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**Scientific validation of Amukkara (*Withania somnifera*)
chooranam - before and after purification**

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Abstract

The herbs and herbal products were commonly used as a medicine to treat various ailments. Chooranam (herbal powder) is one of the herbal formulations used in Siddha system of medicine. As per Siddha text chooranam will be purified before use it an internal medicine. Amukkara chooranam is one of the Siddha medicine purified as per Siddha literature to access what are all the scientific changes occurred before and after purification through physicochemical, HPTLC analysis. Results suggest stability and qualitatively chemical constituents increased after purification of Amukkara chooranam.

Keywords: Siddha, Amukkara (*Withania somnifera*), Physicochemical, HPTLC.

Introduction

Herbs are commonly used as medicine from ancient days onwards. In siddha system single herbal, compound herbal formulation are used to treat various ailments. Amukkara (*Withania somnifera*) as one of the wonderful herb used as a medicine to manage various disease condition and rejuvenation in siddha system⁽¹⁾. Amukkara (*Withania somnifera*) tuber have alkaloids such as withanine, somniferine, perinponyine, steroids⁽²⁾, reducing sugar, phytosterol, Ipuranol⁽³⁾. It has Alterative, Aphrodisiac, Deobsturnent, Diuretic, Tonic, Astringent and Nervine sedative⁽³⁾. Amukkara chooranam with honey used for loss of appetite, obesity and inflammation. Amukkara chooranam with jaggery used for nervine tonic and strengthen the body⁽¹⁾. Amukkara (*Withania somnifera*) is one of the ingredients in various Siddha formulation for Anaemia, General weakness, Arthritis, Respiratory disorders, Infertility and Immune boost.

Various research studies suggest Amukkara (*Withania somnifera*) have Antioxidant⁽⁴⁾, Immuno modulatory⁽⁵⁾, Antistress activity⁽⁶⁾, Anti hypergelsic effect⁽⁷⁾, Anticancer activity⁽⁸⁾ In siddha system chooranam (Powder form) will be purified before prescribed as a medicine^(9,10). In this present study we discussing about what are all the changes occurred before and after purification of Amukkara chooranam through physicochemical and HPTLC analysis.

Materials and Methods

Procurement of raw drug:

Dried Amukkara tubers (*Withania somnifera*) were procured from K.Ramasamy Chetty Country drug shop, Rasappa street, Park town, Chennai and authenticated as Amukkara.

Purification of Raw drug:

Amukkara tubers (*Withania somnifera*) were washed in water and dried under shade.

Preparation of Amukkara Chooranam:

After purification process Amukkara tubers were powdered and sieved by white cloth which was mentioned as vashthirakayam in classical siddha text⁽⁹⁾. Then sieved Amukkara chooranam stored in a clean air tight container.

Purification of Amukkara Chooranam:

Sieved fine powder of Amukkara chooranam (*Withania somnifera*) was purified by pittavial process (Milk steaming process) mentioned in siddha text⁽⁹⁾. Then the purified Amukkara chooranam (*Withania somnifera*) tuber dried under sunlight and again sieved. Finally the purified Amukkara chooranam was stored in a airtight container.

Test sample:

Totally two sample of Amukkara chooranam ('purified-Sample A & Unpurified- Sample B) were subjected for analysis.

Physico – Chemical analysis:

Physico chemical analysis of purified (Sample A) and Unpurified (Sample B) Amukkara Chooranam were carried out as per standard procedures recommended in WHO Guideline (Anonymous 1988).

Loss on drying, Total ash, Acid insoluble ash, Water soluble extractives, Alcohol soluble extractives and P^H were carried out.

HPTLC analysis:

HPTLC of purified (Sample A) and Unpurified (Sample B) Amukkara chooranam assessed as per Standard procedure wagnerH&Bladfs, 1996.

