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# PHYSICO-CHEMICAL AND HPTLC STUDIES OF *ROSA DAMASCENA* MILL

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## ABSTRACT

Herbal medicines have become more popular in the treatment of many diseases due to its availability and less side effects. It is known for various pharmacological activities, and the presence of colored pigments and chemical constituents like flavonoids. It is also valued for their culinary, medicinal, cosmetic and aromatic properties. The physico- chemical studies like foreign matter, loss on drying, ash & extractive values, solubility at room temp, sugar estimation etc. were carried out. The findings of the study reveals that the plant contains negligible amount of silicates; shown by the acid insoluble ash and contains considerable amount of inorganic materials; shown by the higher ash value. The findings also reveal that the plant contains mainly polar compounds soluble in alcohol and water. WHO parameters i.e. heavy metals, pesticide residue, microbial load and aflatoxins were also carried out to determine the safety and toxicity of *R. damascena.*

**KEYWORDS:** *R. damascene.*

## INTRODUCTION

Various medicinal properties have been attributed to natural herbs which constitute the main source of new pharmaceuticals and healthcare products. *Rosa damascena* Mill is commonly known as “Gulab” or “Gul-e-surkh”. It is the hybrid between *R. gallica* and *R. Phoenicia* and is one of the most important species of the Rosaceae family. It is referred to as the king of flowers and is a well- known ornamental plant[1,2] used in parks, gardens and houses. Apart from these uses, rose plants are principally cultivated for its use in perfume, medicine and

food industry.[3] Its major products are rose water and essential oil. This plant contains several phyto-constituents such as terpenes, glycosides, flavonoids, and anthocyanins that have beneficial effects on human health. Literature review shows that Citronellol, geraniol, nerol, phenyl ethyl alcohol, nonadecane, nonadecene, eicosane, heneicosane, tricosane, α-guaiene, geranyl acetate and eugenol are the major chemical constituents reported of *R. damascena*. The medicinal properties of plant are attributed to the abundance of phenolics compound. Phenolics possess a wide range of pharmacological activities, such as antioxidants, free-radical scavengers, anticancer, anti-inflammatory, antimutagenic, and antidepressant. Traditionally, Rose water was used in treatment of different ailments viz. as antiseptic agent for eye washing[4] and mouth disinfecting[5] and as antispasmodic agent for alleviating the abdominal pains, and bronchial and chest congestions. The decoction of dried rose water was used as diuretic and was recommended for reliving the fever, breast pain and menstrual problems. The leaves, stem, and flowers of *R. damascena* have bacteriocidal effects on pathogenic micro-organisms. The tea prepared by brewing of leaves and petals are known to lessen fever and common cold, also acts as a diuretic and uses to remove the toxins from the body. It is also known to relieve chest and bronchial congestion. Due to the nutritional value of rose hip, it is added in cooking for the enhancement of nutrition, and also for beautiful colour and flavour. Oil extracted from rose is used in various skin problems, and also helps to moisturise the skin, makes it smooth and relieve skin irritation.[6-7]

In Unani medicine the flowers of R. damascena are used as chief ingredient in compound formulations; Gulqand Gulab, Majun Dabeedulvard and Sherbat Vard Mukarrer. However, many other Unani formulations, *viz*. Jawarish Tabasheer, Jawarish Tamer Hindi, Anooshdaru Sada and Lului, Arq Sheer Morakkab, Dawaul Misk Motadil Sada, Itrifal Zamani, Majun Sangdana Murgh, Majun Ushba, Qurs Kafoor, Qurs Sartan, Qurs Tabasheer, Safoof Aslussoos, Sherbat Deenar, Mufarreh Aazam, Mufarreh Barid, Jawarish Anaren, Jawarish Mastagi, and locally in Marham Muqil have R. damascena petals as one of the constituent.[8]

The present study was aimed to evaluate the physico-chemical and phytochemical standards of the flower of *Rosa damascena* using standard protocol. This study will help not only in examine adulterants present in raw material as well as for it identification.

| | |
|---|---|
|  | Kingdom : Plantae<br>Division : Magnoliophyta<br>Class : Magnoliolpsida<br>Order : Rosales<br>Family : Rosaceae<br>Genus : Rosa<br>Species : indica |

