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PHARMACOGNOSTIC AND ANALYTICAL PARAMETER EVALUATION OF ROSE WATER

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ABSTRACT

Analysis means the comprehensive assessment, which uncovers the minor yet significant angles concerning the medication. The analytical study of a subject gives a few norms to pass judgment on its quality. It is helpful to choose future work plans and target boundaries to know the precise medication status by directing the near comparative study of different samples during drug formulation. The main aim of the analysis is to check the quality for obtaining desired therapeutic effect. So, it is necessary to control batch-to-batch variation, which is possible only through standardization protocols. The present analytical study has been accomplished to analyze the quality parameters like various organoleptic parameters viz. description, odourphysicochemical parameter viz. clarity, pH, specific gravity, viscosity, total volatile content, HPTLC, heavy metals and microbial limit tests viz. total bacterial as well as total fungal count are described in detail.

Keywords: Analytical study, standardization, volatile content, HPTLC, microbial limit tests.

INTRODUCTION

For centuries India has had a rich legacy of the traditional medicinal system and *Ayurveda* is a fortunate thing to give such a cultured heritage. One such formulation is *Arkakalpana*. As per

Ravana'sArkaPrakash, *ArkaKalpana* is given explicit significance, and it is believed to have more strength than the other *Kalpana*. The strength is due to its *Dosharahithatva* and, in particular, *Gunas*. It has been presented in the pharmacy of *Ayurveda* in the later piece of the *Samhita* period. This is unmistakable in its method of readiness and identity in remedial impact. It has all the volatile active components of the drug with which it is made. For the present study, a critical evaluation of the preparation of Gulab Arka by following the Standard Operating Procedure (S.O.P) will be done by considering suitable physico-chemical parameters which may add considerable input to the existing knowledge.

MATERIAL AND METHODS

PHARMACOGNOSTICAL STUDY

Fresh *Rose* flowers (*Gulab*)were procured from the local market of Haridwar and a pharmacognostical study was carried out at P. G. Department of Dravya Guna, Uttarakhand Ayurved University, Rishikul Campus, Haridwar.

The present study has been planned in the following way:

1. **Macroscopic-** Fresh rose flower has been taken for the study.
2. **Microscopic-** Fresh rose petals and dried powder of rose petals have been taken for the study.

1. Macroscopic-The petals were subjected to macroscopic study, which comprises organoleptic characteristics such as colour, odour, taste, texture, shape, and size of the petals.

Table 01: Macromorphological observation of Damask Rose petals[1]

S. no.	Features	Observation
Organoleptic Characteristics of Fresh petals		
1.	**Colour**	Magenta on base and light yellow near the apex
2.	**Odour**	Pleasant like rose
3.	**Taste**	tongue sensitizing aromatic taste with pleasant mild sweetness
Micromorphology of Fresh petals		
1.	**Width**	1.7-1.8cm
2.	**Length**	2.1-2.4cm
3.	**Sepal**	05 in no.
the appearance of Fresh petals		
1.	**Shape**	Heart/ Pear Shape
2.	**Texture**	Soft and smooth

2. Microscopic

a. Fresh Damask Rose petal[2]

Cross-sectioning of petals was cut by freehand for microscopic sections, temporary mounts of the sections of the petals were stained with saffranine, mounted with glycerin water, and observed under a microscope as mentioned in Figure 1.

Powder microscopy of Damask Rose[3]

Fine powder of petals was poured on the clean glass slide, and it was stained with Phloroglucinol-HCl, and Sodium hypochlorite solution mounted with glycerin water. The slide was then placed and observed under the magnifying lenses of 10x, 40x, and 100x magnification in the microscope.

Observation[4]

The acicular cluster of calcium oxalate, lignified fibres, pollen grains, and oil glands were seen as mentioned in Figure 2.

