HRGS' Ayurveda Journal
Journal of Ayurveda Medical Sciences
Peer Reviewed Journal of Ayurveda and other Traditional Medicines
ISSN: 2456-4990|www.jayumedsci.com|jayumedsci@gmail.com

Original Article
Standardisation and Quality Control - Formulation

Pharmaceutico-analytical Study of *Amrutottara Arka*

Deepthi CP[1*], Basavaraj Y Ganti[2], Vinay R Kadibagil[3], Sunil Kumar KN[4]

[1]Final year PG Scholar; Current: Physician, Oushadhi Agency, Karivellur, Kannur, Kerala, [2]Associate Professor and Head, [3]Professor, Department of Rasashastra and Bhaishajyakalpana, SDM College of Ayurveda and Hospital, Hassan, India - 573201. [4]Senior Research Officer, Department of Pharmacognosy and Phytochemistry, SDM Centre for Research in Ayurveda and Allied Science, Udupi, India – 574118; Current: Research Officer and HOD, Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai, India – 600106.

Correspondence: Email: dr.deepthibiju@yahoo.com, Mobile: +918762416131

ABSTRACT

Introduction: *Amrutottara Kwatha* is a very common formulation used for *Jwara* (Fever). Its taste and shelf life are the drawback of the dosage form to implement this into prescriptions. If the same could be modified into a form which would render same results as that of the *Kwatha* (decoction) it would be a better dosage form. Another dosage form namely *Arka* (distillate) is also an important because the preparation is also water based, it has good patient compliance and long shelf life. ***Methods:*** In this study *Amrutottara* or *Nagaradi Kwatha* consisting of *Nagara* (*Zingiber officinale*) *Amruta* (*Tinospora cordifolia*), *Haritaki* (*Terminalia chebula*) in 2:6:4 ratio) was converted into *Arka*. *Amrutottara Arka* (AA) was prepared in two strengths 1:16 and 1:2. 1:16 that is maximum ratio of *Kwatha* mentioned in *Sharangadhara samhita* and 1:2 that is one of ratio of *Arka* mentioned in *Arka prakasha.* The Pharmaceutical data were observed and recorded. ***Results:*** Analysis of both prepared medicines was carried out as per the protocols laid down by Ministry of AYUSH, Govt of India for *Arka.* Pharmaceutically 1:16 is better than 1: 2 ratio as easy in extraction of *Arka* 1:16 ratio and 1:2 ratio is having more shelf life compared to 1:16 ratio as it was not getting contaminated. ***Conclusion:*** The Analytical studies including HPTLC have helped to generate preliminary standards for both samples of *Arka.*

KEYWORDS

Amrutottara yoga, Preparation of *Amrutottara Arka,* Analysis of *Amrutottara Arka*

Received: 14.10.2016 **Accepted:** 10.01.2017 **DOI:** 10.5530/jams.2016.1.15

Panchavidha Kashaya Kalpana[1] are the primary preparations in Ayurvedic pharmaceutics. *Arka Prakasha* describes *Kalka* (paste), *Churna* (powder), *Rasa* (juice), *Taila* (oil) and *Arka* (distillate) as *Panchavidha Kashaya Kalpana*[2] (primary preparations). Among these, *Arka* is said to be the most potent. *Arka* is a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using the *Arka Yantra* (distillation apparatus).[3] This preparation has specificity in the preparation aspect with increased shelf life and reduced dosage. So *Amrutottara* or *Nagaradi Kwatha*[3] which is widely used formulation in *Jwara* (fever) was converted into *Arka*. The antipyretic activity of *Amrutottara kwatha* is proved through different research work. Chemical constituents Gingenol, Shagaol present in *Nagara* (*Zingiber officinale*),[4] diterpenoid lactones, aliphatic compounds, steroids of *Guduchi* (*Tinospora cordifolia*),[5-7] and flavanoids in *Hareetaki* (*Terminalia chebula*)[8] has shown antipyretic activity. In this study preparation of *Amrutottara Arka* (AA) and its analytical evaluation was carried out.

MATERIALS AND METHODS

The methods followed in this work are divided in to pharmaceutical study and analytical study. In the pharmaceutical study attempts were made to prepare two ratio (1:16 ratio and 1:2ratio) of AA and observations were noted. In analytical study different parameters mentioned for assessment of *Arka* including HPTLC of *Amrutottara kwatha churna* – AKC (powder of 3 ingredients) and *Arka* were carried out.

Plant materials

The drugs required for the preparation, fresh *Amruta* (*Tinospora cordifolia*) was collected from local area. Dry *Haritaki (Terminalia chebula)* and *Nagara* (*Zingiber officinale*) were procured from department of Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Hassan, Karnataka. Authentication of raw drugs was done at department of Dravyaguna, Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Hassan based on macroscopic and organoleptic characters. The preparation of AA was done at Department of Rasashastra and Bhaishajyakalpana, Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Hassan as per the reference of general method of preparation of *Arka.*

Pharmaceutical study

AA 1:16 ratio

It was prepared as per general ratio of *Paneeya Kwatha* (1:16ratio)[9] taking *Nagara* (*Zingiber officinale* – 15 g), *Amruta* or *Guduchi* (*Tinospora cordifolia* – 45 g), *Haritaki* (*Terminalia chebula* – 30 g) and purified water (1440 ml). The crushed drugs were soaked in

sufficient quantity of water (250 ml) for overnight. Next day morning it was transferred to distillation apparatus and remaining water (1190 ml) was added and distillation was done.