## MATERIALS AND METHODS

### Plant Materials and Chemicals

The plant material, dried petals of *R. damascena* were procured from Asia Trading Company, Khari Baoli, Delhi. After the identification and authentication of plant material by Dr H.B.Singh, Head, Deptt. of Raw Materials Herbarium & Museum, a voucher specimen (code: R070107) is deposited at National Institute of Science, Communication & Information Resources (NISCAIR), New Delhi. All chemicals (AR grade) were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

### Sample Preparation for Physico-chemical Analysis

The plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature 30+ 20C and relative humidity 50 + 5%) and powdered in an electric grinder. Dried and powdered flower petals of *R. damascena* were exhaustively extracted with a variety of solvents ranging from non-polar to polar ones, i.e. petroleum ether (PE), methanol (ME) and water (AE) using Soxhlet's Apparatus for 8 hours with each of the solvent separately to obtain different extract of the drug.[9]

### Sample Preparation for HPTLC

Conventional extraction of flowers of *R. damascena* was performed at room temperature ($28^{o}$ ± $3^{o}$C) with both non-polar to polar solvents i.e. Chloroform and Ethanol. Dried and powdered parts of *R. damascena* (10 g each) were extracted three times (3 × 50 mL) for 18 h of each extraction with each of the above-mentioned solvents separately. Each extract was filtered by using Whatman filter paper no. 1 and the solvents were removed under vacuum at 50°C, separately and concentrated up to 10mL to get the sample solution of 100 mg mL-1.5µL of each sample was applied separately to TLC plate for the development of fingerprints.

**HPTLC-UV Detection Method**

High Performance Thin Layer Chromatography was performed on 10 cm × 5 cm TLC plates pre-coated with 0.25 µm thin layers of silica gel 60 F254 (E. Merck). Chloroform extracts was applied on the plates as bands 10 mm wide by employing DESAGA AS30 Automatic sample applicator. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate*: *9: 1 (v/v)* and as mobile phase was performed in a twin-trough glass chamber (20 cm × 10 cm) previously saturated with vapours of mobile phase for 20 min separately . The plate was dried in air and visualized under λ 254 nm and λ 366 nm for ultra violet detection and taken the fingerprints as evident in Figures 1 – 2. Further, the same TLC plate was derivatized with 1% *vanilline-sulphuric acid reagent* and visualized in white light obtained fingerprints were as evident in Figures 3. HPTLC of ethanol extract of was performed with same procedure with the mobile phases of *Toluene: Ethyl acetate 9: 1 (v/v)* and then visualized in λ 254 nm, λ 366 nm and white light as shown in Figure 4-6.[11]

**Physico-chemical Analysis**

Physico-chemical studies like total ash, acid insoluble ash, alcohol and water solubility and loss on drying at 105 ºC methods were carried out as per the WHO guidelines.[12] Preliminary phytochemical tests were done as per the standard methods.[11-12] The fluorescence behavior of the powdered drug in the daylight and ultraviolet light were carried out by moistening the powder in different solutions and viewing under the light of different wavelengths in a UV chamber.[13-15] Determination of total phenolic content, tannin estimation, heavy metal analysis, estimations of aflatoxin and pesticide residues were also carried out by standard procedure for experiments.

**Phytochemical Analysis**

The qualitative phytochemical analysis of different extracts was carried out to identify the organic compounds.[16]

**RESULT AND DISCUSSIONS**

**Morphological Characters**

It is an erect shrub, upto 2 m high, considered to be a native of Asia Minor and widely grown in gardens throughout India. Branches long, arching with large hooked prickles, leaves pinnate, leaflets 3-7, petioles prickly. The flowers are many in a corymb, double, red, pink or white on slender glandular-hispid and prickly pedicles, fruits ovoid or obovate, bristly, bright

red and pulpy. Flowers are very fragrant and sweet scented, contain large amount of volatile oil called the Oil and Otto of Rose.

**Physico-Chemical and Phyto-chemcial Analysis**

Analytical data shows 11.8% of moisture content. Ash content of 3.2% and 2.6% of acid in-soluble ash shows the siliceous matter in the plant. Alcohol soluble extractives represent the extraction of polar constituents like phenols, tannins, glycosides, alkaloids and flavonoids. The water soluble extractive denotes the presence of inorganic contents. It contains considerable amount of tannin viz. 5.4% and the plant can be utilized in leather tanning. The aqueous and methanolic extracts of petals of *R. damascena* were tested qualitatively to analyze the presence of secondary metabolites. The results of physico-chemical parameters and preliminary phyto-chemical analysis are shown in Table 1 & 2.[17]

**Microbial Load Analysis**

The microbial load and pathogens studies are shown in Table 3.