Figure 1: Microscopy of Damask Rose Petal

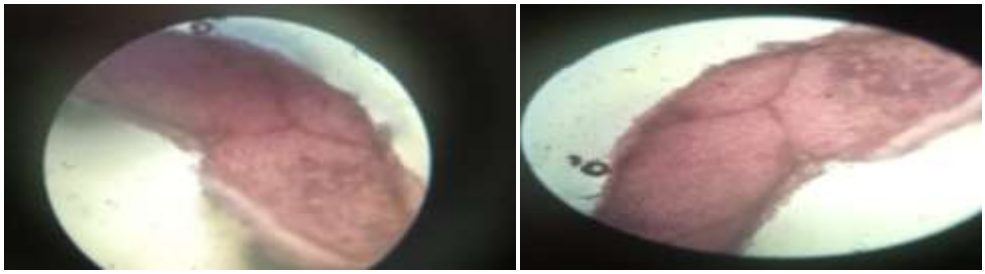
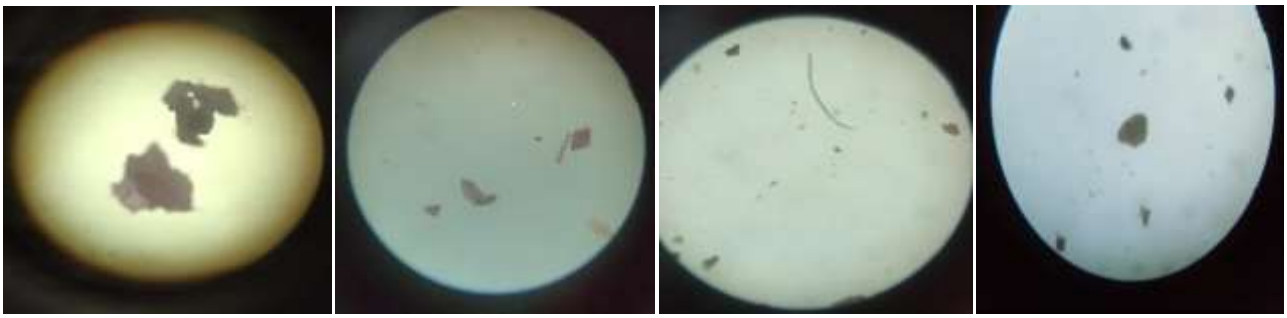


Figure 2: Powder Microscopy of Damask Rose



Acicular cluster of calcium oxalate | **Pollen grains** | **Lignified fibers** | **Oil gland**

ANALYTICAL STUDY

The present study was performed at Vasu Research Centre A2/624-625/2, G.I.D.C., Makarpura, Vadodara (Gujarat)

Aim & Objectives

Aim

- To analyze the *Gulab Arka* by various parameters.

Objectives

- To analyze organoleptic parameters.
- To analyze physicochemical parameters.
- To analyze heavy metals.
- To analyze microbial limits.
- Quantification of HPTLC.

Methodology

1. Organoleptic characters

Organoleptic characters of the samples are obtained by using sense organs, which are beneficial parameters to determine and compare the quality of samples. Here the parameters like Description, colour, odour, etc., are considered.

2. Physicochemical tests

A) Clarity Test[5]

It is important to note that solution clarity is equally dependent on the cleanliness of the intended container and closure. Both container and its closure must be sterile. This means that the container or closure must not contribute 25 particles to the solution during prolonged

contact, such as shelf-life storage. The clarity test involves the visual assessment of formulation in suitable lighting on white and black backgrounds. It is performed for liquid forms, except for suspensions.[6]

B) pH[7]

A number expresses the acidity or alkalinity of a solution on a logarithmic range, lower values indicate a more acidic nature, higher values indicate more alkalinity, and seven indicates neutral. The pH is equalled to $-\log_{10} c$, where c is Hydrogen ion concentration in moles per litre. The pH of a given solution was measured by using a digital pH meter.The first pH meter was standardized. Tablets of different pH were taken, and one tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH 4,7, and 9 (buffer solutions).The buffer solution was gotten held off in the beaker, and the electrode was dipped in it. A similar procedure was repeated for the other buffer solutions after washing the electrode thoroughly with distilled water.The sample was taken (10% aqueous solution), the electrode was dipped in it, and the value of pH was noted.

C) Specific gravity[8]

Specific gravity is termed as the weight of a given volume of liquid at a specific temperature as compared to the same volume of water at the same temperature. The pycnometer was carefully washed and cleaned. Its interior side was dried by hot air oven/dry air.The weight of the Pycnometer or Empty R D Bottle was noted (W1).The pycnometer was filled up with distilled water, and weight was noted (W2).It was dried again, and moisture was evacuated from it.Now it was filled with samples, and weight was recorded. (W3).

