, Heavy metal analysis and HPTLC was also carried out.

The obtained result of analytical parameters shows that the water solubility of Kwatha sample, Soxhlet and market sample are 41.16, 90.16 and 93.63 respectively. Glycoside was found to be present in both classical and Soxhlet sample but absent in Market sample. The % of total Alkaloids content in Kwatha, Soxhlet and market sample are 0.080, 0.500 and 0.305 respectively. The % of Starch content in Kwatha sample, Soxhlet and market sample are 0.506, 0.478 and 0.315 respectively

Key words

Guduchi, Kwatha, Ghana, Soxhlet method, Tinosporaside.

Introduction

Considering the significance of traditional practices in global health care, WHO also has been encouraging and promoting these traditional practices since last few decades. Hence, the standardization of raw drug, in processing, finished products, verification of the claims, mechanism of action and free from heavy metal and microbial contamination etc. become some of the major issues, which have to be taken into consideration in order to increase the world wide acceptability of herbal drugs through out the globe and also prove the clinical efficacy of the old age remedies.

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Guduchi (Tinospora cordifolia), family Menispermaceae[1] is an important medicinal plant recognized as a good Rasayana drug[2] in the classical texts. The active principles and juice of the fresh plant possess a number of pharmacological activities. Dry barks of Tinospora cordifolia have anti-spasmodic, antipyretic[3,] anti-allergic[4], anti-inflammatory[5, 6,] immunomodulatory[7] and anti-leprotic[8] properties.

Guduchi Ghana is the secondary formulation derived from the primary formulation i.e. Kwatha. Acharya Yadavji Trikamji describes first time it as the name of 'Samsamni Vati'[9]. The aqueous extract of Guduchi Ghana is prepared by Soxhlet method. Another sample has been purchased from the market, which is prepared by reactor cum distillation.

An attempt has been made to lay down standards of different samples of Guduchi Ghana prepared from Kwatha and aqueous extract and to compare the data with market samples.

Materials and Methods

Guduchi Ghana was prepared by employing two different methods, prepared from Kwatha and aqueous extract.

Preparation of the Ghana

For the preparation of Guduchi Ghana by classical method, the stem was collected near Jamnagar and authentified by botanist. The freshly collected stem was soaked in four times of water and made decoction of it. The decoction was reheated till it became semisolid and dried in oven at 55°C.

In Soxhlet method[10], the coarsely ground crude drug taken in "thimble" made of porous cotton bag, was placed in central part. The liquid was boiled on maintained temperature of 70°C. The condensed extract dripped into the thimble containing the crude drug, extracting it by contact. When the level of liquid rise to the top of the siphon tube, the liquid contents of central part was run through siphon into Solvent flask. This process was continuous for five times. Then the extract was collected and heated on gas stove. The liquid was attained semisolid type consistency the heating process was stopped .The extract was taken into tray and subjected to oven 55°C for drying. Both the samples were prepared in the practical laboratory of the Department of Rasashastra and Bhaishajya Kalpana, I.P.G.T. & R.A., Jamnagar. The third sample was collected from the market.

Analysis of raw material (Green Guduchi Stem), intermediate and finished product

The raw drug of Guduchi stem was collected from the rural area of Jamnagar. This raw drug was authentified and analyzed before preparing Guduchi Ghana.

Organoleptic Characters and physico-chemical analysis of raw Material (Green Guduchi Stem), intermediate and finished product analysis were carried out (Table no.1).

Qualitative test for various functional groups[11]:

Using the methanol or water-soluble extracts of the samples carried out qualitative tests of Alkaloids, Glycosides, Tannins, Saponins, Starch, Flavonoids, Amines, Phenols, Terpanoids / Sterol

and Anthocynins

Average weight of Guduchi Ghana capsule[12]

Randomly selected 20 capsules were weighed and average weight was calculated.

Disintegration time of Guduchi Ghana capsule[13]

This test determines whether capsules disintegrate within a prescribed time when placed in liquid medium under the prescribed experimental condition.

