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## Original Article:

### Analytical Profile of *Amalaki Rasayana*

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**ABSTRACT:** Number of drugs found mentioned in *Ayurveda* are said to improve the immunity in human body. *Amalaki* (*Emblica officinalis* Gaertn) is one such drug of which crude extracts are proven to have antioxidant and immunomodulatory actions. Plenty of researches have been conducted for analysis of *Amalaki* as a single drug, but works on *Amalaki Rasayana* are not found reported. Considering these, three samples of *Amalaki Rasayana* were studied to evaluate their physicochemical analysis, microbial contamination and HPTLC profile as quality control parameters. Test drugs were prepared and analyzed by following classical guidelines. The results ensure quality of formulations proving their global acceptance. Qualitative study reveals the presence of proteins, tannins, saponins and carbohydrates in all three samples. HPTLC profile of *Amalaki churna* and three samples of *Amalaki Rasayana* with comparison to standard Gallic acid showed selective adsorbing pattern for particular component. All three samples showed antimicrobial and antifungal activities. *Amalaki Rasayana* (ARC) is found to be better antifungal drug.

**KEY WORDS:** *Amalaki*, *Amalaki Rasayana*, HPTLC, Physicochemical analysis, quality control

## INTRODUCTION:

*Ayurveda*, traditional system of medicine in Indian subcontinent uses number of herbal formulations in treatment of various disease conditions without noticeable side effects. Increased side effect of synthetic drugs, lack of remedy for many chronic ailments, microbial resistance raised demand of *Ayurvedic* formulations worldwide. With increasing claim for safer drugs, attention has been drawn towards quality, safety, efficacy, and standards of the *Ayurvedic* formulations. Quality of drug is must for its clinical use. Various exclusive pharmaceutical procedures have been described to develop many dosage forms and qualitative tests to judge proper formation of drug in *Ayurvedic* classics. They have their own scientific rationale but are not efficient to provide quantitative information. Standardization of each step through modern analytical tools is essential for establishing quality control parameters of *Ayurvedic* formulations to avoid ill effects and increase its therapeutic efficacy. *Acharya Charaka* described that potency of the drug will be increased, if the drug is levigated with its own liquid (*Swarasa / Kashaya*)<sup>1</sup>. It results in high therapeutic efficacy even with small doses. *Amalaki Rasayana* is one among herbal formulation containing *Amalaki* as an integral component having *Ayushya* (longevity), *Vaya Sthapana* (anti aging) properties. *Amalaki* is also reported to have anti oxidant<sup>11</sup>, anti ulcerative<sup>8</sup>, cardioprotective<sup>9</sup> and anti diabetic effect<sup>10</sup>. *Amalaki Rasayana* is prepared with levigation of *Amalaki Swarasa* to *Amalaki churna*.

Aim of current study is to provide analytical profile of *Amalaki Rasayana*

prepared with three different methods by following modern methodical parameters.

## MATERIALS AND METHODS:

### Collection of raw material:

Fresh fruits of *Amalaki* were collected from local vegetable market of Jamnagar. Botanical identification was done and it was authenticated at Pharmacognosy lab, I.P.G.T and R.A., Gujarat Ayurved University, Jamnagar.

### Preparation of test drugs:

Three samples of *Amalaki Rasayana* with three different methods were prepared in present study.

**Method I** - 500 gm *Amalaki Churna* was added in mortar and seven *Bhavanas* of *Amalaki Swarasa* (levigation) were given. After complete drying, it was powdered and labeled as AR7.

**Method II** - 500 gm *Amalaki Churna* was subjected to levigation for 21 times, dried and labeled as AR21.

**Method III** - *Amalaki Churna* (500g) was soaked in *Amalaki Swarasa* for 21 days. *Madhu* (Honey-500g), *Ghrita* (clarified butter-500g), *Pippali* (Piper longum Linn-62.5g), *Sharkara* (Sugar candy-125g) were mixed and kept in a box filled with *Bhasma Rashi* (Ash) for four months. It was labeled as ARC.

