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Research Article

PHARMACEUTICO-ANALYTICAL AND ANTIMICROBIAL STUDY OF *AMRITANK RASA*

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ABSTRACT

Amritank Rasa is a herbomineral formulation mentioned in the classical text of *Basavrajiyam* mainly used in the treatment of *Pancha Kasa*. This study aims to prepare the *Amritank Rasa* as per the classical text and conduct analytical and antimicrobial study of the prepared sample. The ingredients of *Amritank Rasa* are *Kajjali, Trikatu, Pippalimoola, Chavya, Chitraka, Vatsanabha* and *Saindhava lavana* all in equal parts and *Bhavana* of *Bhringaraj swarasa.* The pharmaceutical procedure involves the preparation of *Amritank Rasa vati* in the 125mg dosage form. The pharmaceutical, analytical and antimicrobial parameters were compiled and data was recorded. The organoleptic parameters were, dark greyish in colour with pungent odour and taste, appearance was round and uncoated, and smooth in touch. The physiochemical parameters and quantitative parameters were, total ash was 5.5%, acid insoluble ash was 0.5% and loss on drying was 11.8%, average weight was 121mg, uniformity of weight test showed that all the tablets were not within the range of deviation from average weight, friability test was 0.51%, hardness was 5.3kg/cm^2 and disintegration time test showed that any tablet did not dissolve in 60 minutes. The sample was then subjected to advanced analytical parameters i.e., FTIR, PSD, XRF and XRD. The antimicrobial study of *Amritank rasa* showed that it is effective against S.aureus, E.coli, P.aeruginosa and C. albicans. The development of the present study will serve as reference standards for *Amritank Rasa* formulation, quality control and clinical research.

INTRODUCTION

Rasa Shastra and *Bhaishajya Kalpana* is the specialized branch which mainly deals with the identification, purification, processing, analysis, standardisation and quality control of the herbal, mineral and specific marine origin drugs. According to the ancient Acharya of *Rasashastra, Rasa* means mercury. Mercury is fickle is nature but when it is processed properly acts as *Lohasiddhikar* and *Dehasiddhikar.* The herbomineral formulations are the most potent and work effectively in the treatment of acute as well as chronic diseases. The following are the therapeutic qualities of herbomineral formulations:

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- Required in small doses
- Never produce dislike to patient's taste
- Act faster
- Durable and long lasting potency [1]

Amritank Rasa is one such herbomineral formulation which is mentioned in the classical text of *Basavrajiyam, Astam Prakran* in the treatment of all the five types of *Kasa*, twenty types of *Kaphaja vikara* and eighty types of *Vataja vikara*.[2] In Ayurveda classics, *Kasa* is mentioned as a disease as well as the symptom of various diseases. According to Charaka, *Kasa* is divided into five types: *Vata, Pitta, Kapha, Kshataja* and *Kshayaja*.[3]

AIMS AND OBJECTIVES

- To prepare *Amritank Rasa* according to the classical text of *Basavrajiyam.*
- To conduct the analytical study of prepared *Amritank Rasa*.
- To evaluate the antimicrobial activity of prepared *Amritank Rasa*.

MATERIALS AND METHODS

Table 1: Showing ingredients (fig.1) and there proportion for preparing *Amritank Rasa*.[4]

S.No.	Ingredients	Botanical/Chemical name	Classification/Family	Part used	Ratio
1.	*Parada*	Mercury	*Rasa*	NA	1 part
2.	*Gandhaka*	Sulphur	*Uprasa*	NA	1 part
3.	*Pippali*	*Piper longum*	Piperaceae	Dried Fruit	1 part
4.	*Shunthi*	*Zingiber officinale*	Zingiberaceae	Rhizome	1 part
5.	*Maricha*	*Piper nigrum*	Piperaceae	Dried Fruit	1 part
6.	*Pippalimoola*	*Piper longum*	Piperaceae	Root	1 part
7.	*Chavya*	*Piper retrofractum*	Piperaceae	Root	1 part
8.	*Chitraka*	*Plumbago zeylanica*	Plumbaginaceae	Root	1 part
9.	*Vatsanabha*	*Aconitum ferox*	Ranunculaceae	Root	1 part
10.	*Saindhava Lavana*	Rock salt	*Lavana*	NA	1 part
11.	*Bhringaraj*	*Eclipta alba*	Compositae	Whole Plant	Q.S.