Results

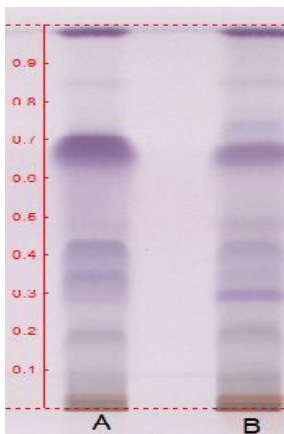
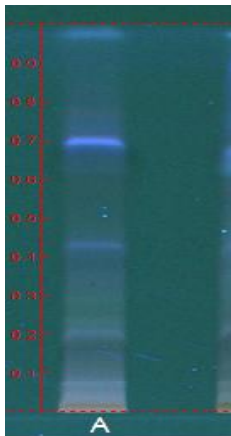
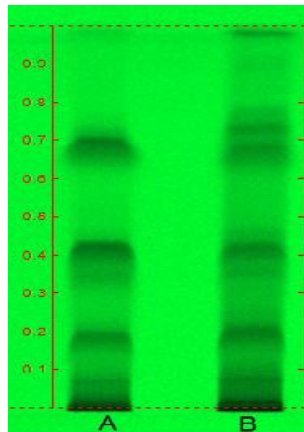
The results of Physicochemical analysis are shown in Table 1. The TLC photo documentation are shown in Figure1, The R^f value and colour spots visible under UV 254 nm, 366 nm and after densitometry with VS reagent are presented in Table 2&3. The HPTLC finger print profile are shown in figure 3, 4, 5 & 7 The table 4,5,6&7 show R^f value and their relative peak area. Figures 5 represent the densitometric chromatogram of alcohol extracts Amukkara chooranam A & B at 254 nm and 366 nm.

Table 1: Physico-chemical parameters:

S. No.	Parameters	Results	
		Sample -B (Before purification)	Sample -A (After purification)
1	LOD	2.96%, 2.94%, 2.98%	1.74%, 1.76%, 1.75%
2	Ash value a. Total ash (w/w)	4.742%, 4.738%	5.424%, 5.425%
	b. Acid insoluble ash (w/w)	4.740%, 0.132%, 0.132%, 0.134%	5.425%, 0.324%, 0.328%, 0.336%
3	Extractive values a. Alcohol soluble (w/v)	2.47%, 2.48%, 2.48%, 23.24%, 23.26%	2.084%, 2.940%, 2.983%, 20.16%, 20.18%
	b. Water soluble (w/v)	23.28%	20.22%
4	pH values (1% solution)	6.24, 6.26, 6.26	6.34, 6.36, 6.36

2.TLC/HPTLC analysis

Figure 1: TLC Photo Documentation of Amukkara Chooranam- Sample A and B



A: After purification; B: Before purification

Table 2: R^f value of Amukkara chooranam –A

Solvent system	R ^f Values		
	UV 254nm (7 spots)	UV 366nm (10 spots)	VS reagent (8 spots)
Toluene : Ethyl acetate (9:1) Colour spots	0.81 Green	0.70 Blue	0.83 Brown
	0.69 Dark green	0.60 Dark violet	0.69 Dark violet
	0.54 Green	0.42 Violet	0.49 Light grey
	0.42 Dark green	0.39 Dark violet	0.42 Grey
	0.32 Green	0.35 Violet	0.32 Grey
	0.18 Dark green	0.29 Light yellow	0.30 Violet
	0.09 Green	0.22 Light yellow	0.19 Light grey
		0.20 Light violet	0.09 Light grey
		0.18 Violet	
		0.09 Light pink	

Table 3: R^f value of Amukkara chooranam –B

Solvent system	R ^f Values		
	UV 254nm (10 spots)	UV 366nm (11 spots)	VS reagent (9 spots)
Toluene : Ethyl acetate (9:1)	0.90 Green	0.89 Blue	0.83 Brown
	0.83 Green	0.72 Blue	0.71 Light yellow
	0.76 Dark green	0.65 Blue	0.69 Dark violet
	0.72 Dark green	0.61 Blue	0.49 Light grey
	0.69 Dark green	0.59 Light violet	0.42 Grey
	0.42 Green	0.42 Light violet	0.32 Grey
	0.40 Dark green	0.35 Violet	0.30 Violet
	0.36 Dark green	0.25 Light violet	0.20 Light grey
	0.20 Dark green	0.20 Blue	0.09 Light grey
	0.09 Green	0.19 Dark violet	
		0.09 Light pink	

Figure 2: Densitometric chromatogram of Amukkara chooranam (A & B) alcohol extract at 254nm