### Evaluation of *Amalaki Rasayana*:

All three samples were analyzed for various analytical parameters. Organoleptic characteristics like color, odor, taste, touch were evaluated. Physico-chemical analysis like loss on drying at 110°C<sup>6</sup>, ash value<sup>2</sup>, acid insoluble ash<sup>3</sup>, pH value<sup>7</sup>, water soluble extractives<sup>5</sup>, methanol soluble extractives<sup>4</sup> were carried out. They were subjected to further higher

analysis namely qualitative tests for various functional groups, quantitative estimation of total alkaloids, Microbial contamination (a bacterial and fungal growth study) and HPTLC profile. Micro Broth Dilution method was adopted to evaluate anti bacterial and anti fungal activities of test drugs.

## RESULTS AND DISCUSSION:

*Amalaki Rasayana* is one of the best examples of pharmaceutical development of dosage forms to overcome drawback of lower shelf life of *Churna*. Three different methods of *Amalaki Rasayana* are found in classics. The organoleptic characters of *Amalaki Rasayana* are summarized at (Table 1). Significant color change, more sourness in taste was observed in different samples of *Amalaki Rasayana*. It may indicate absorption of content from *Amalaki swarasa* during *Bhavana*. All samples were smooth to touch except sample ARC as levigation was not adopted in its preparation. Physicochemical analysis revealed presence of more moisture content in AR21 sample than AR7. It may be due to absorption of moisture during storage. (Table 2)

Qualitative tests were done to detect the presence of functional groups. The study reveals the presence of proteins, tannins, saponins and carbohydrates in all samples of *Amalaki Rasayana*. Other components like flavanoids, steroids, coumarin glycosides etc were absent in all three drug samples. (Table 3)

The Quantitative analysis was carried out for tannin and sugar content in samples. There was no significant difference in tannin content between AR21 & ARC samples. Tannin content in AR7 sample was less than other two samples. Total

sugar was maximum in ARC sample may be because ARC sample containing sugar in it as an ingredient. There was no significant difference in sugar content between AR7 & AR21 samples. Similar observation was found for reducing & non-reducing sugar in all samples. (Table 4)

Observations on microbial overload were found within permissible limits (below 100 CFU/gm). All the three samples of *Amalaki Rasayana* showed anti-microbial activity against *p. aeruginosa*, *s. aureus* and *s. pyogenus*, lesser in comparison to standard drug ampicillin. In addition, sample ARC exerted better anti-fungal activity against *c. albicans* in comparison to greseofulvin. (Table 5-7)

HPTLC profile of *Amalaki churna* and three samples of *Amalaki Rasayana* with comparison to standard Gallic acid showed selective adsorbing pattern for particular component in all the samples of *Amalaki Rasayana*. ARC showed more number of spots (14) in comparison to AR7 (9) and AR21 (10) indicating presence of more therapeutically active ingredients. (Table 8-10, Image 1). Adsorbance pattern indicates different types of components, while area covered in densitogram indicates the load of component in that sample at fraction of specified Rf value. Gallic acid standard was found at 0.28 Rf and area covered by different samples were recorded at 0.28 Rf shows that there is presence of Gallic acid in that much area of particular sample. Graph showed that more area was covered by AR21 than AR7 and ARC. (Graph I)

## CONCLUSION:

The analytical profile of *Amalaki Rasayana* ensures quality of formulation

thereby proving its global acceptance. Qualitative study reveals the presence of proteins, tannins, saponins and carbohydrates in all three samples. All three samples showed antimicrobial, antifungal activities. ARC was found to be better antifungal. HPTLC profile of *Amalaki churna* and three samples of *Amalaki Rasayana* with comparison to standard Gallic acid showed selective adsorbing pattern for particular component. There is a need to validate their exact nature and their respective therapeutic utilities through well stratified analytical, experimental and clinical studies. The results of this study may be used as the reference standard in further research undertakings of its kind.

