

Comparative HPTLC Fingerprinting Study of *Guduchi Kwatha* prepared by using different techniques

Research Article

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Abstract

Kwatha Kalpana is the basic dosage form and standard of its secondary dosage forms are depended upon it. There are various challenges in preparation of *Kwatha Kalpana* in terms of particle size, temperature, proportion of water etc. In order to optimize the process of *Kwatha* the study was planned to assess the quality by HPTLC. For this, *Guduchi* (*Tinospora Cordifolia* Linn) is selected as *Kwatha* drug in its fresh form. *Kwatha* was prepared by classical method using three different proportion of water i.e. 16 parts, 8 parts and 4 parts and reduced to 1/8th, 1/4th and 1/4th part respectively. In other alternative techniques, time-based techniques were used by 1hour, 2 hours, 3 hours and 4 hours boiling. Soxhlet extraction method was been used at three different temperatures i.e. 60^o, 80^o and 100^oC and pressure cooker technique has been used at two different boiling duration i.e. 30 minutes and 60 minutes. Comparative evaluation of HPTLC fingerprinting of all these techniques were done. Conventional method of *Guduchi Kwatha* which used four parts of water observed maximum number of phyto-constituents. In alternative methods, higher spots were noted in *Kwatha* prepared after four hours of boiling and in the Soxhlet extraction method at 60^oC. Also, the Pressure cooker method showed highest spots after 60 minutes of boiling. Presence of common R_f values in all the studied samples of *Kwatha* suggest that there are some stable phyto-constituents which have no impact of types of processing.

Keywords: *Guduchi*, *Kwatha*, Soxhlet, Pressure cooker, Spots.

Introduction

Kwatha preparation is a common practice in Ayurveda pharmaceutical industries. There are various challenges in preparation of *Kwatha Kalpana* in terms of particle size, temperature, proportion of water to be used according to the consistency of drugs etc. Further, various preparation techniques of *Kwatha* hampers the quality of *Kwatha* whether it is small scale or large-scale industrial process as there are no common techniques which are followed universally. Although, the conventional processing of *Kwatha* is gold standard still the addition of water as per consistency of *Kwatha* drugs is the challenge. Often the excessive water addition in *Kwatha* leads to extra fuel consumption which ultimately increases the processing cost. So there needs to look for other alternative *Kwatha* techniques which gives optimum quality with less processing cost. Hence, the various techniques of *Kwatha* were studied. For this purpose, *Guduchi* (*Tinospora Cordifolia* Linn) was selected. Classical references advocate use of fresh *Guduchi* in *Kwatha* preparations. (1) When fresh form of *Guduchi* is used for *Kwatha* purposes, often quantity

of water to be added for *Kwatha* is quite uncertain as it refers to the *Mrudu* (Soft) consistency of drug category. Due to the sticky nature of fresh *Guduchi* most of the time water is added as per the need so, the quality assurance of final product remains the question mark. Hence, the study has been conducted to prepare *Guduchi Kwatha* by using conventional method with various proportion of water. In order to find other alternative techniques which gives same quality as conventional method at the same time save time and fuel, other methods were studied by controlled time duration heating, by soxhlet method at different temperature and pressure cooker method. Previously, research on *Guduchi Kwatha* were reported by the use of 8 times of water, it gives good yield but with the 16 times of water, yield becomes very less which confirmed *Guduchi* as a medium type of drug depending upon hardness (2) whereas in another study which established *Guduchi* as a *Mrududravaya* hence used 4 parts of water in the *Kwatha* for the preparation of *Guduchi Ghana*. (3) So, the present study was planned to see the quality of *Kwatha* by different proportion of water and other alternative techniques.

In order to assess the quality, the prepared *Kwatha* were analyzed by HPTLC study. Comparison among *Kwatha* were done on the basis of presence of highest R_f values which point out the specific phytoconstituents present in the *Kwatha* preparations.

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Methods and Materials

Collection and Authentication of Guduchi Stem:

Fresh *Guduchi* stem was collected from forest region of Yavatmal (Lat N20°22'42.5604", Long E 78° 8' 26.988"). It was certified and authenticated by Botanical survey of India (BSI). 250gm of cut pieces of Fresh *Guduchi* (cut pieces) were taken for *Kwatha* purpose.

