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Physico-chemical Analysis of immune modulatory formulation R9 vati

Research Article

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Abstract

Introduction: The concept of well-being has evolved to include health beyond disease absence. Rasayana refers to the nutritional essence reaching all body tissue components to nourish and replenish them. It enhances longevity, intelligence, health, youth, skin, voice, and motor and sensory strength. Rasayana herbs are used in decoctions or powder to achieve this, enhancing various dhatu (tissues) and promoting overall health. *Ashwagandha*, *Pippali*, *Guduchi*, and *Yashtimadhu* are herbs known for their anti-oxidant properties. *Rasasindura*, a *rasashaudhi* used to treat various illnesses, is a *kupipakwa* preparation with *shuddha parada* and *shuddha gandhaka*. These *Rasaushadhi* are effective in small doses and can enhance the efficacy of herbal drugs when added to formulations. Method: In this study a tablet (R9 *vati*) was prepared with and without *rasasindura*. The prepared samples were subjected to various physico chemical analysis. Observation and result: Increase in the value of Total ash, Alcohol and Water soluble extractive, uniformity of weight, Hardness, and decrease in the value of Loss on drying, Water soluble ash was observed in R9 *vati* prepared with *rasasindura*. HPTLC result shows 8 peaks in both samples of *vati* at Short UV, 6 peaks in both samples of vati at Long UV, and 4 peaks in both samples of *vati* at Post derivatization. Result of Microbial analysis shows absence of Escherichia coli, Staphylococcus aureus, Salmonella sp., Enterobacteriaceae and Pseudomonas aeruginosa in both the samples of vati.

Keywords: *Rasayana, R9 Vati,* Immune Modulator.

Introduction

Agents that cause sickness are able to target any living creature. Even bacteria, which are so tiny that a million of them could fit on the head of a pin, have defense mechanisms against viral infection. As organisms get more complex, this type of defense becomes more advanced. -

The current infections require not just anti-microbial action but also should boost an individual's immunity. This is a strategy and method that can be adopted.

Rasayana herbs and drugs replace lost nutrients and increase virility(1).With the realisation that health is more than just the absence of disease, the idea of well-being has grown in importance.

Although seen from a conventional standpoint, this serves as the fundamental tenet of the idea of functional food as well. *Rasayana* therapy is a centuries-old, tried-and-true method that uses such herbs and herbal ingredients not only to treat illnesses but also to enhance quality of life. Uses of rasayana herbs as decoctions or powders are seen. But a more reasonable approach to the right use of herbs is necessary for modern culture because the process now appears to be somewhat time-consuming and tedious.

Numerous *Rasayana* herbs have been examined and verified by science for their effects on the immune system, endocrinological advantages, antioxidant capabilities, memory and learning behaviour enhancement, etc. Although the idea is well known and widely used, it is necessary to rationalise the strategy and examine the potential phytochemical components that may be responsible for the majority of actions and activity in these herbs(2).

The most appropriate illustration of alternative treatments for health advantages might be found in the *Ayurvedic Rasayana* concept. *Rasayana* literally translates to *Rasa* (elixir) and *Ayana* (home or path), thus signifying the path or direction of the elixir of life., the *Ayurvedic* approach to prevention of disease and health promotion is gaining popularity In the modern world(3). *Rasayana* treatment is one of *Ayurveda*'s eight main sub-disciplines. The word "*Rasayana*" refers to the route used by the nutritional essence (*Rasa-dhatu*) to reach all components of bodily tissue in order to nourish and replenish them. *Rasayana* is described by *Charaka*, the father of *Ayurveda*, as a method of obtaining the best attributes of various *dhatus* (tissues). *Rasayana* therapy enhances longevity, intelligence, health, youth, skin, voice, and motor and sensory strength.

Multiple studies have demonstrated the anti-oxidant properties of *Ashwagandha, Pippali, Guduchi,* and *Yashtimadhu*, which are also referred to as

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rasayana in *Ayurvedic* literatures. *Rasasindura*, commonly known as *rasayana*, is a *rasashaudhi* prescribed for a number of illnesses. This could have a synergistic effect on the formulation in combination with the herbs mentioned above. *Shunti, Tulasi, Maricha*, and *Twak*, which are also referred to as *rasayana* in *Ayurvedic* literature, were used as the *bhavana* of the current formulation. Previous studies have shown that they have anti-oxidant and immuno-modulatory effects as well. The drug's potency is also known to be increased by the process of *bhavana*. As a result, these were chosen for the *bhavana*._Thus, the formulation will be more efficacious and cost-effective as well, hence used for the study.

Since the earliest days, Indian and Chinese traditional medicines have used various kinds of inorganic mercury compounds, primarily mercuric sulphide (HgS) (Williamson, E. M., et al, 2004). *Rasasindura*, *Kajjali*, and *Makardhwaja*_are just a few of the mercury-based medications that are now often used in *Ayurveda* to treat a variety of chronic illnesses, including syphilis, pleural effusion, high fever, and mental disorders (Kamath et al., 2012; Singh et al., 2009). These mercury-based *Ayurvedic* medication's immuno-modulatory function is what gives them their effectiveness (Sinyorita et al., 2011). These medications are utilised as a rejuvenating agent and are thought to be antioxidants (Gholap et al., 2015; Singh et al., 2009).

Despite the fact that Ayurvedic medicine contains Hg,_recent in-vitro and in-vivo research have not discovered any hazardous effects of these Hg-based therapies, (Dwivedi et al., 2013; Kumar et al., 2006; Sathya et al., 2008; Thakur et al., 2014)._Unlike Chinese medicine, which employs cinnabar ore directly, *Ayurvedic kajjali*_and *rasasindura*_remedies are made from processed mercury and sulphur using a time-consuming and laborious process that includes cleaning, combining, and heat treatment. In the process of making *rasasindura*, *kajjali* exhibits the crystalline form of -HgS, which is also used as a medication (Thakur et al., 2014). *Rasasindura*, the finished product, is a single crystalline -HgS phase that is remarkably pure with only a little amount of impurities (Ramanan et al., 2015). *Kajjali* is an intermediate in the *rasasindura* preparation procedure and exhibits β-HgS crystalline form (Thakur et al., 2014) which is also used as a medicine. The final product, *Rasasindura*, is a highly pure single crystalline α-HgS phase (Ramanan et al., 2015) with a trace amount of impurity. These *Ayurvedic* medications contain a portion of particles that are nano-sized (100 nm) and demonstrate a stable crystal structure (crystal defect< 3%) (Ramanan et al., 2015). Therefore, it is reasonable to conclude that the novelties in the *Ayurvedic* preparation techniques are what contribute to the effectiveness of these Hg-based medications(4).