AA 1:2 ratio

It was prepared as per one of the ratio of *Arka* (distillate) mentioned in *Arka Prakasha* (1:2 ratio) taking *Nagara* (90 g), *Amruta* (270 g), *Haritaki* (180 g) and water (1080 ml). The crushed drugs were soaked in sufficient quantity of water (700 ml) for overnight. Next day morning it was transferred to distillation apparatus and water (380 ml) was added and distillation was done. Heating was stopped when the drugs start getting sticking to the apparatus and started slight charring. So 30% (300 ml) of *Arka* was collected.

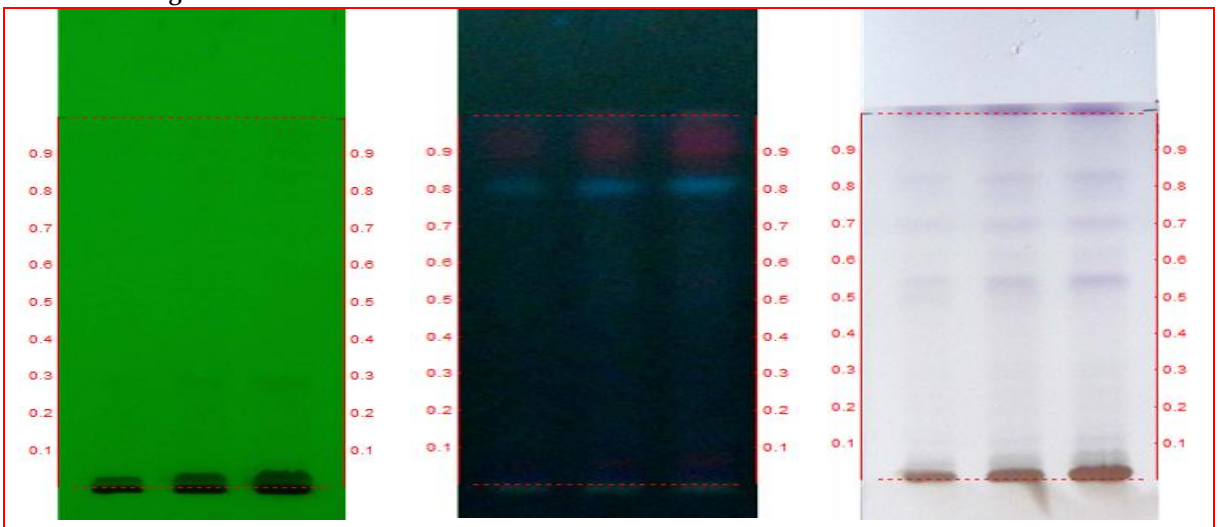
Analytical study

AKC was subjected to HPTLC for standardisation and samples of *Arka* were analysed with organoleptic parameters like colour, taste, odour and physico-chemical characters like pH, volatile matter, specific gravity, boiling point, refractive index, total acidity, viscosity, HPTLC following standard methodology[10] at SDM Research Centre for Ayurveda and Allied Sciences, Udupi 574118.

RESULTS AND DISCUSSION

By TLC photo-documentation of AKC at 254 nm 4 spots were detected, at 366 nm 4 spots were detected and after derivatisation 10 spots of light purple were detected (Figure 1 and Table 1). The same by HPTLC densitometric scan 5, 3 and 13 peaks were detected at 254 nm, 366 nm, and 620 nm (after derivatisation) (Figure 2).

Figure 1. Photo documentation of ethanolic extract of *Amrutottara Kwatha Churna*



Under long UV **Under short UV** **After Derivatisation**

Solvent system - Toluene: Ethyl acetate (6.0: 4.0)

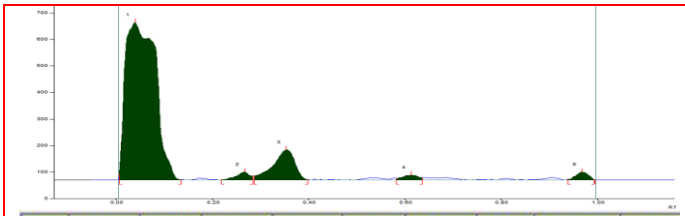
Track 1: *Amrutottara Kwatha Churna* (3µl); Track 2: 6µl; Track 3: 9µl