Calculation:

$$\boldsymbol{SpecificGravity = \frac{weightofliquidsample\left(\frac{g}{ml}\right)}{ml\ volumeofliquidsample\left(1.0\frac{g}{ml}\right)}}$$

D) Viscosity[9]

Viscosity is a quality of a liquid, which is directly related to the resistance to flow.The liquid under test was filled in a U-tube viscometer following the expected viscosity of the liquid so that the fluid level stood within 0.2 mm of the filling mark of the viscometer when the capillary was vertical test liquid was attained by the specified temperature.The liquid was sucked or blown to the specified weight of the viscometer, and the time taken for the meniscus to pass the two specified marks was measured.Absolute viscosity can be calculated directly if the exact dimensions of the measuring instruments were well-known, but it was more common practice to calibrate the instrument with a liquid of documented viscosity and to determine the viscosity of the unidentified fluid by comparison with that of the known.

E) Total volatile content[10]

The determination of volatile oil from the sample was made by distilling the sample with a mixture of water and collecting the distillate in a graduate tube. The aqueous portion of the distilling flask and measuring the volume of the oil. The content of the volatile oil is indicated as a percentage v/w. 250ml of the sample was taken into the distilling flask, which is then connected to the still head.Before attaching the condenser, water is run into the graduate receiver, keeping the tap open until the water flow from its mouth. Any air bubble in the first rubber tabbing is carefully removed by passing the tube.The tap is then closed, and the condenser is attached. The flask contents flask was heated and mixed by frequent agitation until boiling commences. The distillation is sustained at a rate, which keeps the lower end of the condenser cool.At the end of the specified time (4 hrs.), heating is discontinued, the apparatus is allowed to cool for 10 min, and the tap is opened and the second tube lowered slowly; as soon as the layer of the oils completely enters into the graduate part of the receiver the tap is closed, and volume is read.The tap is slowly opened to return the oil to the bulb. The distillation is again continued for another hour, and the volumeof oil is again read after cooling the apparatus as before. Distillation is again continued until successive readings of the volatile oil do not differ. The measured yield of volatile oil is taken.

3. **Heavy MetalAnalysis (Atomic Absorption Spectrophotometry- AAS)**[11]

Heavy metals are commonly occurring substances that are often present in the environment at low levels. Atomic absorption spectrophotometry is used for the conformation of heavy metal elements and some nonmetal elements in the atomic state.The light of characteristic wavelength emitted from a cathode discharge lamp is absorbed when it passes through the atomic vapour generated from the sample containing the element being examined atomized to the ground state.The assay of the component being examined is tested by determining the decreased degree of light intensity of radiation. Atomic absorption obeys the specific rule for absorption spectrophotometry.The assay is carried out by comparing the absorbance of the test preparation with that of the reference preparation.

4. **Microbiological Analysis**[12]

A) Plate count for bacteria: A mixture of 15 ml liquified *casinesoyabean* digest agar and a 1 ml mixture of pre-treated preparation were added to a petri dish having a diameter of 10 cm.Alternatively, the pre-treated preparation was spread on the surface of the solidified medium in a Petri dish of the same diameter.Two such Petri dishes using the same dilution were prepared and incubated at 350ºc for five days until a more reliable count is obtained in a shorter time.The results were calculated using plates with the highest number of colonies but taking 300 colonies per plate as the maximum consistent with proper evaluation.

B) Plate count for fungi: The same procedure as for bacterial counts was followed, but here *Sabouraud dextrose* agar *with antibiotics* was used instead of casein soya bean digest agar.The plates were incubated at 250ºC for five days unless a more reliable count is obtained in a shorter time.The results were calculated using plates with not more than 100 colonies.

5. **Test for Specific Pathogens**[13]

A) *E. coli:* 1 ml of the solution was taken out from the pipette (SCDB) and transferred into 5 ml of McConkey broth (MCB) medium containing Duram's tube. The tubes were then incubated at 37ºC for 18-20 hours. They were checked for the presence of acid and gas production. This shows the preliminary presence of E. coli.

B) *Salmonella:* 1 ml of the solution was taken out from pipetted (SCDB) and transferred into a 5 ml Tetrathionate broth medium. Then the tubes were incubated at 37º C for 18-20 hours. They were then checked for a colour change.

