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Identification and Quantification of Phytochemical Constituents in Rosa Damascena Flower Extract Via GC-MS and HPTLC

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ABSTRACT

Rosa damascena, commonly known as Damask rose has garnered significant attention in the fields of horticulture, biochemistry, and pharmacology, primarily due to the captivating fragrance of its flowers and the abundance of biologically active compounds. Roses hold global economic significance, serving as the focal point of a substantial ornamental shrub in flower industry. It is a plant with a rich history of ethnobotanical use and its traditional applications across various cultures, highlighting its therapeutic properties. Historically, different parts of R.damascena have been employed to address a wide range of ailments, including digestive issues, skin conditions, perfumes, fragrances and emotional distress. The plant's diverse phytochemical composition, encompassing essential oils, flavonoids, and phenolic compounds, underpins its pharmacological action. The present research aims to underline the phytochemical screening of R. damascena using physical evaluation following qualitative and quantitative phytochemical analysis using GC-MS and HPTLC. Preliminary phytochemical analysis reveals the presence of glycosides, flavonoids, saponins, tannins, and phenolic compounds. Furthermore, various essential phytoconstituents, including citronellol, á-pinene, d-limonene, geraniol, and caryophyllene, were detected using GC-MS. Quantitative densitometry analysis using HPTLC revealed the presence of geraniol in concentrations of 3.54±0.23 w/w. Overall, these findings suggest that R.damascena consist diverse essential phytochemicals that holds significant potential for further exploration as a valuable medicinal resource, offering a foundation for future pharmacological and therapeutic studies.

Keywords: *Rosa damascena, Phytochemicals analysis, geraniol, HPTLC, GC-MS*

1. INTRODUCTION

In India, a multitude of plant species are recognized for their medicinal properties. Various parts of these plant species continue to be extensively utilized in traditional Ayurveda and Siddha medicine. More than 60-70% of the rural population continues to depend on traditional medicine as their primary source of healthcare. Recently, there has been a surge in scientific interest in medicinal plants, driven by the enhanced efficacy of plant-derived drugs and growing concerns about the side effects associated with modern medicine [1–3]. Research endeavors are ongoing to investigate the bioactive compounds, providing insights into their sources, seasonal availability, and the biological properties of the isolated compounds [4,5]. R.damascena a key species in the Rosaceae family, commonly known as Damask rose, is renowned for its perfuming properties and holds a special place in various cultures [6–8]. It is not only associated with perfumery but also carries profound symbolism, representing inspiration, purity, love, happiness, and beauty [6]. R.damascena is widely cultivated in countries like Turkey and Bulgaria for its use in perfumery, flavorings, and medicine [9], but also grown in regions such as Iran, India, China, North Africa, and Europe [10].

R.damascena has versatile applications, making it valuable in cosmetics, creams, hand lotions, and perfumes. Commercially, it yields products like rose oil, rose water, dried petals, and rose hips[11]. In addition to its aromatic qualities, R.damascena boasts an array of pharmacological properties, such as antioxidant, astringent, antibacterial, antimicrobial, anti-inflammatory, and analgesic effects. The plant is widely employed in treating conditions like erectile dysfunction, cardiovascular disorders,

and respiratory tract infections. It also aids in alleviating constipation and promoting intestinal movement. Furthermore, it exhibits anti-inflammatory, antibacterial, and anti-HIV activities [9,11,12]. Nevertheless, its significance in healthcare and

medicine dates back centuries, with notable scholars like Avicenna extracting essential oil from it in the 10th century for therapeutic purposes [13]. Traditional Iranian medicine utilized flower decoctions for various ailments, including pain, digestive issues, and menstrual problems, while dried flower decoctions were used for fever, menstrual relief, breast pain, and as a diuretic [13,14].

Chemically, R.damascena consist of various phytochemicals including kaempferol, cyanidin 3,5-O-diglucoside, quercetin, and gallic acid, citronellol, geraniol, rutin, and many more [7,15–18]. Interestingly, studies have revealed variations in the concentration of phenolic compounds across different R.damascena extracts. The total phenolic content is reported to be higher in both the essential and absolute oils compared to the hydrosol, commonly known as rose water. Furthermore, phenyl ethyl alcohol is the dominant compound in rose absolute. In contrast, citronellol and geraniol are identified as the primary volatile constituents of rose water. Notably, rose water exhibits higher concentrations of the valuable fragrance compounds geraniol and nerol when compared to rose oil [19–21].

Several accumulating evidence revealed the presence of numerous flavonoids, polyphenols, terpenoids, glycosides, and fatty acids in the essential oil of R.damascena, which are attributed to diverse pharmacological activities; however, the identification and quantification in the flower extract is limited. Thus, the present study focuses on the identification of bioactive compounds from the hydroalcoholic flower extract of R.damascena. Through a comprehensive analysis involving physical evaluation, and qualitative and quantitative phytochemical assessments, this research aims to provide a thorough characterization and standardization of the plant. Gas Chromatography-Mass Spectrometry (GC-MS) and densitometry analysis using high-pressure thin-layer chromatography (HPTLC) was also utilized to identify and quantify the key phytoconstituent within the plant material with an aim to pave the way for further exploration of its potential therapeutic applications.