Fig. 1: ingredients of *Amritank Rasa*

Pharmaceutical Study

Pharmaceutical study involves preparation of *Amritank Rasa* according to the classical text of *Basavrajiyam.* Purification of *Parada, Gandhaka, Vatsnabhmoola* and *Chitrakmoola* were done according to the method mentioned in *Rasa Tarangini. Kajjali* was prepared in the ratio of 1:1 by *Shodhita Parada* and *Shodhita Gandhaka.* In a mortar fine powder of *Pippali, Shunthi, Maricha, Pippalimoola, Chavyamoola, Shuddha Chitrakmoola, Shuddha Vatsanabhmoola, Saindhava lavana* and *Kajjali* all in equal parts were added, followed by the *Bhavana* of *Bhringaraj kwatha,* until it got the consistency of dough. Then from this mixture, small pellets of *Gunja pramana* i.e., 125mg were prepared. *Bhringaraj kwatha* was prepared in place of *Swarasa*.

Practical No. 1 (Purification of *Parada*)[5]

500g *Ashodhita Parada* and 500g *Sudha Churna* were taken in a mortar and was triturated for 47 hours with the help of pestle. *Shodhita Parada* was collected from *Sudha Churna* by *Vastragalana.* Remaining *Shodhita Parada* was obtained by *Urdhvapatana* method. The amount of *Shodhita Parada* obtained was 474.15gm.

Then 450g *Sudha Shodhita Parada* and 450g *Rasona Kalka* were added in the mortar and 225g of *Saindhava Lavana* was added and triturated for 30 hours. *Prakshalana* of garlic paste was done with lukewarm water for 3 times. Every time, the supernatant water was collected and dried to obtain the *Parada.* The remaining garlic paste was dried in sunlight and *Parada* was obtained by scrapping. *Urdhvapatana* process was performed to obtain the remaining *Parada* from the garlic paste. The amount of *Shodhita Parada* obtained was 427.86g.

Practical No. 2 (Purification of *Gandhaka*)[6]

500g *Ashodhita Gandhaka* was crushed in *Khalva Yantra* to obtain fine powder. The same amount of *Goghrita* was heated and melted in a vessel. Then powdered *Gandhaka* was added in it and stirred continuously till it got completely melted. 1L *Godugdha* was taken in another steel vessel and a cotton cloth was tied over it. Then molten mixture of *Gandhaka* and *Goghrita* was poured in to the vessel containing *Godugdha* through the cloth to filter the impurities. After self cooling a solid mass with some granular part of *Gandhaka* was taken out from the vessel and thoroughly washed with hot water to

remove the *Snehansha.* Same procedure was repeated for 2 times using fresh ghee and milk. 414g *Shodhita Gandhaka* was obtained after the procedure.

Practical No. 3 (Purification of *Vatsnabhmoola*)[7]

500g roots of *Asuddha Vatsanabha* were washed with water and dried and cut into small pieces (*Chanaka* size)**.** Then they were kept in a vessel containing 2L cow urine. The vessel was kept in sunlight. The cow urine was changed every day. On 5th day *Vatsanabhmoola* were taken out from the cow urine and washed with warm water. Their outer shell was scraped using knife and then washed again with warm water. After that needle test was done. In this test, needle was inserted through a piece of *Vatsanabhmoola.* Then pieces were dried completely. *Vatsanabhmoola* which did not pass the test, were again dipped in cow urine. Same above procedure was repeated to obtain the *Vatsanabhmoola* having positive needle test. The amount obtained was 412.5g.