• **Water for Kwatha purpose:** Distilled water was taken for *Kwatha* preparation.

• **Equipment for Kwatha preparation: For Group A and B technique:** Stainless-steel vessel of having capacity of 5 lit (Length – 20cm: Depth -20 cm: Diameter -18cm) was taken for *Kwatha* preparation, LPG gas (Commercial LPG at 2.92kN/m² (30gf/cm²)), For **Group C technique:** Soxhlet Apparatus of capacity 3lit (Extractor 1000ml F.B Flask 3000ml Allihn condenser 300mm Rating watt – 500) For **Group D technique:** Pressure cooker of ISI (IS – 2347), CM/L -3707063(German steel) having capacity of 5 lit (Length – 20cm, Depth – 17cm,Diameter - 23cm)

Images 1: Fresh Guduchi for Kwatha preparation



A. Cut pieces of Guduchi

B. Guduchi Kalka (Paste)

Methods

Categorization of Kwatha techniques: Preparation of *Kwatha* were done by different techniques which were categorized under four major groups i.e. Conventional method (Group A), Time Based method (Group B), Soxhlet method (Group C) and pressure cooker method (Group D). Total 12 batches were prepared by these techniques and codified for the practical purpose.

Table 1: Codification of Guduchi Kwatha Prepared by four different techniques

Group	Kwatha Techniques	Process of the Kwatha	Code Used
A	Conventional Method	1:16 times of water	GC1
		1:8 times of water	GC2
		1:4 times of water	GC3
B	Time Based Method	1 hr. boiling	G1H
		2hrs. boiling	G2H
		3 hrs. boiling	G3H
		4hrs. boiling	G4H
C	Soxhlet Method	@60° C	GS60
		@ 80° C	GS80
		@ 100° C	GS100
D	Pressure cooker method	30 min boiling in PC	GP30
		60 min boiling in PC	GP60

Method of Preparation

Group A: *Guduchi Kwatha* were prepared by fresh *Guduchi Kalka* (250gms) in three batches by using three different proportion of water i.e. 16 parts (4000 ml), 8 parts (2000 ml) and 4 parts(1000 ml) of water and boiled to reduced 1/8th, 1/4th and 1/4th respectively. (3 batches)

Group B: *Guduchi Kwatha* were prepared using four different time duration using *Guduchi kalka* (250G) and 8 Parts (2000ml) of distilled water and boiled. First batch were boiled for 1 hr only(G1H). Similarly, another three batches were prepared by boiling the *Kwatha* for 2 hrs(G2H), 3hrs(G3H) and 4 hrs(G4H) respectively.(4 batches)

Group C: In this technique, three batches of *Kwatha* were prepared by Soxhlet extraction technique using *Guduchi Kalka* (250G) and distilled water (2000ml) at three different temperatures i.e. 60°C, 80°C and 100°C respectively. (3 batches)

Group D: In this method, pressure cooker was used for *Kwatha* preparation. *Guduchi kalka* was then taken into Pressure cooker and 8 Parts (2000ml) distilled water was added into it and boiled at low temperature for 30 mints. Another batch was prepared by boiling the *Kwatha* for 60 mints. (Total 2 batches)

Temperature of *Kwatha* belongs to group A, B and D were maintained between 91°C to 100°C. Frequent stirring were done in these group in order to maintain the temperature of mixture and avoid excessive heating at base. All the 12 samples of *Kwatha* were filtered with muslin cloth and stored in PET bottles.

Analytical study

HPTLC Study of Kwatha

Table 2: HPTLC Study of Guduchi Kwatha

Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Distance Between Tracks	11.8 mm
Sample Application Volume	5.0 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Chloroform : Methanol (9 : 1 v/v)
Visualization	@ 254 nm, @ 366 nm and @ 540 nm (after derivatization)
Spray reagent	Anisaldehyde - sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 Second
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 5°C for 3 minutes

Preparation of Test solution

5 g of *Guduchi Kwatha* sample was weighed accurately in an evaporating dish and evaporated on water bath till complete dryness. The residue thus obtained was dissolved in 1 to 2 mL Methanol and filtered with 45 μ Syringe filter. The solution thus obtained was used for HPTLC fingerprinting. The procedure was repeated for all groups of *Kwatha*.