**Heavy Metal Analysis**

The medicinal plants materials are generally contaminated with arsenic and heavy metals due to environmental pollution. These components even in trace amounts are dangerous and can damage the important human organs such as kidney, liver and heart.[18] The amount of various heavy metals found in the plant material is given in Table 4. The heavy metal contents viz. lead, cadmium, mercury and arsenic as per WHO guidelines were found within the permissible limits viz. 10, 0.3, 1 and 3 ppm respectively. The plant is hence considered non-pollutant in the environment and it cannot cause any illness due to the heavy metals.

**Analysis of Aflatoxins**

The aflatoxin can be acute toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive to the human being if these are found in the plant above the prescribed limits. The various aflatoxins found in the plant material are given in Table 5. The aflatoxins B1, B2, G1 and G2 were found below the detecting limit and the toxic effect of the plant may be considered as nil and hence the use of the plant may be safe.

**Analysis of Pesticide Residues**

The various pesticidal residues α - HCH, β - HCH, γ - HCH, δ -HCH, op-DDT, pp-DDT, op-DDE, α- Endosulfan, β – Endosulfan, op-DDD and pp-DDD were tested in the drugs and

found nil, the results are shown in Table 6. The estimation of heavy metals and analysis of aflatoxins and pesticidal residues will be useful from the toxicity point of view and serve as safety standards.

**Fluorescence Analysis**

This technique utilized for the observation of fluorescence produced by a compound in ultraviolet light for qualitative evaluation of phytochemicals. The ultra light is very active in producing fluorescence in many substances which do not visibly fluoresce in day light.

Fluorescence analysis of drug powders and various extracts of the powder were studied and observations are shown in Tables 7 and 8.

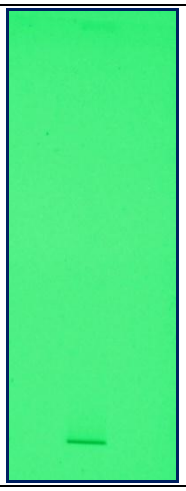
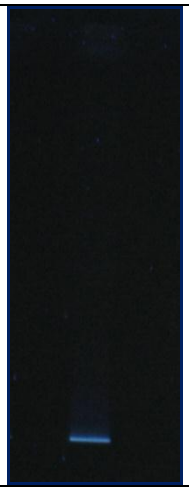
In Table 7, the fluorescence analysis of various extracts of *R. damascena* the absorbance of the petroleum ether at day light (transparent), 254nm (black) and 366nm (violet); Acetone at day light (Transparent), 254nm (Black) and 366nm (Violet); Ethyl Acetate at day light (Transparent), 254nm (Grey) and 366nm (Violet); chloroform at day light (Pinkish), 254nm (Blackish) and 366nm (Violet); Methanol at day light (Straw), 254nm (Blackish) and 366nm (Green); alcohol at day light (Transparent), 254nm (Blackish) and 366nm (Violet) and water at day light (Peach), 254nm (Black) and 366nm (Brown) are also characteristics of the plant by which the plant can be identified.

In Table 8, fluorescence analysis of powder of *R. damascena* the absorption of the powder at day light (pink), 254nm (black) and 366nm (purple); dil. HCl solution of the powder at day light (orange), 254nm (black) and 366m (rust); dil. $HNO_3$ solution of the powder at day light (light yellow), 254nm (grey) and 366m (green); dil. $H_2SO_4$ solution of the powder at day light (deep orange), 254nm (black) and 366m (brown); conc. HCl solution of the powder at day light (dark orange), 254nm (black) and 366m (black); conc. $HNO_3$ solution of the powder at day light (yellow), 254nm (brown) and 366m (green); conc. $H_2SO_4$ solution of the powder at day light (deep red, 254nm (black) and 366m (bluish green); iodine solution of the powder at day light (bright red), 254nm (black) and 366m (black); and glacial acetic acid solution of the powder at day light (pink), 254nm (black) and 366m (purple); are characteristics of the plant.

**HPTLC profile**

Chromatogram of *chloroform* extract shows no major spot under UV 254nm & 366nm. After derivatization, the chromatogram shows seven major spots at $R_f$ 0.13(purple), 0.17(pink), 0.23(pinkish purple), 0.26, 0.30(pink), 0.37 & 0.44(purple) under visible light.