Practical No. 4 (Purification of *Chitrakmoola*)[8]

Churnodaka (lime water) was prepared in the ratio of 1:240 i.e., 8.4g lime and 2L water respectively in a vessel and then was kept still for 12 hours. Then it was filtered through cotton cloth. The obtained clear water was *Churnodaka.* 500g *Chitraka* roots were immersed in *Churnodaka* for 9 hours. Then the roots were washed with lukewarm water for 3 times and dried completely in sunlight.

Practical No. 5 (Preparation of *Kajjali*)[9]

380g *Shodhita Parada* and 380g *Shodhita Gandhaka* in ratio of 1:1 were taken in the *Khalva Yantra* and triturated for 37 hours. Trituration was done until the *Kajjali* became black in color, lusterless and *Varitara.*

Kajjali obtained was 742g.

Practical No. 6 (Preparation of Powder of Herbal Drugs)[10]

All the crude drugs were individually washed thoroughly for 3 to 4 times in order to remove the external impurities. They were then dried completely in sunlight for 3 to 4 days to avoid any moisture content. All the crude drugs were separately weighed. Then they were separately crushed in pulverizer. Then the powders were separately sieved through 85#. All the powders were separately kept in airtight containers.

Practical No. 7 (Preparation of *Bhringaraj Kwatha*)

300g *Bhringaraj panchang* was thoroughly washed for 6 times. Then it was soaked in 2.5L amount of water for overnight. Next day the vessel containing *Brhingaraj* and water was heated to obtain 600ml of *Kwatha.*[11]

Practical No. 8 (Preparation of *Amritank Rasa*)

30g of each ingredient were taken in a mortar (fig.2a). Total amount of the powdered mixture was 270g. *Bhavana* of 600ml *Bhringaraj swarasa* was given to the mixture (fig.2b). *Mardana* of the mixture was done until it attained the consistency of dough (fig.2c). The *Vati* of 125mg weight was manually prepared. It was then dried in shade and kept in airtight container. (fig.2d).

fig. 2a

fig.2b

fig.2c

fig.2d

Analytical study

This part deals with the analysis of organoleptic, physiochemical, quantitative and advanced analytical parameters of the prepared sample of *Amritank Rasa.*

Antimicrobial study

The prepared sample of *Amritank Rasa* was studied for its antimicrobial and antifungal activity on some bacterial (gram negative and gram-positive) and fungal strains by measuring the zone of inhibition using agar well diffusion method.

OBSERVATIONS AND RESULTS

Pharmaceutical Study

Loss of 5.17% and 4.92% of *Parada* were observed during the purification in first and second step respectively. Loss of 17.2% of *Gandhaka* was observed during its purification. Loss of 17.5% of *Vatsnabhmoola* was observed during its purification. Loss of 2% of *Chitrakmoola* was observed during its purification. Loss of 2.36% of *Kajjali* was observed during its preparation. *Bhavana* of *Bhringaraj kwatha* led to negligible increase in the weight of the mixture. About 2150 *Vati* prepared from the 270g of dough.