Table 1. R_f values of ethanolic extract of *Amruthottara Kwatha Churna*

At 254 nm	At 366 nm	After Derivatisation
-	0.07 (FD. red)	0.07 (L. purple)
-	-	0.11 (L. purple)
-	-	0.15 (L. purple)
0.22 (L. green)	-	-
-	-	0.28 (L. purple)
0.30 (L. green)	-	-
-	-	0.51 (D. purple)
-	-	0.54 (D. purple)
-	-	0.63 (D. purple)
-	-	0.71 (D. purple)
-	-	0.79 (D. purple)
-	0.82 (FD. blue)	0.82 (D. purple)
0.86 (L. green)	-	-
0.90 (L. green)	0.90 (FD. red)	-
-	0.95 (FD. red)	-

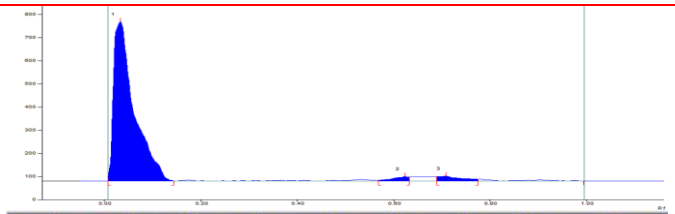
*F- fluorescent; D – dark; L - light

Figure 2. Densitometric scan of ethanolic extract of *Amrutottara Kwatha Churna* - 9µl



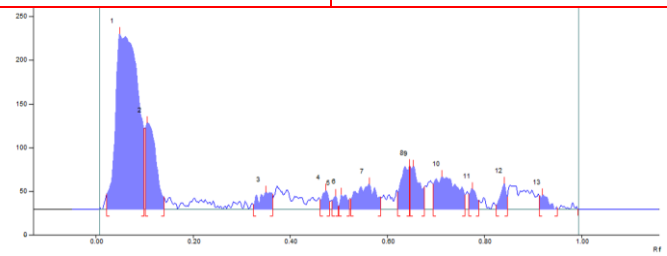
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	23.4 AU	0.04 Rf	593.0 AU	75.42 %	0.14 Rf	1.0 AU	26970.5 AU	83.41 %
2	0.22 Rf	0.9 AU	0.27 Rf	30.3 AU	3.85 %	0.29 Rf	15.1 AU	617.8 AU	1.91 %
3	0.29 Rf	15.3 AU	0.35 Rf	114.1 AU	14.52 %	0.40 Rf	1.3 AU	3719.7 AU	11.50 %
4	0.58 Rf	5.9 AU	0.61 Rf	18.5 AU	2.35 %	0.64 Rf	8.6 AU	450.0 AU	1.39 %
5	0.94 Rf	2.4 AU	0.97 Rf	30.3 AU	3.86 %	1.00 Rf	1.8 AU	578.1 AU	1.79 %

At 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	11.9 AU	0.03 Rf	686.8 AU	94.52 %	0.15 Rf	0.3 AU	21097.7 AU	94.51 %
2	0.57 Rf	3.0 AU	0.62 Rf	19.0 AU	2.61 %	0.63 Rf	16.9 AU	467.4 AU	2.09 %
3	0.69 Rf	18.6 AU	0.71 Rf	20.9 AU	2.88 %	0.78 Rf	7.9 AU	759.1 AU	3.40 %

At 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	17.0 AU	0.05 Rf	200.6 AU	33.02 %	0.10 Rf	92.7 AU	6609.3 AU	50.47 %
2	0.10 Rf	92.9 AU	0.11 Rf	98.9 AU	16.29 %	0.14 Rf	14.2 AU	1583.1 AU	12.09 %
3	0.32 Rf	5.4 AU	0.35 Rf	19.7 AU	3.24 %	0.36 Rf	17.4 AU	380.8 AU	2.91 %
4	0.46 Rf	10.7 AU	0.47 Rf	21.5 AU	3.53 %	0.48 Rf	8.5 AU	204.6 AU	1.56 %
5	0.49 Rf	9.3 AU	0.50 Rf	15.5 AU	2.55 %	0.50 Rf	3.1 AU	98.1 AU	0.75 %
6	0.50 Rf	4.2 AU	0.51 Rf	17.0 AU	2.79 %	0.52 Rf	11.7 AU	179.5 AU	1.37 %
7	0.53 Rf	11.9 AU	0.56 Rf	29.0 AU	4.78 %	0.59 Rf	14.0 AU	790.3 AU	6.03 %
8	0.62 Rf	19.8 AU	0.65 Rf	49.7 AU	8.19 %	0.65 Rf	49.7 AU	620.7 AU	4.74 %
9	0.65 Rf	48.8 AU	0.65 Rf	48.9 AU	8.06 %	0.68 Rf	25.7 AU	672.3 AU	5.13 %
10	0.70 Rf	28.7 AU	0.71 Rf	37.0 AU	6.09 %	0.76 Rf	16.5 AU	1245.8 AU	9.51 %
11	0.77 Rf	17.3 AU	0.78 Rf	23.1 AU	3.81 %	0.79 Rf	10.1 AU	233.6 AU	1.78 %
12	0.83 Rf	5.0 AU	0.84 Rf	29.9 AU	4.92 %	0.85 Rf	14.3 AU	258.6 AU	1.97 %
13	0.91 Rf	15.6 AU	0.92 Rf	16.5 AU	2.72 %	0.95 Rf	1.5 AU	219.1 AU	1.67 %