Antioxidants present in food stuffs operate as health-protecting agents and are essential to human survival. In addition to this function antioxidants are one of the main additives used in fats and oils. Antioxidants have been employed in the food processing sector to delay or avert food spoilage. Medicinal plants have gained tremendous traction as sources of antioxidants against many ailments. Due to its ability to combat free radicals and other damage caused by metabolic disorders and age-related syndromes in both humans and other animals, antioxidants have been hailed as the most significant forces behind human progress and existence(5). Mineral drugs are effective in small doses. The combination of herbal drugs i.e. *Ashwagandha, Pippali, Guduchi, Yashtimadhu, Shunti, Tulasi, Maricha, Twak* with mineral drug *Rasasindura* could give a synergistic effect. This combination of eight herbal and one mineral drug is named as R9 Vati.

In this study R9 *Vati* was prepared with and without *rasasindura* and was subjected to various physico chemical analysis.

Objectives of the study

1. Standardisation of R9 vati samples
2. Assessment of changes in physicochemical parameters

Materials and Methods

Raw drugs required for preparation of R9 *vati*_i.e. *Ashwagandha, Pippali, Guduchi, Yashtimadhu, Shunti, Tulasi, Maricha, Twak, Parada* and *Gandhaka*_were procured from Gadgil vanoushadhi, Belgaum. Herbal drugs were authenticated by department of *dravya guna* and mineral drugs were authenticated by department of *rasashastra* and *bhaishajya kalpana*.

Herbal drugs i.e. *ashwagandha*, *pippali*, *guduchi*, *yashtimadhu*_were converted into fine powder and drugs i.e. *shunti, tulasi, maricha, twak*, were converted to coarse powder. Mineral drugs i.e. *parada* and *gandhaka* were subjected for *shodhana* as per reference. *Kajjali* was prepared by using *shuddha parada* and *shuddha gandhaka* and later *rasasindura* was prepared.

Sample 1 (R9V)- *Bhavana* by *kwatha* of *shunti, tulasi, maricha, twak* was given to mixture of *ashwagandha, pippali, guduchi, yashtimadhu*_and tablet was prepared by Parle ECO-III tablet punching machine.

Sample 2 (R9VWS)- *Bhavana* by *kwatha* of *shunti, tulasi, maricha, twak* was given to mixture of *ashwagandha, pippali, guduchi, yashtimadhu, rasasindhura*_and tablet was prepared by Parle ECO-III tablet punching machine.

Both samples of tablet were subjected for analytical parameters as per reference of CCRAS protocol for testing AYUSH drugs (6) and microbial load as given below.

The prepared samples were subjected to following analytical parameters

Organoleptic characteristics

Colour, Odour, taste which are documented by sensory perception using sense organs.

Particle size by powder microscopy

Using a drop of glycerin-water and a pinch of the sample, a tiny slide was mounted. Under bright field

lighting, characters were examined using a Zeiss AXIO trinocular microscope connected to a Zeiss AxioCam camera. The scale-bars, which were calibrated beforehand using the Zeiss Axio Vision programme, show how magnified the figures are.

Loss on drying at 105°C(7)

A tared evaporating dish was filled with 10 g of sample. It was weighed after drying in a hot air oven for five hours at 105°C. The drying process was continued until the difference in weight between two successive weights, after chilling in the desiccator, was less than 0.01. The sample's weight was used to compute the percentage of moisture.

Total Ash(8)

A tared platinum crucible was used to burn 2 g of the material at a temperature no higher than 450 °C until carbon-free ash was produced. The percentage of ash was determined using the sample's weight as a reference.

Acid insoluble Ash(9)

Add 25ml of diluted HCl to the crucible containing the complete ash, then bring to a boil. On ashless filter paper (Whatman 41), collect the insoluble material and wash with hot water until the filtrate is neutral. To ignite to a consistent weight, transfer the filter paper holding the insoluble material to the original crucible, dry on a hot plate, and ignite. Wait 30 minutes while the residue cools in a suitable desiccator before weighing. Calculate the amount of acid-insoluble ash by using the air-dried medication as a reference.

Water soluble ash(10)

The ash should be boiled for 5 minutes with 25 cc of water, then the insoluble material should be collected on ashless filter paper, washed in hot water, and ignited for 15 minutes at a temperature no higher than 450 °C. To determine the amount of water-soluble ash relative to the air-dried sample, subtract the weight of the insoluble material from the weight of the ash.

Alcohol soluble extractive(11)

Put 4 g of the sample in a glass flask and weigh it precisely. Incorporate 100 ml of distilled alcohol (approximately 95 percent). Shake once in a while for six hours. Give something 18 hours to stand. Quickly filter, being careful not to lose any solvent. Pour 25 ml of the filtrate into a 100 ml beaker that has already been weighed. Evaporate on a water bath until dry. Keep it at 105°C in an air oven for 6 hours, then cool it in a desiccator for 30 minutes before weighing. Determine the sample's extractable alcohol content as a percentage. Take the average value after conducting the experiment twice.

Water soluble extractive(12)

Put 4 g of the sample in a glass flask and weigh it precisely. For six hours, add 100 ml of distilled water and shake occasionally. Give something 18 hours to stand. Quickly filter, being careful not to lose any solvent. Pour 25 ml of the filtrate into a 100 ml beaker that has already been weighed. Evaporate on a water bath until dry. For six hours, bake it at 105°C in an air oven. Weigh after cooling in a desiccator. Double-check the experiment. Consider the average.