Preparation of Spray reagent [Anisaldehyde – Sulphuric acid reagent]:

0.5 mL Anisaldehyde was mixed with 10 mL Glacial acetic acid, followed by 85 mL Methanol and 5 mL Sulphuric acid (98 %).

Observation and Results

Observations of pharmaceutical study

Table 3: Conventional method of *Guduchi Kwatha* (Group A)

Sr. No.	Group Name	Quantity of <i>Kalka</i> (gm)	Quantity of Water (ml)	Obtained Volume (ml)	Total time in minutes
1	GC1	250	4000	550	475
2	GC2	250	2000	460	220
3	GC3	250	1000	275	80

Table 4: Time based method of *Guduchi Kwatha* (Group B)

Sr. No.	Group Name	Quantity of <i>Kalka</i> (gm)	Quantity of Water (ml)	Obtained Volume (ml)	Total time in minutes
1	G1H	250	2000	1610	60
2	G2H	250	2000	1000	120
3	G3H	250	2000	625	180
4	G4H	250	2000	300	240

Table 5: Soxhlet method of *Guduchi Kwatha* (Group C)

Sr. No.	Group Name	Quantity of <i>Kalka</i> (gm)	Quantity of Water (ml)	Obtained Volume (ml)	Total time in minutes
1	GS60	250	2000	1800	465
2	GS80	250	2000	1800	340
3	GS100	250	2000	1900	190

Table 6: *Guduchi Kwatha* by Pressure Cooker Method (Group D)

Sr. No.	Group Name	Quantity of <i>Kalka</i> (gm)	Quantity of Water (ml)	Obtained Volume (ml)	Total time in minutes
1	GP30	250	2000	1850	30
2	GP60	250	2000	1800	60

Images 2: Various *Kwatha* techniques in four different groups



Image 2.1: GC1



Image 2.2: GC2



Image 2.3: GC3



Image 2.4: G1H

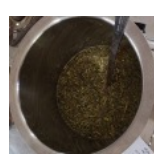


Image 2.5: G2H

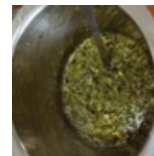


Image 2.6: G3H



Image 2.7: G4H



Image 2.8: GS60



Image 2.9: GS80



Image 2.10: GS100



Image 2.11: GP30



Image 2.12: GP60

HPTLC study of *Guduchi Kwatha*

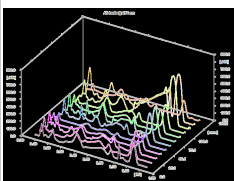
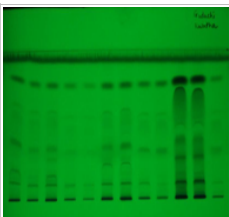
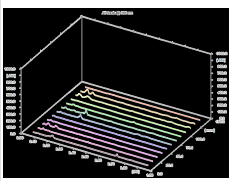
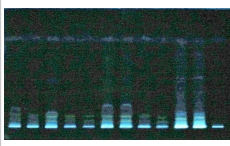
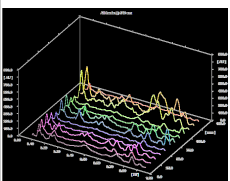
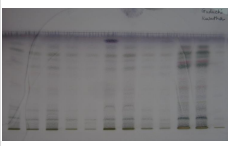
Image 3: 3D HPTLC Chromatogram of <i>Guduchi Kwatha</i> @254nm		Image 4: 3D Overlay HPTLC Chromatogram of <i>Guduchi Kwatha</i> @366nm		Image 5: 3D Overlay HPTLC Chromatogram of <i>Guduchi Kwatha</i> @540nm	
					
HPTLC Chromatogram @254nm	HPTLC Plate @254nm	HPTLC Chromatogram @366nm	HPTLC Plate @366nm	HPTLC Chromatogram @540nm	HPTLC Plate @540nm