At 620 nm post derivatisation

During distillation, in 1:2 ratio, after 15 minutes vapours were seen at the neck of the flask. After 60 minutes first drop was seen. Vapours started condensing and changing into liquid form when they passed through the condensing tube. In 1: 16 ratio, after 15 minutes vapours were seen at the neck of the flask. After 50 minutes first drop was seen. After three hours of distillation there was sticking of drug particles to apparatus due to less quantity of water so there was difficulty in extraction. In the case of 1:16 ratio, 6 hours duration was required for collections of 875 ml *Arka*, while in 1:2 ratio 3 hours was taken for distillation of 300 ml *Arka* (Table 2).

Table 2. Preparation details of different ratios of *Amrutottara Arka*

Parameter	**1:2 ratio**	**1:16 ratio**
Colour	Colourless	Colourless
Odour	Aromatic	Aromatic
Taste	Sweet	Sweet
Drugs Quantity	90 g	540 g
Water for soaking	250 ml	700 ml
Water added at the time of distillation	1190 ml	380 ml
Total quantity of water taken	1440 ml	1080 ml
Total Distillate	875 ml	300 ml
Duration of preparation	6 hrs	3 hrs

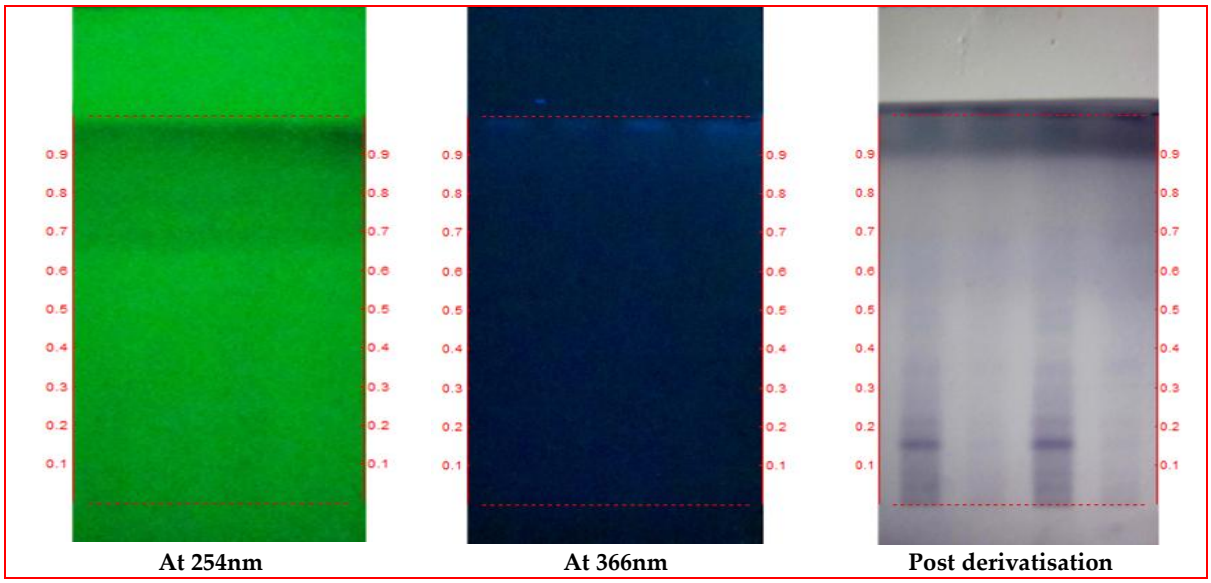
Simple distillation apparatus was used and 60^0C of temperature was maintained for both preparations. Physico-chemical constants like specific gravity were 0.9922 in 1:16 ratio which was more than 0.9915 in 1:2 ratio. The pH of both *Arka* was 6.0, refractive index for both the *Arka* was 1.3318. Volatile matter was 0.14 in 1:16 ratio which was more than 0.11 in 1:2 ratio. Both the samples have boiling point of 101^0C. Total acidity was 1.0198 in 1:16 ratio which was more than 1.0188 in 1:2 ratio. Viscosity was 1.05 in 1:16 ratio which is less than 1.051 in 1:2 ratio (Table 3).

Table 3. Physico-chemical constants of different ratios of *Amrutottara Arka*

Parameter	1:2 ratio	1:16 ratio
pH	6.0	6.0
Refractive index	1.3318	1.3318
Specific gravity	0.9915	0.9922
Volatile matter (%)	0.11	0.14
Boiling point	101ºC	101ºC
Total acidity	0.0188	0.0198
Viscosity	1.055	1.051

Two ratios of AA were compared by HPTLC. By photo-documentation 1:16 ratio showed 6 spots while 1:2 ratio showed 11 spots (Figure 3 and Table 4). By densitometric scan at 254 nm 1:2 ratio showed 9 peaks while 1: 16 ratio showed 3 peaks only; the same plate after derivatisation followed by scanning at 620 nm showed 10 and 7 peaks in 1:2 and 1:16 ratio respectively (Figure 4 and 5). Total bacterial count and total fungal count in both the ratios were found to be nil.