Uniformity of weight(13,14)

20 units were randomly chosen or the contents of 20 separate containers for single-dose preparations were individually weighed, and the average weight was determined. Neither one nor more than two of the individual weights vary from the average weight by a percentage greater than that indicated in the table.

Average Weight of tablet	Percentage deviation
80 mg or less	10
More than 80 mg but less than 250 mg	7.5
250mg or more	5

Hardness test(15)

The hardness of 5 tablets each of R9V and R9VWS was assessed. The tablet came into contact with the lower plunger. The tablet was then broken by rotating a threaded bolt, which pulled the upper plunger up against a spring. The fracture's force was noted.

Disintegration time(16)

The required amount of distilled water was placed in the tank of the disintegration device. Each 1000 ml beaker was filled with 750 cc of distilled water. The device's timer was set for 60 minutes. The water in the main tank was kept at 37.5°C, whereas the water in the beakers was kept at 37°C. Each tube received a single vati, which also added a disc. The apparatus was turned on while the assembly was suspended in the water-filled beaker. It was noticed how long the tablet took to dissolve.

Determination of pH(17)

Preparation of buffer solutions

Standard buffer solution

Dissolve one tablet with pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH

Take 1 g of R9V and R9VWS samples and dilute with distilled water to 100 ml, shake well and filter. The filtrate was used for the experiment. The instrument is on. A period of 30 minutes is allowed to warm up the pH meter. A pH 4 solution was first introduced and the pH was adjusted with a knob to 4.02 for an ambient temperature of 30°C. A pH 7 solution is introduced and the pH meter is set to 7 using the button. Add pH 9.2 solution and check the pH reading without adjusting the knob. The sample solution is then introduced and the reading recorded. I repeated the test four times and took the average result.

HPTLC

Chromatographic analysis of both samples was done at SDM Centre for Research in Ayurveda and

Allied Sciences, using CAMAG Automatic TLC Sampler 4(ATS4).

1.0g of each of **R9V** and **R9VWS** tablet powder were suspended in 10.0ml methanol filtered after 24hrs. 4 and 8µl of each of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7mm using Linomat 5 TLC applicator. The plate was developed under **Toluene: Ethyl acetate (9.0: 1.0)**. The developed plates were visualized in short UV, long UV, White light and then scanned at 254nm, 366nm, derivatised with Vanillin sulphuric acid reagent subsequently scanned at 620nm. Rf, colour of the spots, densitometric scan and 3-D chromatograms were recorded.

Test for Microbial contamination – Direct method- R9v and R9vws

Preparation of Casein Soya bean Digest Agar Medium (CSDAM):

Casein peptone (15 g), Soya peptone (5 g), Sodium Chloride (5 g) were taken and dissolved in 990 ml distilled water and pH was adjusted to 7.3±0.2 and make up the volume to 1000 ml. Finally add 15 g of agar to the media and autoclaved at 121˚C for 20 minutes. The prepared media is poured on to plates and allowed to cool.

Preparation of Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0

Dissolve potassium dihydrogen phosphate (3.56 g), disodium hydrogen phosphate (7.23 g), Sodium Chloride (4.3 g), peptone (1.0 g) were taken and dissolved in 990 ml distilled water. The pH was adjusted to 7.0 and make up the volume to 1000 ml. Then above buffer solution was autoclaved at 121˚C for 20 minutes.

Methods

1gm of test samples were dissolved in 100ml of buffered solution and the solution was poured over the plates containing the above prepared media. The plates were incubated at 35°C for 48hrs and observed for microbial growth.

Test for specific pathogen

Hi Dtect TM Universal Microbial Limit Test disc (DT005-10DS) was used for rapid detection and confirmation of *Escherichia coli*, *Staphylococcus aureus, Salmonella* sp., *Enterobacteriaceae and Pseudomonas aeruginosa* as per kit procedure.

Observations and Results

Table 1: Organoleptic characteristics

Parameters	R9V	R9VWS
Color	Brown	Brown
Odour	Characteristic	Characteristic
Taste	Spicy, Sweet, Bitter	Spicy, Sweet, Bitter

Table 2 : Result of Standardisation parameters

Parameters	*Results n = 3 %w/w*	
	R9V	R9VWS
Loss on drying	10.21±0.01	8.49±0.01
Total ash	6.98±0.14	7.14±0.05
Acid insoluble ash	0.0±0.0	0.0±0.0
Water soluble ash	3.87±0.01	3.52±0.02
Alcohol soluble extractive	6.66±0.01	10.66±0.01
Water soluble extractive	32.43±0.01	54.75±0.02
Tablet weight variation (Avg ± SEM)	Tab ranges from 0.490-0.542	Tab ranges from 0.505-0.559
Tablet average weight	0.516	0.532
Hardness (Kg/cm²)	3.0	3.5
Disintegration time (min)	6	6
pH	6.0	6.0
Particle size	20µm	20-50 µm