Analytical Study

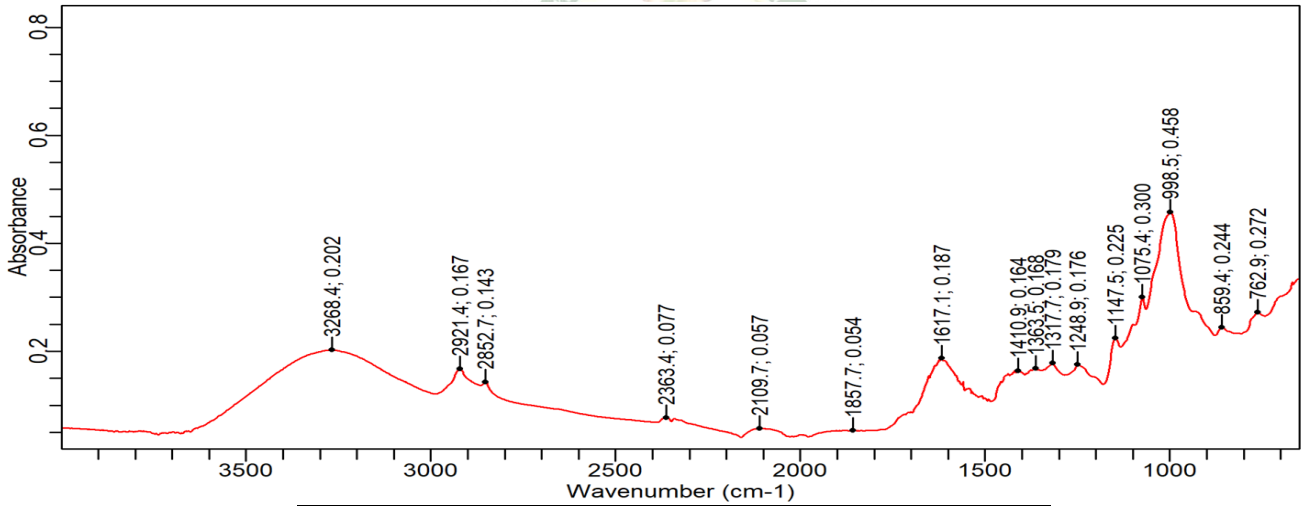
Table 2: Showing Organoleptic test of *Amritank Rasa*

Parameter	Characteristics
Colour	Dark Greyish
Odour	Pungent
Taste	Pungent
Touch	Smooth
Appearance	Round

Table 3: Showing Results of Analytical and Quantitative tests of *Amritank Rasa*

S. No.	Test parameters	Unit	Results
1.	Loss on Drying	%w/w	11.8
2.	Total Ash	%w/w	5.5
3.	Acid insoluble Ash	%w/w	0.5
4.	Average weight	mg	121
5.	Uniformity of weight	mg	All the tablets were not within the range of deviation from average weight
6.	Friability Test	%w/w	0.51
7.	Hardness	kg/cm^2	5.3
8.	Disintegration Time	minute	Any tablet did not dissolve in 60 minutes

FTIR (Fournier Transform Infrared Spectroscopy)



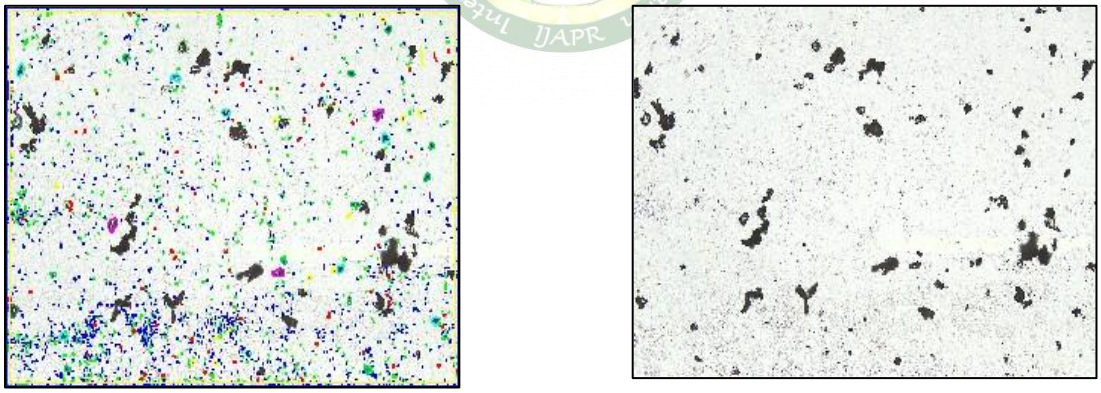
Peak Number	Wave number (cm-1)	Intensity
1	762.87403	0.27229
2	859.42837	0.24428
3	998.53209	0.45783
4	1075.44827	0.30034
5	1147.45490	0.22472
6	1248.91879	0.17558
7	1317.65239	0.17871
8	1363.47479	0.16832
9	1410.93371	0.16391
10	1617.13452	0.18741
11	1857.70213	0.05381