C) *Staphylococcus aureus:*0.1 ml of the solution was taken out from the pipette (SCDB) and streaked onto Vogel-Johnson Agar Medium to check for the presence of Staphylococcus aureus. The plates were incubated at 37º C for 18-20 hours and then observed for typical black colonies that were surrounded by yellow zones.

D) *Pseudomonas aeruginosa:* 0.1 ml of the solution was taken out from the pipette (SCDB) and streaked onto Centrimide agar plates to check for the presence of Pseudomonas aeruginosa. The plates were incubated at 37º C for 18-20 hours and then observed for colonies that show fluorescence when observed under UV.

6. High-performance thin-layer chromatography (HPTLC)[14]

HPTLC is a very useful qualitative analysis method; it combines the art of chromatography with quickness at a moderate cost. It was a major advancement of the thin layer chromatography principle with short time duration and better resolution. It is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid.The adsorbent was a relatively thin, uniform layer of dry fine powdered Silica Gel applied to the glass plate.Separation possibly will be achieved based on the partition or a combination of partition and adsorption, depending on the particular kind of support, it was prepared and used with a different solvent.Recognition can be exaggerated by observing spots of identical R_f Value and about equal magnitude obtained correspondingly, with an unknown and a reference sample chromatographed on the same plate.A visual association of the size and intensity of

the spots generally serves for semi-quantitative esti- mation.

OBSERVATION AND RESULTS

TABLE 02: Organoleptic results of *Gulab Arka*sample

S. No.	Parameters	Sample
1.	Description	Transparent liquid
2.	Colour	Transparent
3.	Odour	Pleasant like rose

TABLE 03: Physicochemical results of *Gulab Arka*sample

S. No.	Parameter	Sample
1.	pH	4.20
2.	Specific gravity at 25°C	0.999
3.	Viscosity by Ostwald (cP)	0.760
4.	Volatile oil content	0.01

TABLE 04: Heavy metals content of *Gulab Arka* samples

S. No.	Parameters (ppm)	Sample	Permissible limit
1.	Lead	ND	10
2.	Cadmium	ND	0.3
3.	Mercury	0.168	1.0
4.	Arsenic	0.253	3.0

TABLE 05: Microbial Limit of different *Gulab Arka*samples

S. No.	Parameters (cfu/mL)	Sample	Possible limit
1.	Total bacterial count	52	10^7
2.	Total fungal count	Absent	10^5

TABLE 06: Test for the specific pathogen of different *Gulab Arka*samples

S.No.	**Parameters**	**Sample**	**Possible limit**
1.	*E. coli*	Absent	-
2.	*Salmonella sp.*	Absent	Absent
3.	*S. aureus*	Absent	-
4.	*P. aeruginosa*	Absent	10

TABLE 07: HPTLC of different *Gulab Arka*samples

S. No.	**Wavelength**	**R_f Value**	**No. of spot**
1.	**254nm**	0.34, 0.81, 0.88	03
2.	**366nm**	0.71, 0.88	02
3.	**540nm**	0.20, 0.39, 0.47, 0.62, 0.71, 0.88	06

DISCUSSION

On the basis of macro-morphological features, the petals were soft and smooth, aromatic odour with a magenta colour on the base and light yellow color-near to apex. The taste of the petal was tongue-sensitizing aromatic with pleasant mild sweetness. Petals were heart and pear-shaped 1.7-1.8cm in length and 2.1-2.4cm in width. Sepal was found 5 in no. in each rose flower. So, the features were found characteristic of each rose flower. On the basis of powder microscopy, fine powder of dry rose petals was identified under the magnifying lenses of the microscope; the acicular cluster of calcium oxalate, lignified fibres, pollen grains, and oil glands were seen. So, the macro-morphological and microscopic features identified that this variety of rose as damask rose. These authenticated and identified damask rose petals were selected for the study. On the basis of organoleptic parameters,thecolour of the samples was transparent, and the odour was pleasant like a rose as mentioned in table no. 2.