Figure 1. Powder microscopy of R9V

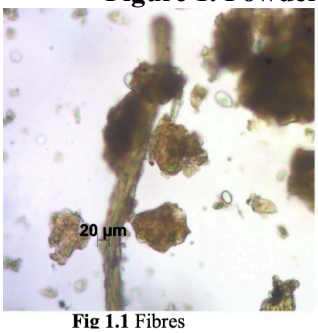


Fig 1.1 Fibres

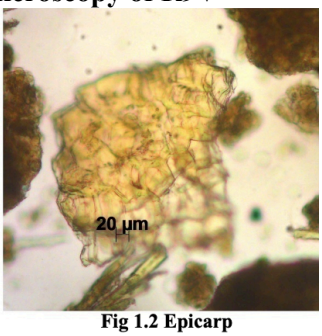


Fig 1.2 Epicarp

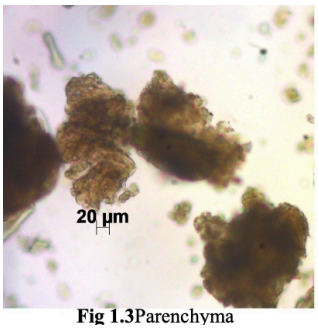


Fig 1.3 Parenchyma

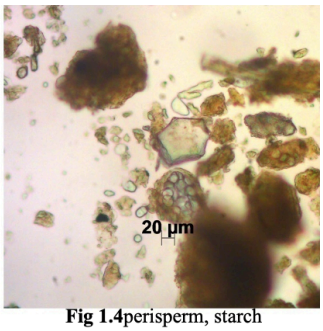


Fig 1.4perisperm, starch

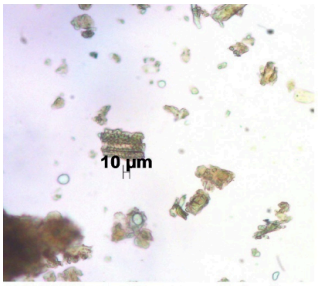


Fig 1.5vessel

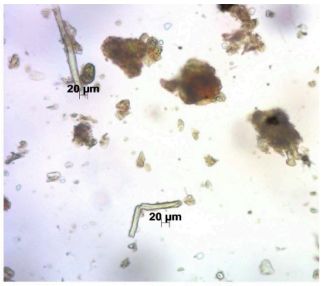


Fig 1.6 Vessel

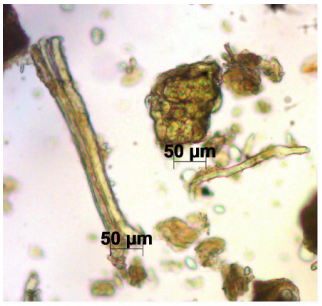


Fig 1.7 Fibres, stone cell in

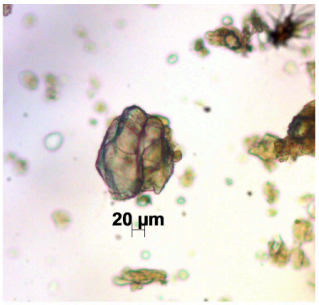


Fig 1.8 Perisperm

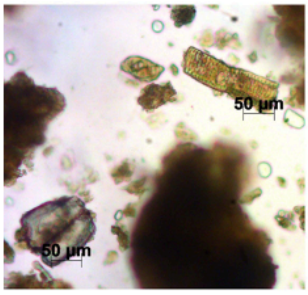


Fig 1.9Perisperm, vessel

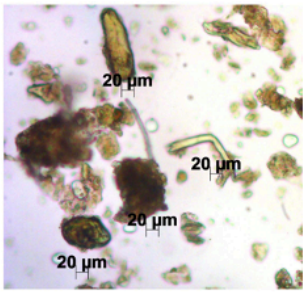


Fig 1.10sclereids, mesophyll with

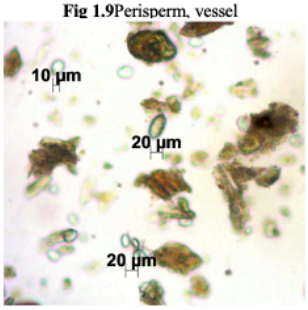


Fig 1.11Starch

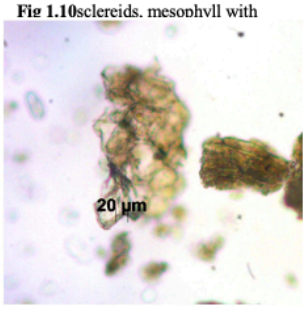


Fig 1.12 Loose

Figure 2. Powder microscopy of R9VWS

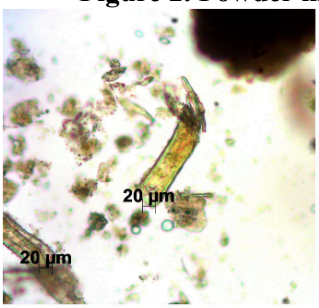


Fig 2.1 Vessels

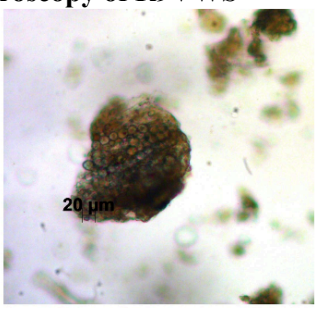


Fig 2.2 Starch in masses

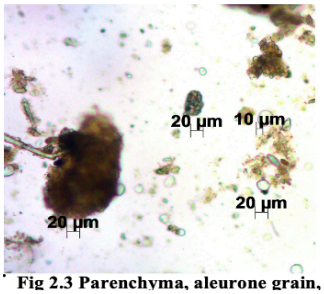


Fig 2.3 Parenchyma, aleurone grain,

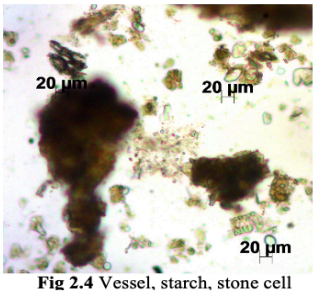


Fig 2.4 Vessel, starch, stone cell

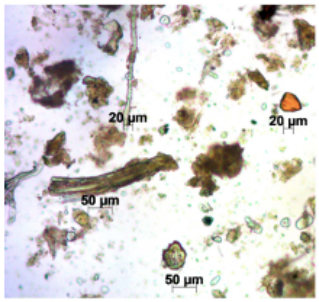


Fig 2.5Fibre, stone cells

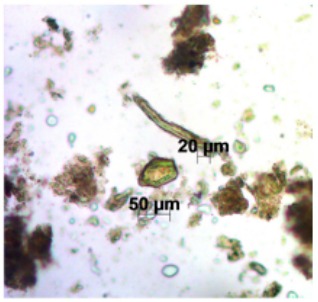


Fig 2.6Stone cell

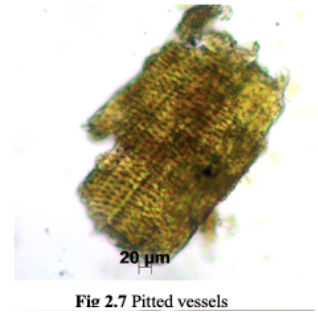


Fig 2.7 Pitted vessels

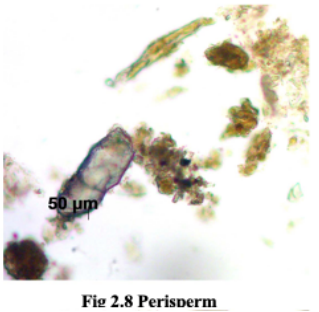