12	2109.72534	0.05737
13	2363.38506	0.07746
14	2852.70285	0.14266
15	2921.43646	0.16728
16	3268.37750	0.20238

Table 4: FTIR interpretation of *Amritank Rasa*

Peaks	Actual	Functional groups	Functional Group Source
770-735	761	C-H out of plane deformation Four adjacent hydrogen atoms	Piper longum, Piper nigrum, Piper refractorum active constituents
998	998	Cyclohexane ring vibrations	*Bhringaraj* active constituents
860-800	859	C-H out of plane deformation Three adjacent hydrogen atoms	*Piper longum* group
1150-1050	1073, 1447	Alkyle- substituted ether C-O stretch	*Trikatu* group
1270-1230	1248	Aromatic ethers, aryl o stretch	*Trikatu* group
1340-1220	1317	Aromatic primary amine	Milk used for *Shodhana*
1360-1310	1363	Aromatic tertary amine, CN stretch	Plumbago and aconite active constituents
1420-1370	1419, 1438	Organic sulphates	Garlic and *Gandhaka*
1800-2100	2109	Transition metal	*Parada*
1680-1620	1617	Alkenyl C=C stretch	*Pippali*, Plumbago active constituents
2140-2100	2117	C=C terminal alkyne (mono)	*Pippali*, Plumbago active constituents
2880-2860/ 2935-3915	2852, 2923	Methylene C-H asym/sym stretch	Aconite active constituents
3570-3200	3278	Hydroxy group, H bonded OH stretch	Gingerol from ginger and *Bhringaraj*

Particle Size Distribution



05-Apr-23, 1:36:01 PM, Calib: 10X 24122021, Unit: microns, X: 1.23, Y: 1.23 {pixels/microns]

Particle Sizing: Results Summary

Fields measured 5

Particles count 25422

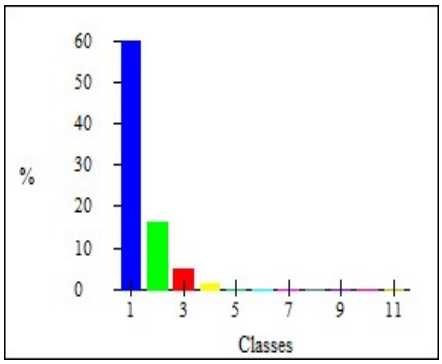
Type	Count microns	d10 microns	d50 microns	d90 microns	D(3,2) microns	D(4,3) microns	D(1,0) microns
Single	20858	0.9173814	1.8348	4.3029	9.4802	17.5489	2.3627
Agglomerates	4564	1.8348	4.1027	8.6546	38.6547	73.133	5.248
Unclassified	0	0	0	0	0	0	0
All(combined)	25422	0.9173814	1.8348	6.0852	26.1853	64.5319	2.8807

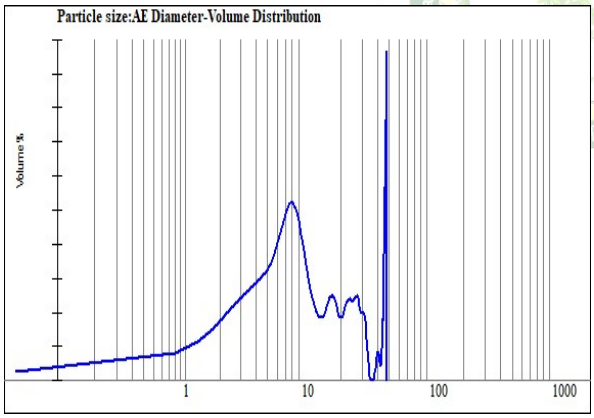
STATS (Excluding: Agglomerates, Unclassified)