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**Tables:****Table 1: Oragnoleptic characters of Amalaki Churna & Amalaki Rasayana**

Parameters	<i>Amalaki Churna</i>	AR7	AR21	ARC
Colour	Yellowish brown	Dark brown	Blackish brown	Black
Taste	Sour, astringent	Sour +	Sour ++	Sour++
Odour	Faint	Faint	Very Faint	Fermented
Touch	Smooth	Smooth	Smooth	Rough

**Table 2: Comparative data of physico-chemical parameters of different samples.**

Parameters	AR7	AR21	ARC
Loss on drying	7.60 w/w%	8.07w/w%	-
Ash value	3.19w/w%	3.08w/w%	-
Acid insoluble Ash	0.20w/w%	0.10w/w%	-
Water soluble extract	77.51 w/v%	81.9 w/v%	51.28 w/v%
Methanol soluble extract	73.61 w/v%	70.95 w/v%	62.08 w/v%
pH	-	-	6.00

**Table 3: Comparative data of qualitative tests of different samples.**

Parameters	AR7	AR21	ARC
Proteins (precipitation test)	+	+	+
Tannins & Phenolics	+	+	+
<b>Test for Glycosides</b>			
Flavanoids	-	-	-
Saponins	+	+	+
Cardiac glycosides	-	-	-
Coumarin glycosides	-	-	-
Antraquinine glycosides	-	-	-
Carbohydrates	+	+	+
Alkaloids	-	-	-
Volatile oils	-	-	-
Steroids	-	-	-
Fats & Oil	-	-	+

**Table 4: Comparative data of quantitative tests of different samples.**

Parameters	AR7	AR21	ARC
Tannin content	15.61w/w%	20.43w/w%	20.85w/ w%
<b>Sugar content</b>			
Total	31.10 w/w%	27.09 w/w%	60.58w/w%
Reducing	16.46 w/w%	18.55 w/w%	57.55w/w%
Non Reducing	14.64 w/w%	8.54 w/w%	3.03w/w%

**Table 5: Results of Microbial Overload test:**

Sample	Test Parameters	Result	Limit
A	<b>Total Microbial Count</b>	50 CFU per gm	100 CFU/gm
	Ø Total Bacterial Count	40 CFU per gm	
	Ø Total Fungal Count	10 CFU per gm	
	<b>Pathogens</b>		Should be Absent/10 gm
	Ø <i>E.coli</i>	Absent	
	Ø <i>Salmonella spp.</i>	Absent	
	Ø <i>Pseudomonas aeruginosa</i>	Absent	
Ø <i>S.aureus</i>	Absent		
B	<b>Total Microbial Count</b>	40 CFU per gm	100 CFU/gm
	Ø Total Bacterial Count	40 CFU per gm	
	Ø Total Fungal Count	00 CFU per gm	
	<b>Pathogens</b>		Should be Absent/10gm
	Ø <i>E.coli</i>	Absent	
	Ø <i>Salmonella spp.</i>	Absent	
	Ø <i>Pseudomonas aeruginosa</i>	Absent	
Ø <i>S.aureus</i>	Absent		
C	<b>Total Microbial Count</b>	60 CFU per gm	100 CFU/gm
	Ø Total Bacterial Count	40 CFU per gm	
	Ø Total Fungal Count	20 CFU per gm	
	<b>Pathogens</b>		Should be Absent/10 gm
	Ø <i>E.coli</i>	Absent	
	Ø <i>Salmonella spp.</i>	Absent	
	Ø <i>Pseudomonas aeruginosa</i>	Absent	
Ø <i>S.aureus</i>	Absent		

**Table 6: Results of Anti-Microbial activity:**

DRUG (µg/ml)	<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S.aureus</i>	<i>S.pyogenus</i>
-	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
GENTAMYCIN	0.05	1	0.25	0.5
AMPICILLIN	100	100	250	100
CHLORAMPHENICOL	50	50	50	50
CIPROFLOXACIN	25	25	50	50
NORFLOXACIN	10	10	10	10
AR7	250	500	125	250
AR21	200	125	250	500
ARC	250	100	250	62.5

**Table 7: Results of Anti-Fungal activity**

DRUG ( $\mu\text{g/ml}$ )	<i>C.albicans</i> MTCC 227	<i>A.niger</i> MTCC 282	<i>A.clavatus</i> MTCC 1323
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100
AR7	1000	1000	>1000
AR21	500	500	500
ARC	250	1000	1000

**Table 8: Comparative Chromatographic separation of Amalaki churna, three samples of Amalaki Rasayana (Methanolic extract) with standard Gallic acid on Silica gel G**