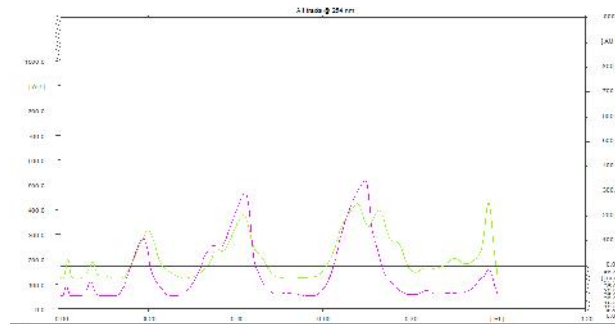


Figure 3: HPTLC finger print of Amukkara chooranam (A) at 254nm

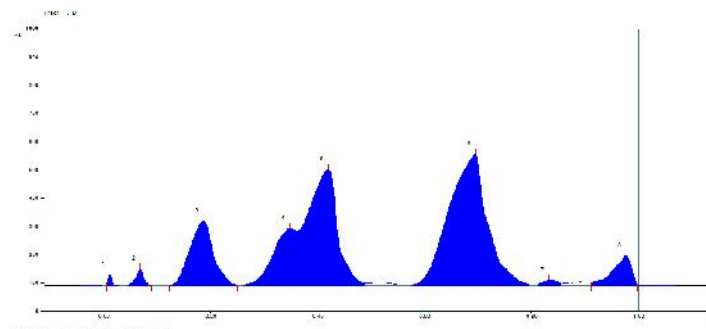


Table 4: R^f value of Amukkara chooranam (A) at 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	5.6 AU	0.01 Rf	40.7 AU	2.65 %	0.03 Rf	0.0 AU	236.9 AU	0.36 %
2	0.05 Rf	0.3 AU	0.07 Rf	58.9 AU	3.84 %	0.09 Rf	0.0 AU	715.2 AU	1.07 %
3	0.12 Rf	0.2 AU	0.19 Rf	229.1 AU	14.93 %	0.25 Rf	0.2 AU	8361.6 AU	12.54 %
4	0.26 Rf	0.1 AU	0.35 Rf	202.2 AU	13.17 %	0.36 Rf	93.4 AU	7110.7 AU	10.66 %
5	0.36 Rf	193.4 AU	0.42 Rf	412.0 AU	26.84 %	0.49 Rf	8.6 AU	19027.4 AU	28.53 %
6	0.58 Rf	0.9 AU	0.70 Rf	466.0 AU	30.37 %	0.80 Rf	5.6 AU	27751.2 AU	41.60 %
7	0.81 Rf	5.8 AU	0.83 Rf	20.1 AU	1.31 %	0.86 Rf	8.8 AU	448.7 AU	0.67 %
8	0.91 Rf	12.1 AU	0.98 Rf	105.9 AU	6.90 %	1.00 Rf	4.9 AU	3050.1 AU	4.57 %

Figure 4: HPTLC finger print of Amukkara chooranam (B) at 254nm

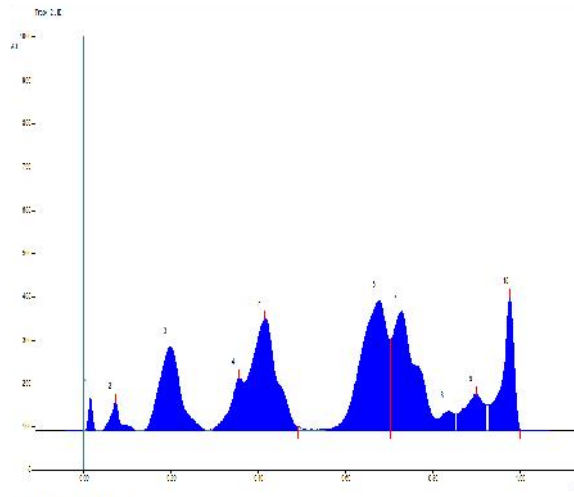


Table 5: Rf value of Amukkara chooranam (B) at 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.0 AU	0.02 Rf	76.1 AU	4.42 %	0.03 Rf	0.1 AU	574.6 AU	0.91 %
2	0.04 Rf	0.1 AU	0.07 Rf	66.4 AU	3.86 %	0.12 Rf	0.1 AU	1127.9 AU	1.79 %
3	0.14 Rf	1.5 AU	0.20 Rf	193.6 AU	11.25 %	0.28 Rf	0.4 AU	7732.9 AU	12.25 %
4	0.30 Rf	3.6 AU	0.36 Rf	121.0 AU	7.03 %	0.37 Rf	09.7 AU	3073.5 AU	4.87 %
5	0.37 Rf	110.2 AU	0.42 Rf	256.7 AU	14.92 %	0.49 Rf	7.9 AU	12079.7 AU	19.14 %
6	0.56 Rf	2.2 AU	0.68 Rf	301.0 AU	17.49 %	0.70 Rf	10.4 AU	14897.6 AU	23.61 %
7	0.70 Rf	210.7 AU	0.73 Rf	274.0 AU	15.92 %	0.81 Rf	25.2 AU	12456.6 AU	19.74 %
8	0.81 Rf	25.2 AU	0.84 Rf	43.4 AU	2.52 %	0.85 Rf	38.8 AU	1162.9 AU	1.84 %
9	0.86 Rf	38.9 AU	0.90 Rf	81.3 AU	4.72 %	0.93 Rf	59.0 AU	3148.1 AU	4.99 %
10	0.93 Rf	59.2 AU	0.98 Rf	307.5 AU	17.87 %	1.00 Rf	9.9 AU	6849.6 AU	10.85 %