Apparatus used: Basket & pedal apparatus Introduce one capsule into each tube and add a disc to each tube, suspend the assembly in beaker containing the liquid at specified temperature for dispersible capsules for specified time. Remove the assembly from the liquid. The capsule passes the test all of them have disintegrated. If one or two capsules fail disintegrate, repeat the test on additional capsules. If the capsules adhere to the disc and preparation being examined fails to comply, repeat the test omitting the discs.

Estimation of alkaloids content[14]

Accurately weight 2.5g of sample was weighed accurately, 50 ml of Methanol was added to it and it was kept overnight. Next day, it was filtered in an evaporating dish and kept on a water bath for evaporation. Then in the concentrated extract 30 ml conc. H_2SO_4 was added and made the solution acidify. To basify acidic layer, NH4 solution was added till it become alkaline (ph 10 by the use of litmus paper). Then the liquid was transferred into separating funnel and 60 ml Ether was added. Ether layer was separated on the upper part and lower layer was thrown. Ether layer was collected in accurately weight evaporating dish and kept on water bath to evaporate till complete drying. Then it was weighed and calculated % total alkaloid.

Estimation of starch (Mont. Gomery, 1957)[15]

Sample solution was prepared in 80 % methanol centrifuge at 2000 rpm for 15 min. In this residue 4 ml distilled water was added and heated on water bath for 15 min.3 ml of 52 % perchloric acid was added in the sample and centrifuged at 2000 rpm for 15 min. Then 0.1 ml of 8 % phenol and 0.8 ml distilled water were added in 0.1 ml aliquot. 1 ml solution and 8 ml conc. H_2SO_4 were collected and read the absorbance at 490 nm.

Heavy metal analysis[16]

For the determination of heavy metals, 0.1-0.2 g of dry material was weighed into digestion vessel. Add 5 ml conc. HNO_3, close vessel, and placed it in the vessel holder, place vessel holder in microwave oven and closed door carefully, set oven programme and started digestion. Wattage should be selected upon the number of vessels being used for digestion. Full wattage was used. Removed digestion vessel from microwave oven then let it cool thoroughly before opening them. Transferred solution to 50 ml volumetric flask and diluted to mark with deionized water. Then transferred solution to plastic container. Treat blanks in the same as test samples. One blank should be included in every set.

Considering it 1 g each of the three groups of Guduchi Ghana was taken for digestion and rest procedure followed as

mentioned.

Take all necessary precaution to avoid possible contamination of the sample. Analyze prepared samples with atomic absorption spectrophotometer.

Microbiological Study[17]:

The determination of Escherichia coli and moulds may indicate the quality of production and harvesting practices.

Culture medium was prepared by following method:

As per requirement weighed solid ingredients was dissolved in appropriate distilled water and agar* was added. The solution was heated and final volume was made and the medium was distributed in flasks and sterilized by autoclaving at 121°C for 15 min. In the sterilized area, the solution was poured into plates and kept for cooling. After that weighed sample i.e. 1 g Guduchi Ghana was spreaded on plates in sterilized area. Plates were kept downwards. Plates were observed after 24 h for bacteria and 36 to 48 h for yeast and moulds.

For bacteria :Mac conkey Agar

For yeast and mould: Sabroud's Agar

Chromatographic study (TLC & HPTLC)

A comparative study of Guduchi Ghana from Kwatha and aqueous extract and market sample of Guduchi extract were carried out through thin layer chromatographic studies by using different condition and finally by using suitable conditions chromatographic patterns were developed. Here on attempt has been made to develop TLC patterns according to presence of chemical constitutes of the samples can be checked. Hence for the comparison of Kwatha, aqueous extract and market sample were used. For chromatographic patterns the methanolic extract of Kwatha, aqueous extract and market sample were used. Standard sample of **Tinosporaside** was used for the comparative study of the samples. After developing the chromatographic pattern on TLC, results were developed on photograph and same results were also recorded on HPTLC.