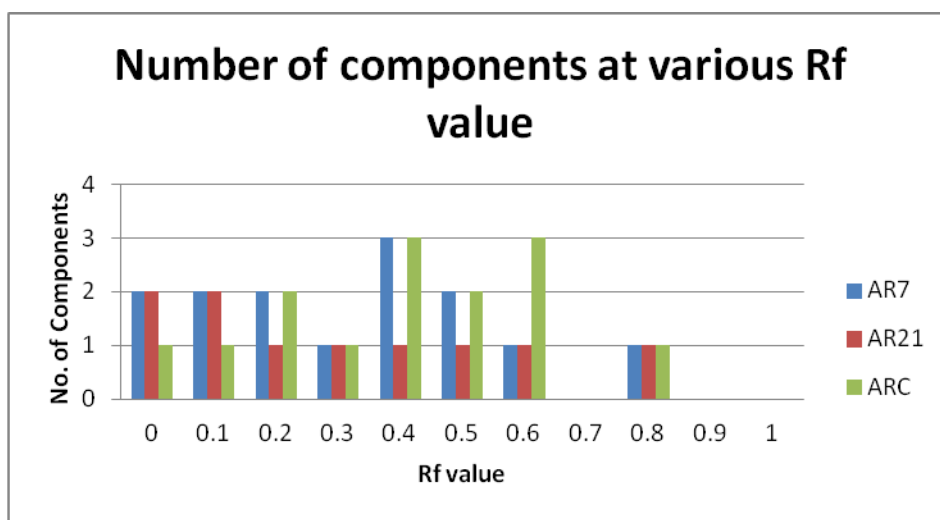
Toluene : Ethyl acetate: Formic acid (5: 3.5: 0.5 v/v)				
Sr. No.	Samples	Conditions	No. of spots	Rf
1	<i>Amalaki Churna</i>	Short UV - 254 nm	14	0.02, 0.05, 0.13, 0.19, 0.20, 0.28, 0.37, 0.42, 0.44, 0.46, 0.50, 0.55, 0.67, 0.82
		Long UV – 366 nm	10	0.02, 0.07, 0.13, 0.20, 0.28, 0.37, 0.44, 0.46, 0.50, 0.55
2	AR7	Short UV - 254 nm	9	0.02, 0.04, 0.14, 0.28, 0.35, 0.42, 0.56, 0.69, 0.84
		Long UV – 366 nm	4	0.02, 0.14, 0.29, 0.35
3	AR21	Short UV - 254 nm	10	0.02, 0.07, 0.14, 0.19, 0.28, 0.33, 0.42, 0.54, 0.67, 0.83
		Long UV – 366 nm	3	0.02, 0.14, 0.28
4	ARC	Short UV - 254 nm	14	0.02, 0.14, 0.22, 0.28, 0.33, 0.41, 0.43, 0.44, 0.50, 0.54, 0.62, 0.65, 0.69, 0.83
		Long UV – 366 nm	11	0.02, 0.14, 0.28, 0.33, 0.41, 0.43, 0.44, 0.50, 0.52, 0.59, 0.83
5	Gallic acid	Short UV - 254 nm	7	0.02, 0.11, 0.17, 0.21, 0.28, 0.42, 0.83
		Long UV – 366 nm	4	0.02, 0.11, 0.21, 0.28

**Table 9: Comparative data of Quantitative tests of Gallic acid% in different samples at 254nm**

Toluene : Ethyl acetate: Formic acid (5: 3.5: 0.5)					
	Standard	<i>Amalaki powder</i>	AR7	AR21	ARC
Max. Height	489.3	430	479.3	505.9	377.1
Gallic Acid %	83.41	61.31	54.74	54.11	52.31

**Table 10: Comparative data of Quantitative tests of Gallic acid% in different samples at 366nm**

Toluene : Ethyl acetate: Formic acid (5: 3.5: 0.5)					
	Standard	Amalaki powder	AR7	AR21	ARC
Max. Height	302.6	283.1	288.3	294.2	280
Gallic Acid %	88.97	59.01	53.66	54.43	50.70

**Graph I- Number of components at various Rf value****Image 1- HPTLC-three samples of Amalaki Rasayana with comparison to standard Gallic Acid**