Figure 3. HPTLC photo documentation of *n*-hexane fraction of different ratios of *Amrutottara Arka*



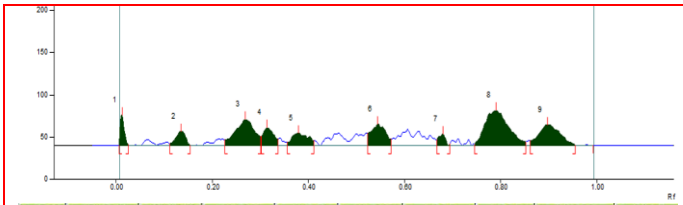
Track 1: *AA 1:2* -8µl; Track 2: *AA 1:16* -8µl; Track 3: *AA 1:2* -12µl; Track 4: *AA 1:16* -12µl
Solvent system - Toluene: Ethyl acetate (8.0:2.0)

Table 4. R_f values of *n*-hexane fraction of different ratios of *Amrutottara Arka* at 620 nm post derivatisation

AA 1:2	AA 1:16
0.04 (L. purple)	0.04 (L. purple)
0.08 (L. purple)	0.08 (L. purple)
0.16 (D. purple)	0.16 (L. Purple
0.22 (L. purple)	0.22 (L. purple)
0.27 (L. purple)	-
0.37 (L. purple)	0.37 (D. purple)
0.42 (L. purple)	-
0.47 (L. purple)	-
0.52 (L. purple)	-
-	0.70 (D. purple)
0.72 (L. purple)	-

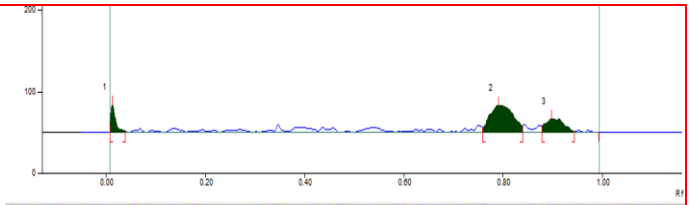
*F- fluorescent; D – dark; L - light

Figure 4. Densitometric scan of different ratios of *n*-hexane fraction of different ratios of *Amrutottara Arka* - 12µl



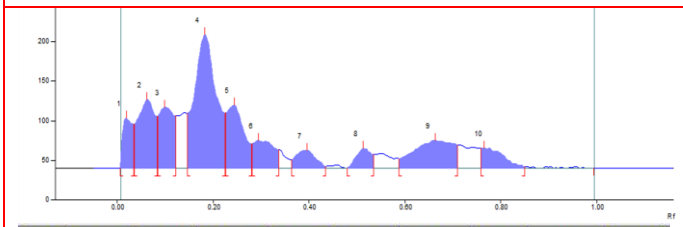
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.01 Rf	36.2 AU	16.13 %	0.03 Rf	0.6 AU	217.9 AU	4.41 %
2	0.11 Rf	2.3 AU	0.14 Rf	16.9 AU	7.50 %	0.15 Rf	0.1 AU	242.3 AU	4.90 %
3	0.23 Rf	6.8 AU	0.27 Rf	30.7 AU	13.67 %	0.30 Rf	10.4 AU	869.4 AU	17.58 %
4	0.30 Rf	10.8 AU	0.31 Rf	21.0 AU	9.33 %	0.34 Rf	7.5 AU	330.8 AU	6.69 %
5	0.36 Rf	3.7 AU	0.38 Rf	14.7 AU	6.55 %	0.41 Rf	5.5 AU	360.5 AU	7.29 %
6	0.52 Rf	14.4 AU	0.55 Rf	25.6 AU	11.40 %	0.57 Rf	7.3 AU	554.4 AU	11.21 %
7	0.67 Rf	6.7 AU	0.68 Rf	13.7 AU	6.10 %	0.70 Rf	1.7 AU	142.0 AU	2.87 %
8	0.75 Rf	1.3 AU	0.79 Rf	41.5 AU	18.48 %	0.85 Rf	4.3 AU	1450.3 AU	29.34 %
9	0.86 Rf	5.7 AU	0.90 Rf	24.3 AU	10.83 %	0.96 Rf	0.1 AU	776.4 AU	15.70 %