On the basis ofPhysico Chemical Parameters Samples were clear at 0 months, indicating a good sterile container or solution and pH suggested the acidic nature of the Sample. Specific gravity was measured with the help of the Spengel Ostwald Pycnometer. the value lies between the standard value of specific gravity of *Gulab Arka* samples mentioned in API. It means it complies with the standard. Viscosity can be determined by the Ostwald viscometer to an extent. values suggest that it is less viscous, which means the rate of absorption is very high. Hence, absorption is best. Because viscosity is inversely proportional to the rate of absorption.[15] The volatile content of the prepared sample of *GulabArka*was evaluated with the help of the Soxhlet apparatus. The results of total volatile content were the same in all the samples, i.e., 0.01%. Test for heavy metals for *Gulab Arka* determined that Lead was not detected in the sample; Mercury was detected in the sample as 0.168ppm. Arsenic was detected in sample I as 0.253ppm. All the above-stated values were found within permissible limits suggesting *Gulab Arka* is safe for therapeutic use. On the evaluation of *Gulab Arka*, the total bacterial count was found as 52cfu/mL and the total fungal count was found absent. This implies that all the sample was within the permissible limit, ensuring the safety of *Gulabarka* for therapeutic use. HPTLC using Toluene: Ethyl acetate (9:1 v/v) at wavelength 254 nm, 366nm, 540nm shows 3 (0.34, 0.81, 0.88), 2 (0.71, 0.88), 6 (0.20, 0.39, 0.47, 0.62, 0.71, 0.88) peaks respectively. A wavelength 254 nm, 366nm, 540nm R_f value 0.88 found identical major area peaks. With a wavelength of 366nm and 540nm, two identical major area peaks corresponding to R_f values 0.71 and 0.88 were found. It is not feasible to cite to which component these R_f values correspond.The chromatogram shows 03, 02, and 06 spots at wavelengths 254 nm, 366nm, and 540nm, respectively. So, the higher susceptibility was observed at wavelength540nm because of the maximum concentration of separated materials. The wavelength,540nm found the maximum concentration of separated materials.

CONCLUSION

Ayurveda is a principle among the traditional health practice in the world hence traditional inspired practical approach should be made in preparing prime quality preparations.Macro-morphologically the species of Rose was identified as Damask Rose. It is the verity of roses extensively utilized worldwide for aroma creation. The Physico-chemical parameters were assessed at zero months and this data revealed that the sample was stable. Microbial count and heavy metal results were within the permissible limit, ensuring the safety of *Gulabarka* for therapeutic use.

REFERENCES

1. Fathima, S. N., & Murthy, S. V. (2019). Pharmacognostic study of petals Rosa damascena. *Asian J Pharm Pharmacol*, *5*, 779-85.
2. Harinarayan SC, Pathak AK, Mukul T. 2011. Characterization of Some Herbal Antidiabetic Drugs in Polyherbal Formulation by Microscopy. Research Journal of Pharmacy and Technology, 4 (1):131-145.
3. Zhongzhen Z. 2010. Application of microscopic techniques for the authentication of herbal medicines. Microscopy: Science, Technology, Applications and Education, 803-812 www.

4. Fathima, S. N., & Murthy, S. V. (2019). Pharmacognostic study of petals Rosa damascena. *Asian J Pharm Pharmacol*, *5*, 779-85.
5. Michael, R. Richards, E. 2009, 'Ophthalmic Products', in Winfield. A. J. Rees, J. A. Smith, I (ed.). Pharmaceutical Practice, 4th edition, Churchill-Livingstone, Edinburgh, pp 429–446.
6. Nanjwade, B. K., Sonaje, D. B., &Manvi, F. V. (2011). Preparation and evaluation of eye-drops for the treatment of Bacterial Conjunctivitis. *IJPI's Journal of Pharmaceutics and Cosmetology*, *1*(2), 43-49.
7. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 3.23-page no. 191.
8. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 3.2-page no. 190
9. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 2.2.13 page no. 144
10. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 2.2.11 page no. 15-16
11. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 2.3.3-page no. 256-259
12. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 2.4.1-page no. 45-46.
13. API, Government of India Ministry of Health and Family Welfare, Departement of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homoeopathy, 2007, Volume 1, part 2, Appendix 2.4.2-page no. 277.
14. Nurok, David (1989). "Strategies for optimizing the mobile phase in planar chromatography". Chemical Reviews. 89 (2): 363–375.
15. Sharma A. (2020). MD dissertation; *"*A Comparative Pharmaceutico - Analytical Study ofDadrughnLepaand Its Cream W.S.R. To Antifungal Activity inDermatophytosis*"* (U.A.U. Rishikul campus, Haridwar).

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