OBSERVATIONS AND RESULTS:

* The Organoleptic characters of the Green Guduchi stem, Kwatha, wet Ghana & dried Guduchi Ghana sample are tabulated as Table no. – 2
* The Physico- chemical parameters of the Green Guduchi stem sample are tabulated as Table no. –3
* The comparative analytical parameters of Kwatha are tabulated as Table no. – 4.
* The average physico-chemical parameters of the Guduchi Ghana are tabulated as Table no. –5.
* The qualitative test for various functional groups is tabulated as Table no. – 6
* The % of Total Alkaloid Content is tabulated as Table no. – 7.
* The comparative results of % of Starch Content is tabulated as Table no. – 8.
* The comparative results of I.C.P.M.S. Results are tabulated as Table no. – 9.
* The total microbial count Results are tabulated as Table no. – 10.
* The average weights of both the

groups of capsules are tabulated as Table no.- 11.

* The average disintegration time of both the groups of capsules are tabulated as Table no.- 12.
* The R_f values and colour of the samples of Guduchi Ghana in under 366 nm and daylight are tabulated as Table no.- 13.
* The Percentage content of tinosporoside estimated using HPTLC is tabulated as Table no.- 14.

Discussion

The present analytical study has been carried out to compare the formulation Guduchi Ghana prepared by different methods and market sample of Guduchi Ghana and to establish the quality of the finished product. Organoleptic and physico-chemical analysis were carried out at three different stages of manufacturing i.e. raw, intermediate process and finished product level to reveal the changes occurred during the processing and to establish the standard quality parameters for the formulation. Qualitative tests for various chemical moieties carried out to reveal any possible changes from raw to finished product level. Spectroscopic and Chromatographic study – HPTLC and HPLC were carried out to establish the fingerprinting profile for the formulation and to reveal the possibly active phyto-constituents compare them both at raw and finished product level. Also the microbiological study and heavy metal content of the three samples of Guduchi Ghana were carried out to finally fulfill and establish the quality standard at finished product level.

The organoleptic characters of Green Guduchi stem were as shown in Table No.2, which was performed at three stages of production because these parameters were changed at different stages. The colour was cremish brown while soft and slimy in touch and bitter in taste. The organoleptic characters of Guduchi Kwatha were brownish green in colour and sticky in touch as shown in table No.2., which differs from the organoleptic characters of different samples of Guduchi Ghana The colour varies from cream to dark brown due to the effect of heat on the sample. The odour of Guduchi Ghana – Kwatha and Soxhlet sample were found no specific, but Aromatic smell was found in market sample. While in texture and taste, no specification was found.

pH was taken of Kwatha and different samples of Guduchi Ghana. Table No. 4 shows the pH of Kwatha that was 5.65, 5.64, and 5.53 in batch-1, batch-2, and batch-3 respectively. Table No.5 shows the pH of different samples of Guduchi Ghana. Those values show not much difference in the pH.

Data pertaining to Table No.4 shows the slight variation in specific gravity of Guduchi Kwatha. On the basis of the average specific gravity can be calculated as 1.0126, 1.019 and 1.0213 for Kwatha of batch-1, batch-2, and batch-3 respectively.

Data pertaining to Table No.4 shows the slight variation in total solid content of Guduchi Kwatha. The average T.S.C. comes 5.01, 4.29 and 6.53 in batch-1, batch-2, and batch-3 respectively. In batch-3 T.S.C. comes slight more in comparison to other batches due to more

water-soluble extractive comes in Kwatha on heating.

Table No. 3 shows loss on drying value of Green Guduchi stem is 73.4 % w/w. It is because of the herb is in green state. Table No. 5 shows the variation in loss on drying values of different samples of Guduchi Ghana. The average value of loss on drying is 8.84 % w/w, 11.26 % w/w, and 16.50 % w/w in GGK, GGM and GGS samples respectively. Loss on drying values was high in all the samples may be due to hygroscopic nature of the starch. The value was more in the sample prepared by Soxhlet method is indicating presence of more water-soluble starch.