Fig 2.8 Perisperm

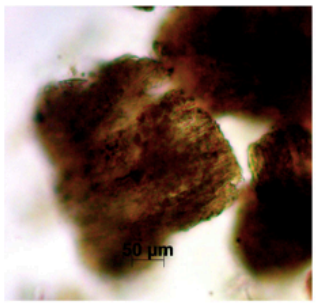


Fig 2.9Parenchyma

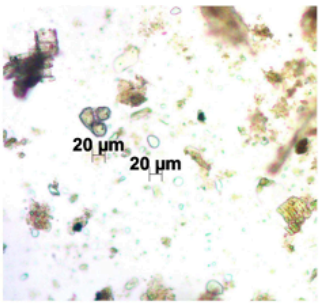


Fig 2.10Starch in group and isolated.

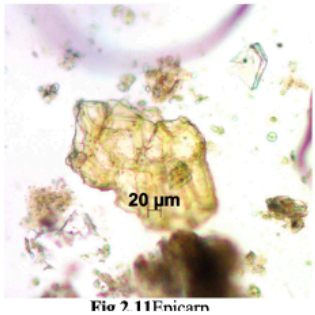


Fig 2.11Epicarp

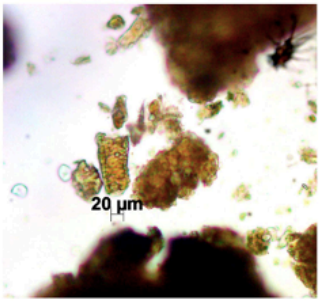
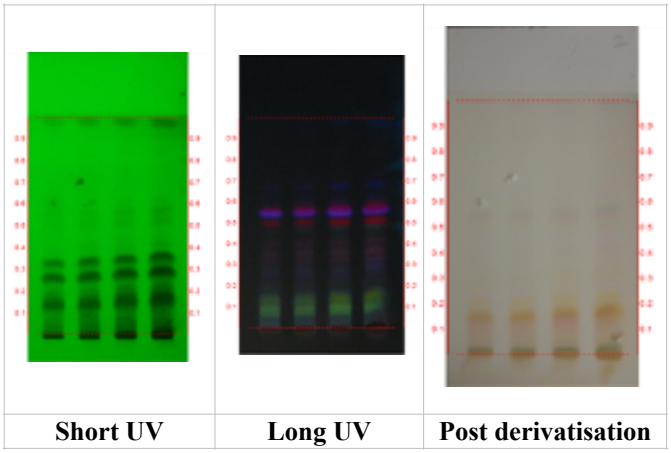


Fig 2.12Vessel

Figure 3. HPTLC photo documentation of ethanol extract of R9V and R9VWS



Short UV	Long UV	Post derivatisation

Track 1 –**R9V** – 4µl
Track 2 –**R9VWS** – 4µl
Track 3 –**R9V** – 8µl
Track 4 –**R9VWS** – 8µl

Solvent system – Toluene: Ethyl acetate (9.0: 1.0)

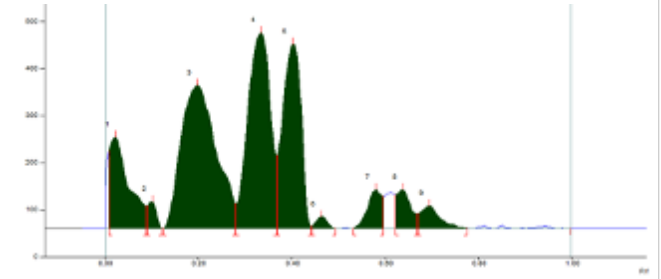
Table 3:R_f values of sample of Ethanol extract of R9V *and* R9VWS

Short UV		Long UV		Post derivatisation	
R9V	**R9VWS**	**R9V**	**R9VWS**	**R9V**	**R9VWS**
0.08 (Green)	0.08	0.09 (F. green)	0.09 (F. green)	-	-
-	-	0.14 (F. green)	0.14 (F. green)	-	-
0.16 (Green)	0.16 (Green)	-	-	0.16 (Orange brown)	0.16 (Orange brown)
0.21 (Green)	0.21 (Green)	-	-	-	-
0.28 (Green)	0.28 (Green)	-	-	-	-

0.35 (Green)	0.35 (Green)	0.35 (F. blue)	0.35 (F. blue)	-	-
0.40 (Green)	0.40 (Green)	0.40 (F. blue)	0.40 (F. blue)	-	-
-	-	-	-	0.45 (Purple)	0.45 (Purple)
0.51 (Green)	0.51 (Green)	0.51 (F. blue)	0.51 (F. blue)	-	-
-	-	-	-	0.55 (Pink)	0.55 (Pink)
0.57 (Green)	0.57 (Green)	0.56 (F. blue)	0.56 (F. blue)	0.57 (Purple)	0.57 (Purple)