Figure 5: Densitometric chromatogram of Amukkara chooranam (A &B) alcohol extract at 366 nm

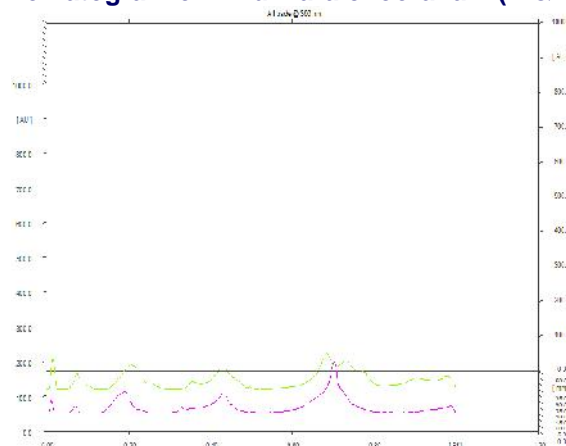


Figure 6: HPTLC finger print of Amukkara chooranam (A) at 366nm

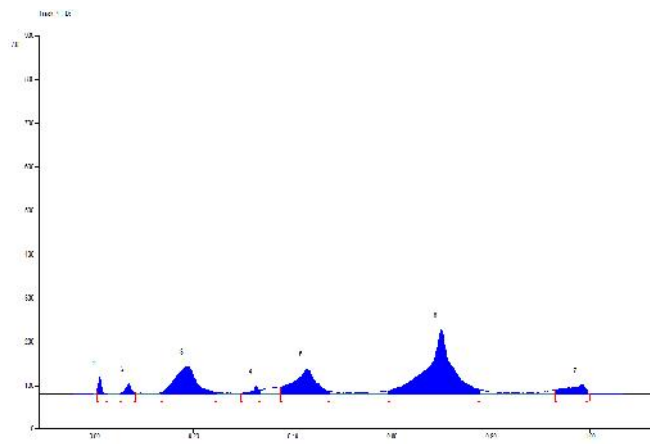


Table 6: Rf value of Amukkara chooranam (A) at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	6.5 AU	0.01 Rf	37.4 AU	10.30 %	0.03 Rf	0.0 AU	203.5 AU	1.76 %
2	0.05 Rf	0.8 AU	0.07 Rf	23.0 AU	6.35 %	0.08 Rf	0.9 AU	211.5 AU	1.83 %
3	0.13 Rf	1.8 AU	0.19 Rf	62.1 AU	17.12 %	0.25 Rf	2.1 AU	2217.5 AU	19.14 %
4	0.30 Rf	0.2 AU	0.33 Rf	17.7 AU	4.89 %	0.34 Rf	7.6 AU	176.7 AU	1.53 %
5	0.38 Rf	15.2 AU	0.43 Rf	56.5 AU	15.57 %	0.48 Rf	5.2 AU	2026.2 AU	17.49 %
6	0.59 Rf	6.8 AU	0.70 Rf	146.6 AU	40.37 %	0.78 Rf	9.0 AU	6101.4 AU	52.67 %
7	0.93 Rf	8.6 AU	0.98 Rf	19.6 AU	5.41 %	1.00 Rf	4.6 AU	648.3 AU	5.60 %