1:2 ratio at 254 nm



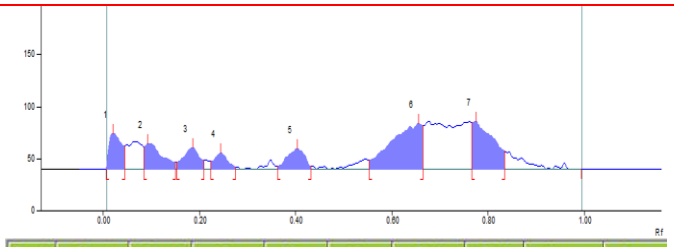
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	8.3 AU	0.01 Rf	34.0 AU	40.77 %	0.04 Rf	1.6 AU	243.8 AU	13.42 %
2	0.76 Rf	6.1 AU	0.79 Rf	33.2 AU	39.73 %	0.84 Rf	8.3 AU	1125.1 AU	61.92 %
3	0.88 Rf	8.0 AU	0.90 Rf	16.3 AU	19.51 %	0.95 Rf	1.1 AU	448.0 AU	24.66 %

1:16 ratio at 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	62.8 AU	10.17 %	0.04 Rf	55.6 AU	961.7 AU	5.43 %
2	0.04 Rf	55.8 AU	0.06 Rf	86.3 AU	13.98 %	0.08 Rf	64.8 AU	2173.8 AU	12.28 %
3	0.09 Rf	65.4 AU	0.10 Rf	77.1 AU	12.50 %	0.12 Rf	65.8 AU	1724.6 AU	9.75 %
4	0.15 Rf	69.4 AU	0.18 Rf	168.0 AU	27.22 %	0.23 Rf	69.5 AU	5521.7 AU	31.20 %
5	0.23 Rf	69.7 AU	0.25 Rf	79.8 AU	12.93 %	0.28 Rf	30.5 AU	2038.6 AU	11.52 %
6	0.28 Rf	30.7 AU	0.30 Rf	34.9 AU	5.66 %	0.34 Rf	23.0 AU	1084.6 AU	6.13 %
7	0.36 Rf	10.3 AU	0.40 Rf	22.4 AU	3.64 %	0.44 Rf	2.0 AU	675.0 AU	3.81 %
8	0.48 Rf	0.2 AU	0.51 Rf	24.9 AU	4.03 %	0.54 Rf	17.1 AU	555.9 AU	3.14 %
9	0.59 Rf	12.0 AU	0.66 Rf	35.2 AU	5.70 %	0.71 Rf	28.7 AU	2096.7 AU	11.85 %
10	0.76 Rf	25.1 AU	0.77 Rf	25.8 AU	4.17 %	0.85 Rf	1.6 AU	863.9 AU	4.88 %

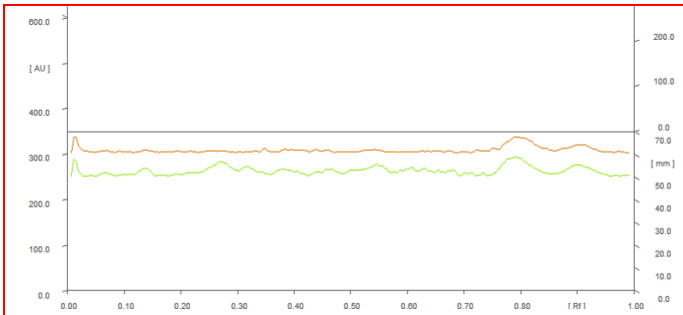
1:2 ratio at 620 nm post derivatisation



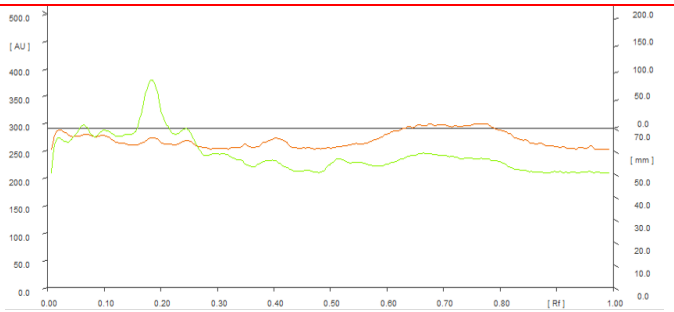
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	34.3 AU	16.80 %	0.05 Rf	22.1 AU	635.5 AU	10.58 %
2	0.09 Rf	21.3 AU	0.09 Rf	24.3 AU	11.91 %	0.15 Rf	6.7 AU	644.3 AU	10.73 %
3	0.15 Rf	6.8 AU	0.19 Rf	20.6 AU	10.09 %	0.21 Rf	8.5 AU	483.4 AU	8.05 %
4	0.22 Rf	7.7 AU	0.25 Rf	15.6 AU	7.63 %	0.28 Rf	1.7 AU	307.6 AU	5.12 %
5	0.36 Rf	2.7 AU	0.40 Rf	19.7 AU	9.63 %	0.43 Rf	2.8 AU	512.4 AU	8.54 %
6	0.55 Rf	8.3 AU	0.66 Rf	43.6 AU	21.36 %	0.67 Rf	41.6 AU	2002.5 AU	33.35 %
7	0.77 Rf	44.7 AU	0.78 Rf	46.1 AU	22.59 %	0.84 Rf	17.4 AU	1418.1 AU	23.62 %