*F – Fluorescent; L –Light; D – Dark

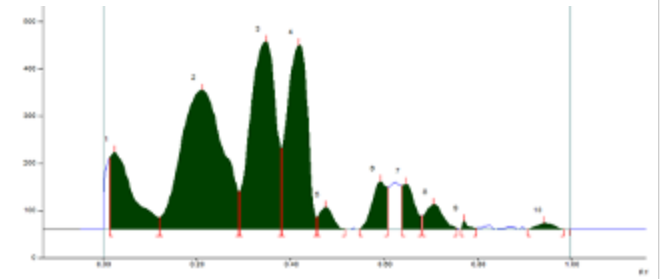
Figure 4: Densitometric scan at 254nm



Track 7, ID: R9V

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	184.6 AU	0.02 Rf	193.5 AU	12.12 %	0.09 Rf	47.5 AU	5838.0 AU	10.76 %
2	0.09 Rf	47.6 AU	0.10 Rf	56.6 AU	3.56 %	0.12 Rf	0.1 AU	762.4 AU	1.40 %
3	0.13 Rf	0.6 AU	0.20 Rf	303.8 AU	19.02 %	0.28 Rf	52.6 AU	16574.7 AU	30.53 %
4	0.28 Rf	52.8 AU	0.34 Rf	415.2 AU	25.99 %	0.37 Rf	54.2 AU	14415.3 AU	26.56 %
5	0.37 Rf	154.6 AU	0.40 Rf	391.1 AU	24.48 %	0.44 Rf	4.4 AU	11156.4 AU	20.55 %
6	0.44 Rf	4.5 AU	0.46 Rf	25.1 AU	1.57 %	0.50 Rf	0.1 AU	431.9 AU	0.80 %
7	0.53 Rf	0.4 AU	0.58 Rf	82.3 AU	5.15 %	0.60 Rf	88.2 AU	1823.3 AU	3.36 %
8	0.62 Rf	71.5 AU	0.64 Rf	81.7 AU	5.12 %	0.67 Rf	31.1 AU	1632.1 AU	3.00 %
9	0.67 Rf	31.2 AU	0.70 Rf	47.7 AU	2.99 %	0.78 Rf	1.1 AU	1446.1 AU	2.66 %

Fig 4a. R9V

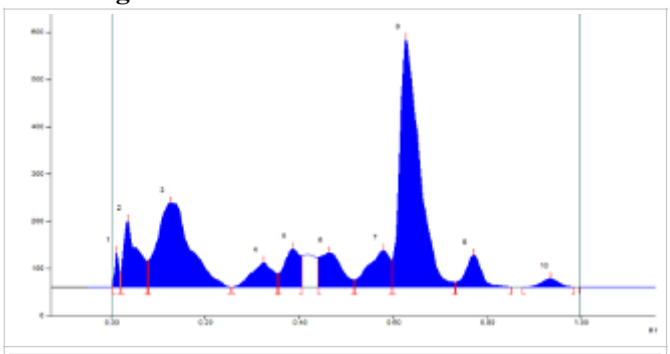


Track 9, ID: R9VWS

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	151.0 AU	0.02 Rf	182.8 AU	10.39 %	0.12 Rf	24.1 AU	5803.2 AU	9.58 %
2	0.12 Rf	24.3 AU	0.21 Rf	293.5 AU	16.73 %	0.29 Rf	76.8 AU	19914.6 AU	32.27 %
3	0.29 Rf	79.2 AU	0.35 Rf	386.8 AU	25.32 %	0.38 Rf	70.5 AU	15226.9 AU	25.96 %
4	0.38 Rf	170.8 AU	0.42 Rf	383.5 AU	24.86 %	0.46 Rf	25.1 AU	12054.8 AU	20.57 %
5	0.46 Rf	25.3 AU	0.49 Rf	46.6 AU	2.97 %	0.52 Rf	0.7 AU	940.6 AU	1.61 %
6	0.55 Rf	0.4 AU	0.59 Rf	109.8 AU	8.43 %	0.61 Rf	88.2 AU	2174.0 AU	3.71 %
7	0.64 Rf	92.5 AU	0.65 Rf	95.0 AU	6.06 %	0.68 Rf	28.4 AU	1828.7 AU	3.12 %
8	0.68 Rf	28.6 AU	0.71 Rf	52.1 AU	3.32 %	0.76 Rf	2.0 AU	1387.4 AU	2.36 %
9	0.78 Rf	0.4 AU	0.77 Rf	17.5 AU	1.12 %	0.80 Rf	2.2 AU	138.4 AU	0.24 %
10	0.91 Rf	0.0 AU	0.94 Rf	12.6 AU	0.80 %	0.99 Rf	0.4 AU	327.6 AU	0.56 %

Fig 4b. R9VWS

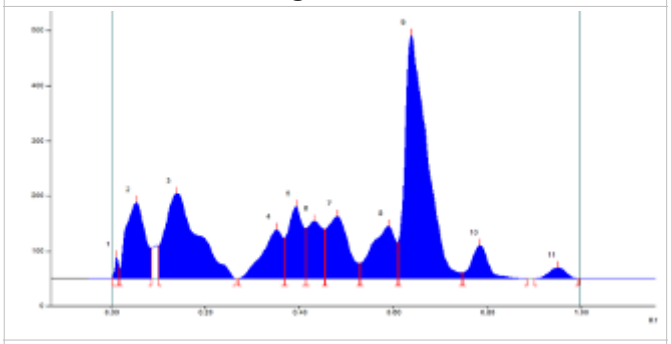
Figure 5: Densitometric scan at 366nm



Track 7, ID: R9V

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.2 AU	0.01 Rf	73.4 AU	5.70 %	0.02 Rf	25.5 AU	473.5 AU	1.21 %
2	0.02 Rf	36.0 AU	0.04 Rf	140.0 AU	10.88 %	0.08 Rf	55.0 AU	3033.0 AU	7.76 %
3	0.08 Rf	55.7 AU	0.13 Rf	178.4 AU	13.87 %	0.25 Rf	0.1 AU	8954.4 AU	22.95 %
4	0.26 Rf	0.1 AU	0.33 Rf	52.5 AU	4.08 %	0.35 Rf	26.0 AU	1703.9 AU	4.37 %
5	0.36 Rf	29.0 AU	0.39 Rf	81.0 AU	6.30 %	0.41 Rf	88.3 AU	1980.2 AU	5.08 %
6	0.44 Rf	62.0 AU	0.46 Rf	72.4 AU	5.63 %	0.52 Rf	15.9 AU	2470.5 AU	6.35 %
7	0.52 Rf	16.1 AU	0.58 Rf	78.2 AU	6.08 %	0.60 Rf	58.9 AU	2541.0 AU	6.51 %
8	0.60 Rf	58.2 AU	0.63 Rf	524.1 AU	40.76 %	0.73 Rf	10.7 AU	15684.3 AU	40.21 %
9	0.75 Rf	10.8 AU	0.77 Rf	67.8 AU	5.27 %	0.85 Rf	0.1 AU	1672.2 AU	4.29 %
10	0.88 Rf	0.0 AU	0.94 Rf	18.2 AU	1.41 %	0.99 Rf	0.2 AU	487.1 AU	1.25 %