Similarly chromatogram of *ethanol* extract shows no spot under UV254nm. Under UV366nm, one major spot is observed at $R_f$ 0.48 (dark blue). After derivatization, the chromatogram shows five major spots at $R_f$ 0.15(pinkish purple), 0.24(purple), 0.31(pinkish purple), 0.39(pink) & 0.44(purple) under visible light as evident in Table 9.

| **HPTLC of *Chloroform* extract** | | |
|---|---|---|
|  |  |  |
| UV 254nm | UV 366nm | Visible Light After derivatization |
| Figure:1 | Figure:2 | Figure:3 |
| Solvent system: *Toluene: Ethyl acetate*: *9: 1 (v/v)* | | |

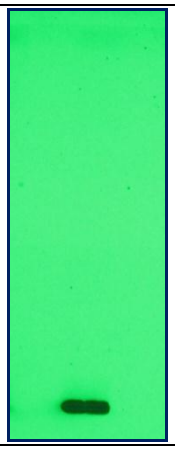
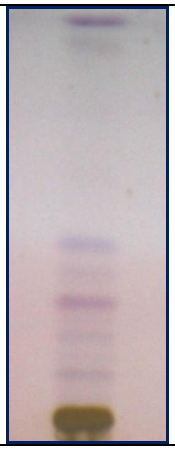
| **HPTLC of *Ethanol* extract** | | |
|---|---|---|
|  |  |  |
| UV 254nm | UV 366nm | Visible Light After derivatization |
| Figure:4 | Figure:5 | Figure:6 |
| Solvent system: *Toluene: Ethyl acetate*: *9: 1 (v/v)* | | |

**Table 1: Physico-chemical analysis of *Rosa Damascena* Mill. –Flower.**

| S. No. | Parameters | Results in % (n = 3) |
|---|---|---|
| 1. | **% Foreign matter** | Nil |
| 2. | **% Loss on drying at 105ºC** | 11.8 |
| 3. | **% Ash** | 3.2 |
| 4. | **% Water soluble ash** | 3.3 |
| 5. | **% Acid insoluble ash** | 2.6 |
| 6. | **% Sulphated Ash** | 6.3 |
| 7. | **% Extractive values:** | |
| | **A. Cold Extraction:** | |
| | a. Petroleum ether | 1.0 |
| | b. Chloroform | 1.7 |
| | c. Alcoholic | 27.3 |
| | d. Aqueous | 10.6 |
| | **B. Hot Extraction:** | |
| | a. Petroleum ether | 1.6 |
| | b. Chloroform | 8.4 |
| | c. Alcoholic | 32.5 |
| | d. Aqueous | 41.3 |
| | **C. Successive Extraction:** | |
| | a. Petroleum ether | 0.62 |
| | b. Chloroform | 0.67 |
| | c. Alcoholic | 19.8 |
| | d. Aqueous | 4.63 |
| 8. | **%Fixed oil** | 1.36 |
| 9. | **% Tannin** | 5.4 |
| 10. | **Total phenolics (%)** | 7.4 |
| 11. | ***PH* values (1% aqueous solution)** | 5.47 |

**Table 2: Preliminary Phytochemical Tests of *R. Damascena* Mill.**

| S.No | Qualitative tests | Phyto-chemical Analysis | Aqueous extract | Methanolic extract |
|---|---|---|---|---|
| 1. | Test for Saponins | • Foam Test | -ve | - |
| | | • Heamolysis test | -ve | - |
| 2. | Test for Flavanoids | • Shinoda test | +ve | +ve |
| 3. | Test for Tannins | • Lead acetate test | +ve | +ve |
| | | • Ferric chloride reagent | +ve | +ve |
| | | • Potassium dichromate test | +ve | +ve |
| 4. | Test for Protiens | • Biuret test | +ve | +ve |
| | | • Xanthoproteic test | -ve | -ve |
| 5. | Test for Alkaloids | • Mayer's reagent | -ve | -ve |
| | | • Wagners's reagent | -ve | -ve |
| | | • Dragendroff's reagent | -ve | -ve |

**Table 3: Analysis of Microbial load of the whole plant *R. Damascena* Mill.**

| S.N0. | Parameter Analyzed | Results | WHO Limit |
|---|---|---|---|
| 1 | Total Bacterial Count | 200 cfu/gm | $10^5$cfu/gm |
| 2 | Total Fungal Count | 100 cfu/gm | $10^3$cfu/gm |
| 3 | *Escherichia coli* | Absent | Absent |
| 4 | *Salmonella typhaiSpp.* | Absent | Absent |
| 5 | *Staphylococcus aurous* | Absent | Absent |