1:16 ratio at 620 nm post derivatisation

Figure 5. 3-D chromatogram of Amrutottara Arka 1:2 and 1:16 ratio



At 254 nm



At 620 nm

1:2

1:16

In this study drugs of *Amrutottara Kwatha Churna* were taken and made in to *Arka*. The fresh *Amruta* was crushed and *Nagara* and *Haritaki* were coarsely powdered using mortar and pestle. Some quantity of water was added to the drugs for soaking and kept over-night. Soaking is suggested because increased duration of contact of drug with water and some constituents of drugs leaches out to water. On the following morning the soaked contents were shifted into *Arka Yantra* (distillation apparatus) and the remaining quantity of water was added and heat was applied. The vapour condensed and collected in a receiver. In the beginning the vapour consists of only steam and may not contain the essential principles of the drugs. So it was discarded. The last portion also may not contain therapeutically essential substance and it was discarded. 1:16 ratio was selected for *Arka* (distillate) preparation because the basic drugs used was ingredients of Amrutottara *Kwatha*. 1:2 ratio was prepared as per one of the general ratio for *Arka Kalpana* (distillation preparation) as per *Arka Prakasa* which is an authenticated book of *Arka Kalpana*.

Boiling helps for easy extraction of water soluble principles into water. In distillation due to *Toyadhara*, (water flow) the developing *Bhashpa* (vapours) will turn to *Arka* by the *Sheetata* (cold nature) of *Jala*. The *Arka* thus formed will be collected through the condenser into the *Grahana Patra* (receiver). *Uttama Arka lakshana* (the best quality features) like characteristic aroma of the constituent drugs especially *Nagara (Zingiber officinale)*, clear liquid with oil droplets on the surface was appreciated.

As a preliminary way of standardization different analytical parameters mentioned for *Arka kalpana* were performed and logical reasoning was carried out. The present analytical study has been carried out to know the quality of the finished product.

Organoleptic characters were alike in both the samples. Specific gravity revealed that 1:2 ratio is a little denser than other ratio. The pH of both *Arka* was 6.0, indicating the slightly acidic nature of *Arka*, as pH influences the rate of oxidation. Refractive index for both the *Arka* was 1.3318, as usually *Arka* samples are colourless this parameter may be used to identify and differentiate different *Arka* samples. Refractive index indicates how light propagates through that medium, refractive index of water is 1.33, meaning that light travels 1.33 times slower in water than it does in vacuum as *Arka* contains some dissolved substances in it the value slightly differed from that of water. Volatile matter indicates the volatile active principles in the formulation, as *Arka* is type of volatile distillation it will certainly contain some volatile principles, volatile matter was found to be more in 1:16 ratio than 1:2 ratio, the higher value volatile matter may contribute to higher density and specific gravity to preparations. Boiling point is the temperature at which the liquids start boiling, it has its effect on dissolved substances present in a liquid, here both the samples have boiling point of 101^0C which is almost equivalent to water. The reason behind it may be the *Arka* contains mostly water and no other liquids were added to it. Viscosity is the property of fluids to resist flow it was found be very nearby values, the slight change may be due to concentration of 1:2 ratio. This enables the formulation to remain in the area longer and gives more time for the drug to exert its therapeutic activity or undergo absorption. Total acidity is a representation of acid concentration in the liquid, however the acidity also indicates the chance of decomposition, 1:16 ratio is more chance of decomposition as it had higher acidity.

Standarisation and quality control is an important research for brining traditional medicine into limelight. Several traditional medicinal formulations have been attempted for standardisation by research development scientists working on traditional medicines. This kind of research with possible advancements in the testing protocols are essential for development of Indian Systems of Medicine which in turn improve the strengthening of Pharmacopoeias.[11,12]

CONCLUSION

Amrutottara Arka was prepared in two ratio 1:16 and 1:2. Considering yield and therapeutic effect 1:16 is better than 1:2 ratio and 1:2 ratio will be having more shelf life as it this ratio was not contaminated. The analytical studies including HPTLC have helped to generate preliminary standards for both ratios of *Arka.* Specific gravity and viscosity was slightly more for 1:2 ratio. Volatile matter and total acidity for *Arka* 1:16 ratio were a little more than 1:2 ratio. The study suggests that there is difference in physic-chemical constants and HPTLC when it is prepared in two different ratios. However, detailed compositional analysis by GCMS and some pharmacological actions may be further imperative in deciding which ratios of the two classical references works better in a biological system.

ACKNOWLEDGEMENT

The authors are thankful to Department of Bhaishajya Kalpana and Rasashastra, SDMCA, Hassan, Karnataka, India for providing the raw material and gave permission to conduct the pharmaceutical study and SDM Centre for Research in *Ayurveda* and Allied Sciences, Kuthpady, Udupi for performing analytical study.