Table 7: R_f Value of Guduchi Kwatha@254

Spot No.	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
1	0.12	--	0.12	--	--	0.12	0.12	0.12	--	0.12	0.12	0.12
2	0.17	0.17	0.17	--	--	0.17	0.17	0.17	0.17	0.17	0.17	--
3	0.23	--	0.23	--	--	0.23	0.23	--	--	--	--	--
4	--	0.28	0.28	--	--	0.28	0.28	--	--	0.28	0.28	--
5	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	--	--	0.33
6	0.41	0.41	--	0.41	--	0.41	--	--	--	0.41	0.41	--
7	0.45	--	0.45	0.45	0.45	--	0.45	--	--	--	--	--
8	--	0.52	0.52	--	--	0.52	0.52	0.52	0.52	0.52	0.52	0.52
9	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57
10	0.65	--	0.65	--	--	--	0.65	--	--	--	--	--
11	0.74	--	--	--	--	--	--	--	--	0.74	0.74	--
12	--	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	--	--	--
13	0.80	--	--	--	--	--	--	--	--	0.80	0.80	0.80
14	--	--	--	--	--	--	--	--	--	--	--	0.86

Table 8: R_f Value of Guduchi Kwatha@366nm

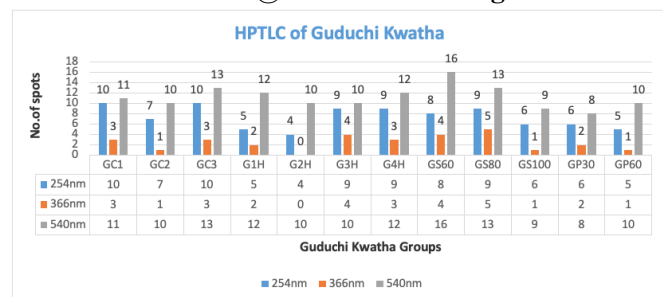
Spot No.	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
1	--	--	0.06	--	--	--	--	--	--	--	0.06	--
2	--	0.12	0.12	0.12	--	0.12	0.12	0.12	--	0.12	0.12	--
3	0.17	--	0.17	--	--	--	0.17	--	--	0.17	0.17	--
4	0.23	--	--	--	--	0.23	0.23	--	--	--	--	--
5	--	--	--	--	--	--	--	0.57	--	--	--	--
6	0.74	--	--	--	--	0.74	--	--	--	--	--	--
7	--	--	--	--	--	--	--	--	--	0.80	0.80	--
8	--	--	--	--	--	--	--	--	--	0.93	0.93	--
9	--	--	--	0.97	--	0.97	--	--	0.97	--	--	0.97

Table 9: R_f value of Guduchi Kwatha @ 540 nm (after derivatization)

Spot No.	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
1	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	--	0.12	0.12	--
2	0.17	--	0.17	--	--	--	0.17	--	--	0.17	--	--
3	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
4	0.28	0.28	0.28	0.28	--	--	--	--	0.28	0.28	0.28	--
5	--	0.33	0.33	0.33	--	0.33	0.33	--	0.33	0.33	0.33	0.33
6	0.38	0.38	0.38	0.38	0.38	0.38	0.38	--	0.38	0.38	--	--
7	--	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
8	0.45	--	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
9	--	--	--	--	--	--	--	--	--	0.52	0.52	--
10	0.57	0.57	0.57	0.57	0.57	--	0.57	0.57	0.57	0.57	0.57	0.57
11	--	--	--	--	--	--	--	--	--	0.62	0.62	0.62
12	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	--	--
13	0.71	--	0.71	0.71	0.71	0.71	0.71	--	--	0.71	0.71	0.71
14	--	--	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	--
15	0.80	0.80	--	--	--	--	--	--	--	0.80	0.80	0.80
16	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86

Track T1: GC1; Track T2: GC2; Track T3: GC3; Track T4: G1H; Track T5: G2H; Track T6: G3H; Track T7: G4H; Track T8: GP30; Track T9: GP60; Track T10: GS60; Track T11: GS80; Track T12: GS100.