**Table 4: Heavy metal analysis of the whole plant *R. Damascena* Mill.**

| S. No | Name of the Elements | *R. Damascena* (ppm) | Permissible Limits (API, 2008) (ppm) |
|---|---|---|---|
| 1. | Lead | 0.0498 | 10 |
| 2. | Cadmium | 0.0170 | 0.3 |
| 3. | Mercury | Nil | 1 |
| 4. | Arsenic | 0.0030 | 3 |

ppm : parts per million.

**Table 5: Analysis of aflatoxins of the whole plant *R. Damascena* Mill.**

| Aflatoxins | Results |
|---|---|
| $B_1$ | BDL (DL: 1.0 ppb) |
| $B_2$ | BDL (DL 0.5 ppb) |
| $G_1$ | BDL (DL 1.0 ppb) |
| $G_2$ | BDL (DL 0.5 ppb) |

BDL: Below Detectable Limit DL: Detectable Limit ppb: Parts per billion

**Table 6: Analysis of pesticidal residues of the whole plant *R. Damascena* Mill.**

| Pesticides | ppm |
|---|---|
| α – HCH | ND |
| β - HCH | ND |
| γ – HCH | ND |
| δ –HCH | ND |
| *op*-DDT | ND |
| *pp*-DDT | ND |
| *op*-DDE | ND |
| α- Endosulfan | ND |
| β - Endosulfan | ND |
| *op*-DDD | ND |
| *pp*-DDD | ND |

Detection limit - 0.01 ppm; ppm : parts per million; ND: Not detectable

**Table 7: Fluorescent Analysis.**

| S.No | Solvent System used | Ordinary Light | UV light | |
|---|---|---|---|---|
| | | | (254 nm) | (366) |
| 1. | Petroleum Ether | Transparent | Black | Violet |
| 2. | Acetone | Transparent | Black | Violet |
| 3. | Ethyl Acetate | Transparent | Grey | Violet |
| 4. | Chloroform | Pinkish | Blackish | Violet |
| 5. | Methanol | Straw | Blackish | Green |
| 6. | Alcohol | Transparent | Blackish | Violet |
| 7. | Water | Peach | Black | Brown |

**Table 8: Fluorescent Analysis.**

| S. No. | Treatment | Day light | UV light | |
|---|---|---|---|---|
| | | | 254nm | 366nm |
| 1. | Powder | Pink | Black | Purple |
| 2. | Powder + Dil. HCL | Orange | Black | Rust |
| 3. | Powder + Dil. HNO3 | Light Yellow | Grey | Green |
| 4. | Powder + Dil. H2SO4 | Deep Orange | Black | Brown |
| 5. | Powder + Conc. HCL | Dark Orange | Black | Black |
| 6. | Powder + Conc. HNO3 | Yellow | Brown | Green |
| 7. | Powder + Conc. H2SO4 | Deep Red | Black | Bluish Green |
| 8. | Powder + Iodine Solution | Bright Red | Black | Black |
| 9. | Powder + Glacial Acetic Acid | Pink | Black | Purple |

**Table 9: Rf value of phyto-chemicals present in chloroform and ethanol extract of *R. Damascena* at different wave-lengths.**

| Wave-length(nm) | Chloroform extract | Ethanol extract |
|---|---|---|
| **254** | - | - |
| **366** | - | 0.48 (dark blue) |
| **Visible light after derivatization in vanillin-sulphuric acid reagent** | $R_f$ 0.13(purple), 0.17(pink), 0.23(pinkish purple), 0.26, 0.30(pink), 0.37 & 0.44(purple) | 0.15(pinkish purple), 0.24(purple), 0.31(pinkish purple), 0.39(pink) & 0.44(purple) |

## CONCLUSION

This study has provided a scientific evaluation of *R. Damascena* on physico-chemical standards. The study on physico-chemical parameters and results of analysis of heavy metals, microbial load, aflatoxins and pesticide residue provide a quality standard for further reference and helps to distinguish the original drug from the spurious ones. The TLC phytochemical fingerprint profiling of chloroform and ethanol extracts of flower petals of *R. Damascena* gives an idea about the presence of various phytochemicals in their reported part and provided valuable clue regarding its identification.

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