CONFLICTS OF INTEREST

Nil

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ABOUT AUTHORS

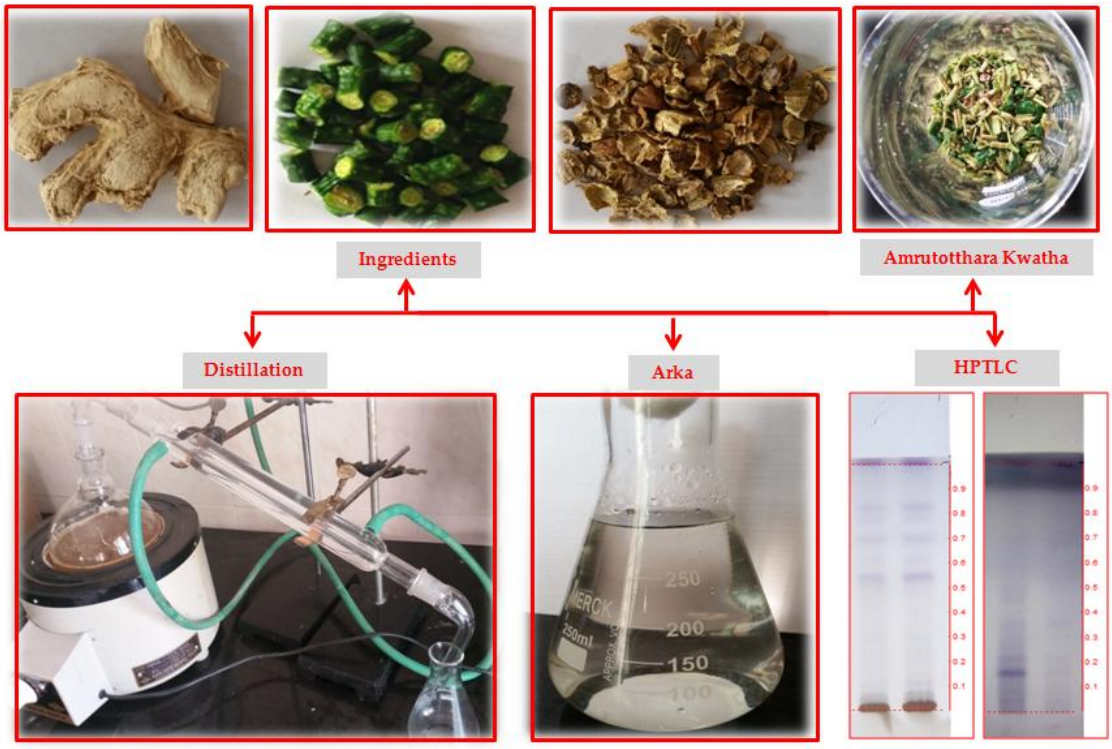
Dr. Deepthi CP MD (Ayu) completed PG in November 2016. Currently she is working as physician at Oushadhi Agency, Karivellur, Kannur, Kerala. She is having working experience as lecturer at Vaidyaratnam PS Varier Ayurveda College, Kottakal, Kerala and Govt Ayurveda College Kannur, Kerala. She is author of three articles published in different journals.

Dr. Basavaraj Y Ganti MD (Ayu) is working as associate professor and Head in the Dept of Rasashastra and Bhaishajya kalpana. Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Hassan, Karnataka. Now pursuing PhD from Tilak Maharashtra Vidyapeeth, Pune under the guidance of Vd SS Savrikar sir. He is author of 18 articles published in various journals. He is serving as editorial board member in Ayurpub (a bimonthly peer reviewed open access journal) and Punarnav (International Peer Reviewed Journal). He has rich experience of manufacturing medicine in bulk and guided 6 post research works.

Dr. Vinay R Kadibagil MD (Ayu) is working as Professor in the Department of Rasashastra and Bhaishajya kalpana, Sri Dharmasthala Manjunatheswara College of Ayurveda and Hospital, Hassan, Karnataka. He is author of 15 articles published in various journals. Also doing PhD from BLDE'S AVS Ayurveda Mahavidyalaya, Bijapur.

Dr KN Sunil Kumar MSc PhD is working as Senior Research Officer in Pharmacognosy and Phytochemistry at SDM Ayurveda and Allied Sciences, Udupi, India 574118. Current: Research Officer and HOD, Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai, India – 600106. He obtained Senior Research Fellowship from ICMR, Young Scientist Award, VGST, Govt. Of Karnataka and Dr. PD Sethi award for 5 best HPTLC papers. He is investigating projects on standardization of Ayurvedic formulation from agencie like UGC, VGST, RGUHS and PCIM (AYUSH). He is Author of 69 research papers and 55 monographs on pharmacognosy, phytochemistry and standardization of medical plants/products. He is also serving as Chief editor Journal of Ayurvedic and Herbal medicine and subject editor Pharmacognosy Ayu-An international Quarterly Journal of Research in Ayurveda.

GRAPHICAL ABSTRACT



Cite this article as: Deepthi CP, Basavaraj Y Ganti, Vinay R Kadibagil, Sunil Kumar KN. Pharmaceutico-analytical Study of *Amrutottara arka*. J Ayu Med Sci 2016;1(1):73-9. DOI: 10.5530/jams.2016.1.15