Chart 1: Showing number of Spots in Guduchi Kwatha @ various wavelength



Discussion

There are many challenges in *Kwatha Kalpana* in terms of exact particle size, Vessel to be used, Temperature, the proportion of water to be added, etc. in spite of various specifications of *Kwatha* mentioned by most of our ancient seers. Hence, there need to optimize the process by studying various factors which validate the *Kwatha* procedure. Present study is addressing the issue regarding proportion of water to be added in *Kwatha* and other optional alternative techniques which can save time and fuel. It also studies the nature or

consistency of *Guduchi*. HPTLC fingerprinting study gives qualitative data of phytoconstituents present in the *Kwatha*. Conventional methods are gold standard hence the comparative study with other alternative techniques were carried out.

Guduchi was taken in fresh form (Image:1) in order to follow drug collection principle hence its *Kalka*(coarse paste) was prepared for *Kwatha* purpose. The bolus was prepared by adding suitable proportion of water.

Present study compared the three conventional practices of *Kwatha* preparation along with other alternative techniques in order to find the suitable technique. The HPTLC fingerprinting were done.

Conventional technique GC1(16 parts of water) needed more time and fuel as compared to GC2 and GC3 as the proportion of water added for *Kwatha* was more. One of the research study validates the indications of *Sharangdhara Samhita* that for the preparation of *Kwatha* of *Mrududravaya* (soft consistency drugs) 4 times, for *Madhyamdravya* (Medium consistency drugs) and *Kathindravaya* (Hard consistency) 8 times, and for *Atyantakathin dravya* (Very hard consistency) 16 times of the water should be used in order to sustain the potency. (4) Wet *Guduchi* were considered under a category of *Mrududravaya* so, 4 times of water is suitable for *Guduchi Kwatha*.

In HPTLC study of conventional method of *Guduchi Kwatha*, it was observed that GC1 showed 10, 03, and 11 spots@254nm, 366nm, and 540nm respectively. In GC2 method, it showed 07, 01, and 10 spots@254nm, 366nm and 540nm respectively whereas GC3 showed 10, 03, and 13 spots@254nm, 366nm and 540nm respectively. It showed that a greater number of phyto-constituents are observed in GC3 group which advocates the use of four times water for *Kwatha* of *Guduchi*. Also, maximum R_f values are observed @540nm wavelength after derivatization. (Table:7.8 & 9) (Images 3,4 &5) One of the study also validated that the *Guduchi Kwatha* prepared by four parts of water in which more phytoconstituents in HPTLC study and more peaks in HPLC were reported. (5)

Group B *Kwatha* technique was based on the rationality that the more the time of heating, the more will be the concentration of phyto-constituents in *Kwatha*. Also, it may give substitute to the conventional method if the quality is same at different hours of boiling. So, to check out whether the quality is achieved with less or more heat consumption, 1hr, 2hr, 3hr, and 4hrs heating was done with controlled temperature. Duration of boiling is crucial factor which decides remaining of important and essential phyto-constituents at final product. Also, excessive heat may hamper thermo-labile constituents of *Kwatha* so, time-based heating may impact the quality of product. With increase in duration of time, G4H type of the *Kwatha* was more concentrated as compared to G1H.

The HPTLC study of Time-based method of *Guduchi Kwatha* have shown varied number of spots. In G1H method, it observed 05,02,12 spots, G2H showed 04,00,10 spots G3H showed 09,04,10, and G4H showed 09,03,12 spots @254, @366 and @540nm respectively.

G3H and G4H have a greater number of R_f values which suggest number of phyto-constituents are directly related to the heating duration of *Kwatha*. (Table 7,8 &9) (Images 3,4&5) Temperature and time may affect chemical constituents of plant materials during *Kwatha* preparation. (6) So, in this group, it confirmed that duration of boiling has impact on presence of phytoconstituents and 3 to 4 hours of boiling is needed for appearance of maximum phytoconstituents.

In Soxhlet method of *Kwatha*, three *Kwatha* were prepared i.e. GS60, GS80, and GS100. Extraction in Soxhlet offers the use of the same solvent for extraction of solute by repeated cycles under control temperature. Soxhlet extraction is very commonly practiced as it is more resembles *Kwatha*(Decoction) due to the hot continuous extraction. It required very little solvent and temperature regulation can be easily achieved with uniform percolation In this study, three samples of the *Kwatha* were prepared at 60°C, 80°C, and 100°C. (Table 5) So, in all these three extractions, it was observed that time required for GS100 method was less as compared to GS60 and GS80 are highest spots appears in GS60 Temperature is the most important factor in Soxhlet extraction which ensures extraction of phyto-constituents in particular solvent. One of the basic principles of high extraction rate is that "Extraction should be performed at high temperature because of increasing temperature, normally viscosity of solvent and the extract decreases and on the other hand the solubility of extract in the solvent increases."(7) In order to check the impact of temperature, mild heating pattern i.e. 60°C, and 80°C which is frequent practiced in Soxhlet extraction were studied.