Ash value of Green Guduchi stem is 1.75 % w/w as shown in Table No. 3. The average ash values (% w/w) are 12.33, 17.33 and 28.26 in GGK, GGM and GGS samples respectively. Ash value of GGM and GGS samples were higher than GGK samples is indicative of presence of more inorganic materials (impurities like sand) in those samples. In GGS sample, presence of more inorganic materials may be due to porcelain pieces those are put in solvent flask (Table No. 5).

The average acid insoluble ash (% w/w) values are 0.50, 1.51 and 2.03 in GGK, GGM and GGS samples respectively. Acid insoluble ash values were also higher in GGM and GGS samples indicating the same reason (Table No. 5).

The water-soluble extractive value of Green Guduchi stem is 7.00% w/w (Table No. 2). The average water-soluble extractive values are 41.16, 93.63 and 90.16 in GGK, GGM and GGS samples respectively (Table No. 5). Here,

The water-soluble extractive value of Green Guduchi stem is less than the different samples of Guduchi Ghana, which indicate the role of temperature in the extraction. The water soluble extractive data indicate that by modern extraction method only water soluble portion is extracted, but by Ayurvedic extraction method some soluble as well as insoluble part is also separated due to straining.

The alcohol soluble extractive value of Green Guduchi stem is 14.00% w/w(Table No. 2). The average alcohol soluble extractive values are 1.86, 19.29 and 32.76 in GGK, GGM and GGS samples respectively (Table No. 5). Here, also

The alcohol soluble extractive value of Green Guduchi stem is less than the different samples of Guduchi Ghana, which indicating the same reason as mentioned in W.S.E. More alcohol soluble extractive in Soxhlet and Market sample indicates presence of more alcohol soluble starch in those samples.

Table 6 shows the results of the qualitative test carried out for various functional groups in the finished products. Presence of major known of raw drugs into the finished product suggests the extraction of these moieties in the formulation. Glycoside was found to be present in both classical and Soxhlet but absent in Market sample. It may be due to destruction of Glycosides during preparation of extract of market sample.

The % total alkaloids content are 0.08, 0.305 and 0.500 in GGK, GGM and GGS respectively as shown in (Table No. 7). They are insoluble sparingly in water, but pass readily into solution on treatment with dilute acids, with formation of soluble salts. From aqueous solution of

their salts the free alkaloids are precipitated by basification (e.g. by alkali carbonates). In organic solvents such as ether or chloroform, alkaloids themselves dissolve freely, but their salts only sparingly.

The % starch are 0.506, 0.315 and 0.478 in GGK, GGM and GGS respectively as shown in (Table No. 8). Starch occurs in two forms. a- amalase and amylopectin. a- Amalase is not truly soluble in water but amylopectin is soluble in water. Thus the variation in percentage may be due to solubility of starch in water. In Kwatha sample both forms of starch that are soluble in water as well as insoluble in water may be present because during straining some water insoluble starch may come.

All most all the sample were found B.D.L. (Below detection limit) for heavy metal components (Table No. 9).

Data pertaining to the table- 10 shows that in all the samples pathogens i.e. E. coli, Salmonella spp., Staphylococcus aureus, and Pseudomonas aureus were absent, while total microbial count were 605 cfu. /g, 725 cfu. /g and 340 cfu. /g in GGK, GGM and GGS respectively. So, total microbial count was higher in market sample may be due to more chances of contamination in the drug during packaging and also during powder microscopy fibers was found, whereas less in Soxhlet samples.

The average weight of capsules of GGK sample was 255.3 mg, while capsules of GGM sample was 254.8 mg. (Table No. 11).

These two samples were carried out for disintegration test using IP standard tablet disintegration test apparatus and 500 ml distilled water as medium. The capsules should be disintegrated within 15 minutes according to IP (Table No. 12).