	Area Sq microns	Aspect	Axis(Major) microns	Axis(Minor) microns	AreaEqv Dia microns	Circularity(Eqv)
Min	0.6609822	0	0.8129883	0.5748696	0.9173814	0
Max	1690.792	24.6491	86.1942	37.2774	46.3981	3.9633
Mean	8.2048	1.2907	2.6645	1.7618	2.3627	1.2248
Sum	171135.6	26921.35	55575.68	36747.82	49282.07	25547
Std.Dev	31.6987	1.0531	3.1919	1.9899	2.2055	0.8320136

Classes (Excluding: Agglomerates, Unclassified)

Classes	Counts	% Count	% Count(All)
#1: 0 to 2.5	15244	73.0847	59.9638
#2: 2.5 to 5	3999	19.1725	15.7305
#3: 5 to 10	1381	6.621	5.4323
#4: 10 to 15	139	0.666411	0.5467705
#5: 15 to 20	54	0.2588935	0.2124144
#6: 20 to 25	22	0.1054751	0.000000001
#7: 25 to 30	13	0.0623262	0.000000001
#8: 30 to 40	4	0.000000001	0.0157344
#9: 40 to 50	2	0.000000001	0.000000001
#10: 50 to 100	0	0	0
#11: 100 Above	0	0	0





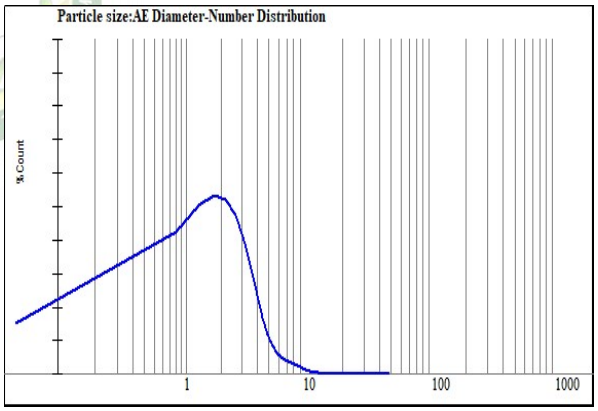
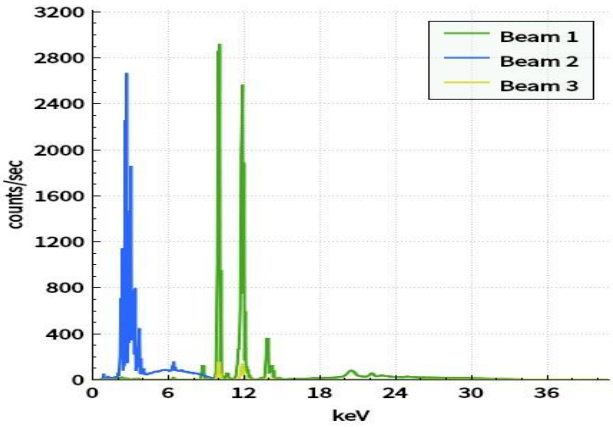


Table 5: Particle size interpretation of *Amritank Rasa*

1.	There are 20858 no. of single particles in 5 felid
2.	4564 no. are agglomerates
3.	Total no of particles count are 25422
4.	d10 Out of 20858 particles 10% of single particles are of size 0.9173814 microns Out of 4564 particles 10% of agglomerates are 1.8348 size microns
5.	d50 Out of 20858 particles 50% of single particles are of size 1.8348 microns Out of 4564 particles 50% of agglomerates are of size 4.1027 microns
6.	d90 Out of 20858 particles 90% of single particles are of size 4.3029 microns Out of 4564 particles 90% of agglomerates are of size 8.6546 microns