Fig 5a. R9V

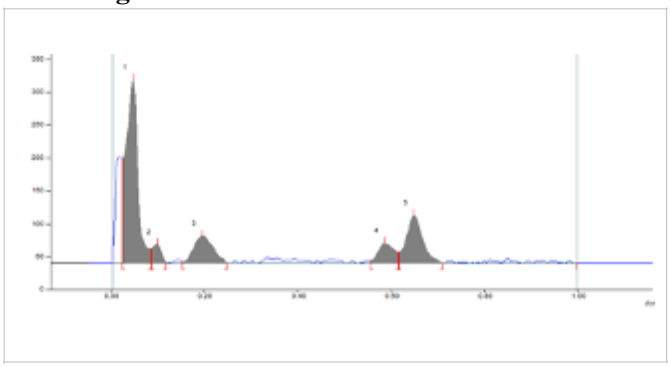


Track 8, ID: R9VWS

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.0 AU	0.01 Rf	39.4 AU	2.84 %	0.02 Rf	22.3 AU	214.7 AU	0.49 %
2	0.02 Rf	16.6 AU	0.05 Rf	138.4 AU	9.98 %	0.09 Rf	55.6 AU	3972.9 AU	9.05 %
3	0.10 Rf	59.2 AU	0.14 Rf	154.5 AU	11.14 %	0.27 Rf	0.2 AU	7793.4 AU	17.75 %
4	0.27 Rf	0.2 AU	0.35 Rf	88.9 AU	6.41 %	0.37 Rf	72.9 AU	2793.5 AU	6.36 %
5	0.37 Rf	73.9 AU	0.40 Rf	131.3 AU	9.47 %	0.41 Rf	90.8 AU	2946.5 AU	6.71 %
6	0.42 Rf	91.2 AU	0.43 Rf	104.5 AU	7.54 %	0.45 Rf	89.7 AU	2430.3 AU	5.53 %
7	0.46 Rf	89.9 AU	0.48 Rf	112.8 AU	8.13 %	0.53 Rf	27.8 AU	3604.6 AU	8.21 %
8	0.53 Rf	27.9 AU	0.59 Rf	94.9 AU	6.84 %	0.61 Rf	65.6 AU	3400.6 AU	7.74 %
9	0.61 Rf	67.6 AU	0.64 Rf	441.3 AU	31.82 %	0.75 Rf	11.0 AU	14687.9 AU	33.45 %
10	0.75 Rf	11.2 AU	0.79 Rf	60.5 AU	4.36 %	0.89 Rf	0.0 AU	1541.0 AU	3.51 %
11	0.90 Rf	0.0 AU	0.95 Rf	20.4 AU	1.47 %	1.00 Rf	0.1 AU	528.2 AU	1.20 %

Fig 5b. R9VWS

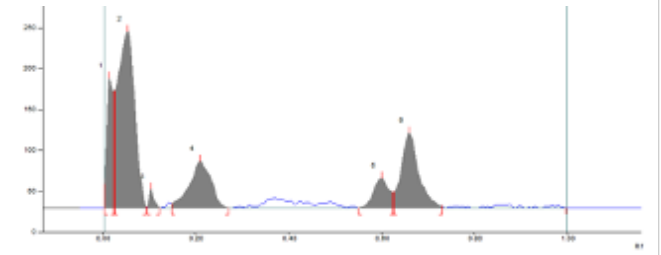
Figure 6: Densitometric scan at 620nm



Track 7, ID: R9V

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	159.2 AU	0.05 Rf	277.9 AU	61.98 %	0.09 Rf	21.8 AU	5246.1 AU	55.70 %
2	0.09 Rf	22.2 AU	0.10 Rf	27.6 AU	6.16 %	0.12 Rf	0.9 AU	392.3 AU	4.17 %
3	0.15 Rf	1.7 AU	0.20 Rf	41.4 AU	9.23 %	0.25 Rf	0.4 AU	1229.3 AU	13.05 %
4	0.56 Rf	3.8 AU	0.59 Rf	29.6 AU	6.60 %	0.62 Rf	16.0 AU	736.6 AU	7.82 %
5	0.62 Rf	16.1 AU	0.65 Rf	71.9 AU	16.03 %	0.71 Rf	0.0 AU	1814.9 AU	19.27 %

Fig 6a. R9V



Track 8, ID: R9VWS

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	29.3 AU	0.01 Rf	158.7 AU	27.21 %	0.02 Rf	42.0 AU	1628.4 AU	12.80 %
2	0.03 Rf	142.9 AU	0.05 Rf	215.5 AU	36.95 %	0.09 Rf	0.8 AU	5677.6 AU	44.62 %
3	0.10 Rf	1.6 AU	0.10 Rf	23.2 AU	3.97 %	0.12 Rf	0.2 AU	171.1 AU	1.34 %
4	0.15 Rf	5.2 AU	0.21 Rf	57.4 AU	9.84 %	0.27 Rf	0.1 AU	1912.5 AU	15.03 %
5	0.55 Rf	0.1 AU	0.60 Rf	36.4 AU	6.24 %	0.63 Rf	19.1 AU	869.5 AU	6.83 %
6	0.63 Rf	19.5 AU	0.66 Rf	92.0 AU	15.78 %	0.73 Rf	2.2 AU	2466.0 AU	19.38 %