Table 13 shows the presence of compound Tinosporoside in all the samples of Guduchi Ghana and Guduchi Kwatha. The maximum no. of spots were found in GGS sample i.e. 9 spots, while in rest of the samples 8 spots were found under the UV light 366 nm. In Guduchi Ghana prepared by Soxhlet method, one spot was found more in comparison to other samples i.e. green in colour and R_f value was 0.76. It indicates one more active constituent may be present in this sample. Under the daylight 6 spots were found in all the samples (Fig. a and b).

Table 14 shows the percentage of compound Tinosporoside was higher in Guduchi Ghana prepared by Kwatha in comparison to both samples of Guduchi Ghana. The peak areas and absorption spectra were recorded and the amount of Tinosporoside was calculated using its calibration curve (Fig. c).

Conclusion

The Kwatha preparation may be taken as better extraction process as more active constituents i.e. Tinosporoside are found in classically prepared Guduchi Ghana.

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Tables

Table no.1 Organoleptic and Physicochemical Parameters of raw (Green Guduchi stem), intermediate product (Kwatha & wet Ghana) and finished product;

Raw (Green Guduchi Stem)		
1.	Organoleptic Characteristic	Colour, Odour, Taste, Touch
2.	Physico-chemical parameters	- Loss on drying[18] - Ash value[19] - Water soluble extractives[20] -Ethanol soluble extractives[21]
Intermediate product (Kwatha & Green Ghana)		
1.	Organoleptic Characteristic of Kwatha & wet Ghana	Colour, Odour, Taste, Touch
2.	Physico-chemical parameters (Kwatha)	- pH value[22] - Specific gravity[23] - Total solid content[24]
Finished product		
1.	Organoleptic Characteristic	Colour, Odour, Taste, Touch
2.	Physico-chemical parameters	- pH value - Loss on drying - Ash value - Acid insoluble ash[25] - Water soluble extractives -Ethanol soluble extractives

Table no. 2 organoleptic characters of raw (Green Guduchi Stem), intermediate product (Kwatha & wet Ghana) and finished product

Sr. No.	Parameter	Raw (Green Guduchi stem)	Intermediate product		Finished product		
			Kwatha	Wet Ghana	GGK	GGM	GGS
1.	Colour	Creamish brown	Brownish green	Dark green	Creamish brown	Cream	Dark brown
2.	Odour	No specific	Characteristic	No specific	No specific	Aromatic	No specific
3	Touch	Soft, Slimy	Liquid, Sticky	Soft, Slimy	Bitter	Bitter	Bitter
4	Taste	Bitter	Bitter	Bitter	Smooth	Smooth	Smooth

Table no. 3 Physicochemical Parameters of Green Guduchi stem

Sr. No.	Parameter	Values
1.	Loss on drying	73.4% w/w
2.	Ash value	1.75 % w/w
3	Water soluble extractive	7.00% w/w
4.	Alcohol soluble extractive	14.00% w/w

Table no. 4 Analytical data of Kwatha

Sr.no.	Parameter	B-1	B-2	B-3
1.	pH	5.65	5.64	5.53
2.	Sp. Gravity	1.0126	1.019	1.0213
3.	Total solid content (%w/w)	5.01	4.29	6.53

Table no.. 5 Average Values of Physico-Chemical Parameters of Different Samples of Guduchi Ghana

Sr. No.	Parameter	GGK	GGM	GGS
1.	pH value	5.50	5.53	5.52
2.	Loss on drying	8.84	11.26	16.50
3.	Ash value (%W/w)	12.33	17.33	28.26
4.	Acid insoluble ash (%w/w)	0.50	1.51	2.03
5.	Water soluble extract (%W/w)	41.16	93.63	90.16
6.	Alcohol soluble extract (%W/w)	1.86	19.29	32.76