The HPTLC study of Soxhlet extraction method shown that in GS60 there was 08, 04,16 spots, in GS80 there was 09,05,13 spots and in GS100 showed 06, 01, 09 spots @254,@366 and @540nm respectively. (Table no 7,8 & 9) (Images 3,4 & 5) It suggests that maximum extraction of phyto-constituents was observed at 60°C and 80°C which helps to understand low heating pattern that stimulate maximum phytoconstituents in the solvents and hence needs to follow *Kwatha Kalpana* at low temperature. Highest number of spots i.e. 16 spots are observed at 60°C which could be the lead for further study with marker compounds.

The Pressure cooker principle is based on increasing the boiling point of water with an increase in pressure, hence very less energy is required to cook. Also, the loss of nutrients is less, due to the close procedure. As the procedure is quick as compared to other conventional procedures of *Kwatha*, this technique for *Kwatha* preparation was selected. Although use of lid is not recommended for therapeutic efficacy of *Kwatha*, but if the thermolabile constituents are saved in this process and the principle is also practicing at large scale industries by using packed steamed jacket pot for *Kwatha* preparations, hence, this process was studied.

In Pressure cooker Method of *Kwatha*, *Kwatha* was boiled for 30 minutes (GP30) and second batch was boiled for 60 minutes (GP60) on low flame by controlled whistling. The reduction volume of *Kwatha*

obtained was more in this group. (Table No.6). The HPTLC study of pressure cooker method of GP30 showed 06,02,08 spots and in GP60, 05,01,10spots @ 254, @ 366, and @ 540nm respectively. (Table no 7,8 &9) (Images 3,4 &5) It suggests that there is some difference if duration of boiling is increased in pressure cooker. There are some common spots i.e. spot no 3,7,8,10 and 16 in pressure cooker made *Kwatha*. Hence this method needs to be explored further analytically and therapeutically in further studies.

It was revealed from HPTLC fingerprinting of *Guduchi Kwatha* that the GC3 method is surely recommended for *Kwatha* preparation as it showed the maximum number of phyto-constituents. The G4H has also shown highest R_f values which ensured *Guduchi* has heat-resistant phyto-constituents at 100°C. The Soxhlet method suggested lower heating pattern for extraction as GS60 group showing highest phyto-constituents. Also, Pressure cooker method showed common R_f value of *Guduchi Kwatha*. In terms of common R_f values, it was observed that spot no 16 (0.86) and spot no 3 (0.23) R_f values @540nm are found in all groups, whereas R_f value 0.57 @254nm and @ 540nm was common in all *Kwatha* except the G3H group. (Table no.7,8, & 9.) So, in case of *Guduchi Kwatha*, there are some stable phyto-constituents and the types of processing have no impact on them. Further, a greater number of spots in alternative techniques may signify the presence of unwanted phytoconstituents which needs to find out by taking selective markers for *Guduchi*.

Conclusion

Conventional methods of *Kwatha* preparation ensures the quality of *Guduchi Kwatha* by using four parts of water for *Kwatha* preparation. In other alternative techniques, Soxhlet method extraction at 60°C having promising results which also regaining maximum yield of *Kwatha*. Long duration boiling have impact on appearance of phytoconstituents in *Kwatha* as in case of time-based technique. Presence of higher spots in alternative techniques point out that the very less impact of time span of boiling as in case of pressure cooker technique, method, however long duration

boiling have impact on appearance of phytoconstituents in *Kwatha* as in case of time-based technique. In order to validate present study, further with a higher number of batches needs to carry out so that statistically significant results can be assessed. Also, the use of therapeutic or active markers of *Guduchi* in HPTLC study could be the best parameters to decide the therapeutic potential of these *Kwatha*.

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