Fig 6b. R9VWS

Figure 7: 3-D Chromatogram

Fig 7a. At 254nm

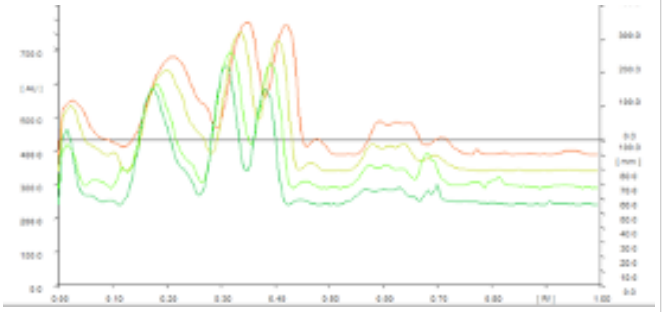


Fig 7b. At 366nm

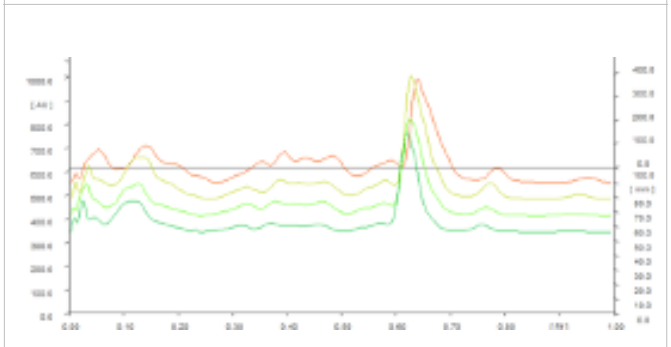
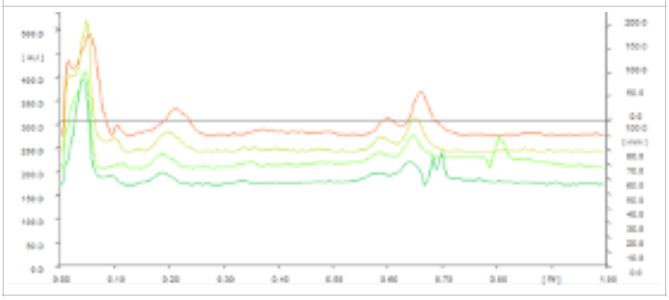


Fig 7c. At 620nm



CFU - Colony Forming Units *TNTC-Too Numerous to Count

Table 4: Microbial load-Direct method of sample R9v

Sl. No.	Dilutions	Number of Colonies		CFU/ml
1	Direct	>300	>300	TNTC*

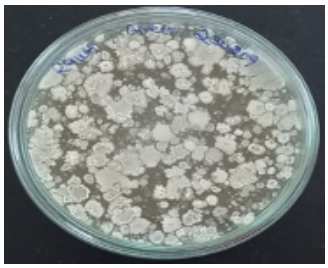
Table 5: Microbial load - Direct method of sample R9vws

Sl. No.	Dilutions	Number of Colonies		CFU/ml
1	Direct	>300	>300	TNTC*

CFU- Colony Forming Units *TNTC-Too Numerous to Count

Conclusion: The Sample R9VWS was contaminated with microbial colonies

Conclusion: The Sample R9V was contaminated with microbial colonies



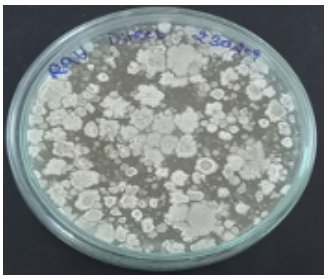


Table 6: Results of Test for specific pathogen

		Organisms				
Sl.	Drug	*E.*	*S.*	*P.*	*Salmonella*	*Enterobacteria*
1	R9v	Absen	Absent	Absent	Absent	Absent
2	R9ws	Absen	Absent	Absent	Absent	Absent

Discussion

The color of both R9 vati samples is brown. They also have a characteristic odor and a taste that is described as spicy, sweet, and bitter. Loss on drying is more in R9 *vati* prepared without *rasasindura* compare to R9 *vati* prepared with *rasasindura* and may be due to more inorganic matter. There is a slight increase in the value of total ash in R9 *vati* prepared with *rasasindura* which may be due addition of *rasasindura*.

Both samples of *vati* have zero acid in-soluble ash. *Vati* with *rasasindhura* has slightly more water-soluble ash compare to *vati* prepared with only herbal drugs. Solubility in alcohol is more in vati prepared with *rasasindhura* compare to *vati* prepared with only herbal drugs. Solubility increases with addition of *rasasindura* in formulation.

There is no much difference in Tablet weight variation and Tablet average weight in both samples of *vati*. Hardness of *vati* sample prepared with *rasasindhura* is more compared to vati prepared with only herbal drugs. *Rasasindhura* helped to increase the hardness of tablet which will beneficial when tablet will be prepared with machine.

There is no difference in the values of Disintegration time and pH both samples of *vati*. Sample of *vati* prepared with *rasasindhura* has more particle size compared with sample prepared with only herbal drugs.

HPTLC result shows 8 peaks in both samples of *vati* at Short UV, 6 peaks -in both samples of *vati* at Long UV, and 4 peaks in both samples of *vati* at Post derivatization. There is no difference in number of peaks of both samples. HPTLC is usually used for herbal drugs, so mineral drugs are not detected.

Microbial contamination report shows contamination of both samples which is may be due to manual handling of samples. But result of Microbial analysis shows absence of *Escherichia coli, Staphylococcus aureus, Salmonella sp., Enterobacteriaceae* and *Pseudomonas aeruginosa* in both the samples of vati.

Conclusion

In organoleptic characters there is no change in the both samples. But in standardization parameters it is observed that increase in the value of Total ash, Alcohol soluble extractive, Water soluble extractive, Tablet average weight, Hardness, and decrease in the value of —Loss on drying, Water-soluble ash in R9 *vati* prepared with *rasasindura*. HPTLC result shows same number of peaks in both samples of *vati*. Result of microbial contamination shows contamination of both samples but test for specific pathogen shows absence of Escherichia coli, Staphylococcus aureus, Salmonella sp., Enterobacteriaceae and Pseudomonas aeruginosa in both the samples of vati. These analytical parameters can be considered as preliminary values of standardization.

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