Figure 7: HPTLC finger print of Amukkara chooranam (B) at 366nm

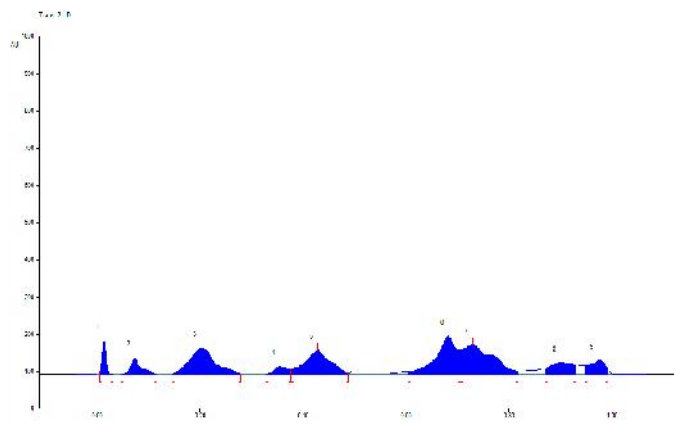


Table 7: Rf value of Amukkara chooranam (B) at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.8 AU	0.02 Rf	90.5 AU	16.53 %	0.03 Rf	0.0 AU	617.9 AU	3.63 %
2	0.05 Rf	0.1 AU	0.08 Rf	45.2 AU	8.26 %	0.12 Rf	0.7 AU	769.2 AU	4.52 %
3	0.15 Rf	0.2 AU	0.20 Rf	71.8 AU	13.13 %	0.28 Rf	2.4 AU	2951.0 AU	17.34 %
4	0.33 Rf	0.8 AU	0.36 Rf	21.4 AU	3.92 %	0.38 Rf	14.2 AU	463.6 AU	2.72 %
5	0.38 Rf	14.4 AU	0.43 Rf	65.8 AU	12.03 %	0.49 Rf	4.0 AU	2671.9 AU	15.70 %
6	0.61 Rf	8.0 AU	0.68 Rf	103.0 AU	18.81 %	0.71 Rf	64.6 AU	3468.8 AU	20.38 %
7	0.71 Rf	64.7 AU	0.73 Rf	80.4 AU	14.70 %	0.82 Rf	10.0 AU	3942.5 AU	23.16 %
8	0.87 Rf	16.7 AU	0.90 Rf	32.1 AU	5.86 %	0.93 Rf	25.5 AU	1157.6 AU	6.80 %
9	0.95 Rf	24.4 AU	0.98 Rf	37.0 AU	6.77 %	0.99 Rf	17.7 AU	980.4 AU	5.76 %

Discussion

Loss on drying of sample A & B were 1.75 % and 2.98 %. Total ash value of Sample A & B were 5.425 % and 4.740 % respectively. Acid insoluble ash (W/W) of Sample A & B were 0.336% and 0.134 %. Alcohol soluble of sample A & B were 2.983% & 2.48%. water soluble of Sample A & B were 20.22% and 23.28%. P^H value of Sample A & B is 6.36 & 6.26. These results suggest stability, Alcohol solubility of the test drug increased after purification than before purification. p^H value of the test drug is increased after purification than before purification.

The value of Total ash, Acid insoluble ash & Alcohol soluble extractive are within the limit⁽¹¹⁾ before and after purification of Amukkara Chooranam (Sample A & Sample B). It indicates the purity and strength of the Amukkara Chooranam.

The TLC photo and finger print profile shows Amukkara chooranam 2 µl concentration of the extract is optimum for the better separation of analysis.

The TLC photo documentation of the sample A under UV 254 nm shows 4 major spots at Rf 0.70 (30.37%), 0.42 (26.84%), 0.35(13.17%) & 0.17 (14.93 %) and other spots are minor. The TLC photo documentation of the sample B under UV 254 nm shows 15 major spots at Rf value 0.68(23.61%), 0.73(19.24%), 0.42 (19.14%), 0.20 (12.25 %), 0.98 (10.85 %) and other spots are minor.

The TLC photo documentation of the sample A under UV 366 nm shows 3 major spots at R^f 0.70(52.67%), 0.19 (19.14%), 0.43 (17.49 %) and other spots are minor. The TLC photo documentation of the sample B under UV 366 nm shows 4 major spots at R^f value

0.73(23.16%), 0.68(20.8%), 0.20 (17.34%) & 0.43 (15.70 %) respectively and other spots are minor.

The HPTLC finger print of the sample A shows 7 spots in UV 254 nm, 10 spots in UV 366 nm and 8 spots in VS reagent. Sample B shows 10 spots in UV 254 nm, 11 spots in UV 366 nm and 9 spots in VS reagent.

Conclusion

The stability of the drug increased and the presence of chemical constituents was increased qualitatively after purification of Amukkara chooranam. TLC and HPTLC documentation of Amukkara chooranam will be useful as tool for standardization. Finally the author conclude the importance of purification of chooranam mentioned in Siddha system has been scientifically validated through this study.

Further research studies need for quantification of active principles after purification of chooranam for better understanding of science behind the purification of chooranam as per siddha literature.

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Conflict of interest: Nil.

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