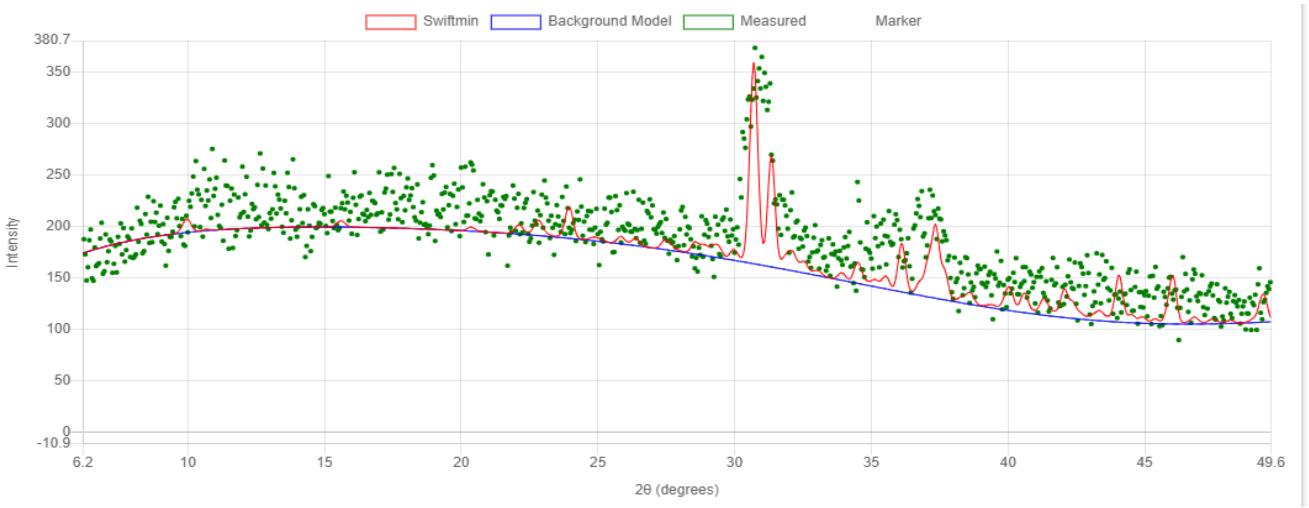
XRF (X- Ray Fluorescence)



Elapsed time: 60.0s

El	PPM	+/-2σ
Si	2010	210
P	1714	82
S	3.242%	0.034
Cl	6.605%	0.061
Ca	6489	98
Mn	76	25
Fe	797	35
Cu	31	9
As	19	8
Se	23	7
Rb	99	7
Nb	54	6
Mo	135	10
Hg	5.352%	0.048
Bi	127	46
LE	83.640%	0.081
El	**PPM**	**+/-3σ**
Mg	ND	<29000
Al	ND	<5500
Ti	ND	<920
V	ND	<61
Cr	ND	<38
Co	ND	<19
Ni	ND	<11
Zn	ND	<39
Sr	ND	<9
Y	ND	<4
Zr	ND	<3
Ag	ND	<0.1
Cd	ND	<15
Sn	ND	<18
Sb	ND	<25
Ba	ND	<2600
La	ND	<4500
Ce	ND	<5900
Pr	ND	<7300
Nd	ND	<10000
W	ND	<160
Pb	ND	<11

XRD (X-Ray Diffraction)



Mineral Configuration

Sample Name	AMTRJ01
Info1	0
Info2	0
Test	15_AMTRJ01_0_0-film.txt
Arsenic	4.63

Arsenopyrite	0.46
Azurite	0
Biotite	2.02
Bismuth	3.16
Calcite	0
Cassiterite	0.09
Cerussite	0
Chalcopyrite	0.46
Cinnabar	2.23
Copper	0
Dickite	0
Ferrosilite	11.93
Fluorite	0.3
Galena	0.12
Gold	0.06
Hematite	0
Iron	0.78
Kaolinite	0
Magnetite	0.2
Malachite	0
Marcasite	2.91
Muscovite	22.96
Natrolite	0
Orpiment	0
Pyrite	1.16
Quartz	27.44
Realgar	0

Siderite	5.43
Silver	0
Smithsonite	3012
Stibnite	0
Talc	1011
Tetrahedrite	0.16
Topaz	6.58
Zincite	2.71