Table no.6 the results of qualitative test for various functional groups

Sr.no.	Tests for	GGK	GGM	GGS
1.	Alkaloids	+Ve	+Ve	+Ve
2.	Glycosides	+Ve	-Ve	+Ve
3.	Starch	+Ve	+Ve	+Ve
4.	Tannin	-Ve	-Ve	-Ve
5.	Saponin	+Ve	+Ve	+Ve
6.	Flavanoids	+Ve	+Ve	+Ve
7.	Amine	+Ve	+Ve	+Ve
8.	Phenols	-Ve	-Ve	-Ve
9.	Terpanoids/ sterol	-Ve	-Ve	-Ve
10.	Anthocyanin	-Ve	-Ve	-Ve

Table no. 7 % of total Alkaloid content

Sample	% Total Alkaloid content
GGK	0.080
GGM	0.305
GGS	0.500

Table no. 8 % of Starch content;

Sample	% of Starch content (mg / ml)
GGK	0.506
GGM	0.315
GGS	0.478

Table no. 9 I.C.P.M.S. the results;

Sample/Element	Hg	Ar	Pb	Cd
GGK	Not detected	Not detected	Not detected	Not detected
GGM	Not detected	Not detected	Not detected	Not detected
GGS	Not detected	Not detected	Not detected	Not detected

Table no. 10 Pathogen and total Microbial count different samples of Guduchi Ghana;

Sr. No.	Samples	Pathogen				Total microbial count cfu./ g
		E. coli	*Salmonella spp.*	*S. aureus*	*Pseudomonas aureus*	
1.	GGK	Absent	Absent	Absent	Absent	605
2.	GGM	Absent	Absent	Absent	Absent	725
3.	GGS	Absent	Absent	Absent	Absent	340

Table no. 11 average weight of both the groups of capsules

Sr.No.	Samples	Average weight (mg)
1.	GGK	255.3
2.	GGM	254.8

Table no.12 disintegration time of both samples

Sr.No.	Samples	Disintegration Time (min)
1.	GGK	5.90 min
2.	GGM	6.16 min

Table no. 13 R_f values and colour of the samples of Guduchi Ghana in under 366 nm and daylight

Samples	Under 366 nm			Under daylight		
	No. of spot	R_f values	Colour of the band	No. of spot	R_f values	Colour of the band
Guduchi Kwatha	**8**	0.18	Light blue	**6**	0.11	Light green
		0.25	Light green		0.16	Light violet
		0.35	Fl green		0.38	Brown
		0.38	Reddish brown		0.49	Light purple
		0.49	Light blue		0.58	Light purple
		0.59	Light blue			
		0.64	Light blue		0.66	Light purple
		0.68	Light yellow			
Guduchi Ghana-Kwatha method	**8**	0.18	Light blue	**6**	0.11	Light green
		0.25	Light green		0.16	Light violet
		0.35	Fl green		0.38	Brown
		0.38	Reddish brown		0.49	Light purple
		0.49	Light blue		0.58	Light purple
		0.59	Light blue			
		0.64	Light blue		0.66	Light purple
		0.68	Light yellow			
Guduchi Ghana-market sample	**8**	0.18	Light blue	**6**	0.11	Light green
		0.25	Light green		0.16	Light violet
		0.35	Fl green		0.38	Brown
		0.38	Reddish brown		0.49	Light purple
		0.49	Light blue		0.58	Light purple
		0.59	Light blue			
		0.64	Light blue		0.66	Light purple
		0.68	Light yellow			
Guduchi Ghana-Soxhlet sample	**9**	0.18	Light blue	**6**	0.11	Light green
		0.25	Light green		0.16	Light violet
		0.35	Fl green		0.38	Brown
		0.38	Reddish brown		0.49	Light purple
		0.49	Light blue		0.58	Light purple
		0.59	Light blue			
		0.64	Light blue		0.66	Light purple
		0.68	Light yellow			
		0.68	Light yellow			

Table no. 14. percentage content of Tinosporoside estimated using HPTLC

Sr. no.	Sample	Tinosporoside (% w/w)
1	Extract	0.114
2	Kwath	0.067
3	Batch-II	0.123
4	Market Sample	0.112