Antimicrobial Study

S.No.	Sample Name	Zone of inhibition (in mm) (Values are mean of Qudaripulate)					
		Microorganisms studied (sample in DMSO)					
		Sample in mg	DMSO (ml)	S. aureus	P. aeruginoa	C. albicans	E.coli
1.	***Amritank Rasa***	10	1	15	15	19.5	26.5
2.	**Streptomycin**	10	1	21.6	15	NA	31.4
3.	**Fluconazole**	10	1	NA	NA	19.5	NA
4.	**DMSO**	-	-	8	8	8	8

Fig.3a Amritank Rasa S. aureus

Fig. 3b Streptomycin control S. aureus

Fig 4a *Amritank Rasa* P. aeruginosa

Fig 4b Streptomycin control P. aeruginosa

Fig. 5a *Amritank Rasa* E. coli

Fig. 5b Streptomycin control E.coli

Fig. 6a *Amritank Rasa* C.albicans

Fig. 6b Flucanozole control C.albicans

DISCUSSION

Amritank rasa is a herbomineral formulation. The dark greyish colour of the final product might be impacted by *Kajjali.* The odour and taste was pungent because the majority ingredient drugs are *Katu, Ushna* and *Teekshna* in nature. *Vati* was smooth in touch without any use of excipient. Percentage loss on drying of *Amritank rasa* was greater than the standard limit. It might be due to the presence of many herbals drugs and salt in the formulation. Total ash and acid insoluble ash shows presence of siliceous particles in the prepared drug, which might came from minute dirt particles remained even after thorough washing of herbal drugs. All the tablets were not within the range of deviation from the average weight. The tablets were manually prepared. Hardness of *Vati* was little bit more than that of standard parameter. Disintegration time of *Vati* is more than 1 hour, so excipient should be added or proper *Anupana* should be administered with the *Vati.* A variety of functional groups were detected in FTIR analysis. The results of particle size distribution showed single particles and agglomerates are of size less than 10 micron, so the powder of *Amritank Rasa* sample was of super fine consistency. A super fine powder has at least 90% of its particles that are less than 10 microns in size. XRF reports of sample showed presence of Si that might came from *Kharal*, clay pot used in *Urdhvapatana* or minute soil particles remained in herbal drugs even after washing. P from milk, ghee or herbal drugs, Cl from water or rock salt, Ca from lime, milk, ghee, herbal drugs or *Churnodaka,* Mn, Fe and Cu from herbal drugs or *Kajjali*, As from herbal drugs, *Kajjali* or *Kharal*, other elements from herbal drugs, water or *Kajjali*. Lighter elements (LE) were maximum in quantity which includes the

elements from atomic number 1 to 11 and HgS from *Kajjali.* XRD shows the siliceous minerals which might had come from the minute soil particles that remained in the herbal drugs although their thorough washing.

The antimicrobial activity results of *Amritank Rasa* were significant when compared to the negative control DMSO. When compared to the positive control standard antibiotic drug Streptomicin, the antibacterial activity of *Amritank Rasa* was less significant towards Staphylococcus aureus and E.coli and equally significant towards Pseudomonas aeruginosa. When compared to the positive control standard antifungal drug Flucanozole, the antifungal activity of *Amritank Rasa* was equally significant towards Candida albicans.

CONCLUSION

Amritank Rasa is a herbomineral formulation and a *Kharaliya rasayana*. The analysis of the literature shows that most of the ingredients of the drug are *Katu, Teekshna* and *Ushna* in nature, so making it *Shrotoshodhak, Laghu, Deepan* and *Amapachan.* Hence acts as *Kasaghn* drug. The pharmaceutical and analytical study were intended to develop the preliminary standards for *Amritank Rasa* as no pharmacopoeial standards are available for this formulation. The antimicrobial study of *Amritank rasa* shows that it is effective towards strains of both bacteria and fungus. The preliminary data of pharmaceutical, analytical and antimicrobial studies can be used to develop a standard profile for the formulation *Amritank Rasa*. The results of this study will be used as benchmarks for clinical research, quality control and drug formulation

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