2. 2. MATERIALS AND METHODS:

2.1. Collection of plant:

The flower petals of R. damascena were collected from the herbal garden of Maharshi Dayanand University, Rohtak, India. The collected leaves were washed thoroughly with distilled water to remove contaminants and air-dried in the shade at room temperature for three days until completely dry. After grinding into a fine powder using a mechanical grinder, the obtained powder (100g) was extracted using water and ethanol (70:30) as solvents following the maceration process and lyophilised. The lyophilized hydroalcoholic extract thus obtained was then stored in airtight containers at 4°C until further use.

2.2. Physical analysis of hydroalcoholic extract of R. damascena:

The physical evaluation of the dried R. damascena petals extract was done. This evaluation involved assessing its appearance, color, odor, solubility, pH, and the amount of moisture lost upon drying [22].

2.3. Qualitative and Quantitative preliminary phytochemical screening of hydroalcoholic extract of R.damascena:

The hydroalcoholic petals extract of R.damascena underwent qualitative chemical screening to identify the presence of different types of active compounds. This screening, performed according to established standard protocols aimed to detect alkaloids, glycosides, saponins, terpenoids, flavonoids, tannins, and phenolic compounds [22–24].

For quantitative analysis of phytochemicals, both total phenolic and total flavonoid content was determined. For estimation of total phenolic content, Folin-Ciocalteu assay was employed and determined using gallic acid as the standard, and results were expressed in terms of gallic acid equivalent (mg/g of dried sample) [25]. Similarly, total flavonoid content was determined using the aluminum chloride colorimetric assay, with rutin as the standard [26]. All the experiments were performed in triplicate and results were expressed in mean ± SEM.

2.4. GC-MS analysis of the hydroalcoholic extract of R.damascena:

Phytochemical analysis of R.damascena was conducted using a Thermo Trace 1300 GC system coupled with a Thermo TSQ 8000 Triple Quadrupole Mass Spectrometer and pure helium as the carrier gas at a flow rate of 1 mL/min with a split flow. Samples were injected at 250°C and separated using a BP-5MS capillary column (30m × 0.25 mm, 0.25µm). The mass spectrometer scanned a range of 35-650 m/z at 0.5s/scan, with an ion source temperature of 230°C. Compound identification was achieved by comparing retention indices and mass spectra to the NIST 2.0 and Wiley library databases [27].

2.5. HPTLC fingerprinting profile of hydroalcoholic extract of R.damascena:

2.5.1. Instrumentation: The High-Performance Thin Layer Chromatography (HPTLC) analysis was conducted using a CAMAG system. This system included a Linomat V Sample Spotter for applying samples onto the TLC plates, a 100 µl Hamilton Syringe for precise sample spotting, and a TLC chamber for developing the plates. Visualization and quantitative

analysis were performed using a Visualizer and a TLC Scanner 3, both connected to WinCATS software for densitometric evaluation.

2.5.2. Preparation of standard solutions of geraniol: 10 mg of geraniol marker compound was accurately weighed and dissolved in a volumetric flask filled with 10 ml of ethanol to obtain a stock solution of 1mg/ml, which further diluted 10X to obtained the working standard solution.

2.5.3. Preparation of sample solutions of plant extract: 5mg/ml solution of R.damascena extract was prepared using ethanol as solvent by accurately weighing 50 mg of plant extract and dissolving it in 10 ml of ethanol using a volumetric flask. The prepared solution was diluted ten times to obtain a concentration of 500µg/ml.

2.5.4. Conditions for HPTLC: The experiment was carried out using silica gel 60F254 HPTLC plates (20 x 10 cm) with a thickness of 0.2 millimeters, without any pre-washing. Samples were applied to the plates as 6mm bands, spaced 8mm apart, using a LINOMAT-5 applicator. The plates were developed in a stainless steel-topped glass chamber using an ascending method with a mobile phase composed of Toluene: Ethyl Acetate::9:1 (v/v). A 20-minute chamber saturation period was maintained before development. Following development, the plates were dried on a TLC hot plate, visualized under a UV cabinet, and then scanned using a CAMAG TLC scanner connected to Win CATS software (version 3.5) at wavelengths of 254 and 366 nm and further derivatized using anisaldehyde [28].

2.5.5. Preparation of calibration curve: To create calibration curves for quantification, the applied volumes (1, 2, 3, 4, and 5 µl) of the working standard solution onto pre-activated HPTLC plates using a CAMAG Linomat-5 Sample Spotter. This resulted in sample amounts of 100, 200, 300, 400, and 500 ng per spot. Densitometric analysis was then carried out as detailed previously. Finally, a calibration curve for each flavonoid was generated by plotting the measured peak areas against the corresponding applied standard concentrations (in nanograms per spot).

2.5.6. Quantitative estimation of Geraniol in the extract of R.damascena: 10 µl of 500µg/ml (5000ng/spot) of prepared extract solution was applied on pre-activated TLC plates using CAMAG Linomat-5. Peak regions and absorption spectra were captured, and the quantity of geraniol was determined using the corresponding linear standard curves.

3. 3. RESULTS AND DISCUSSION:

3.1. Physical evaluation of hydroalcoholic extract of R.damascena:

Results of the physical evaluation of R.damascena hydroalcoholic extract are listed in Table 1. The hydroalcoholic extract of R. damascena obtained was found to be a reddish brown, crystalline solid with a strong and pungent odour. It is soluble in alcohol and has a slightly acidic pH of 4.0 - 6.0. The loss on drying is determined to be 2.7 ± 0.3% w/w.

Table 1: Physical evaluation of hydroalcoholic extract of R.damascena

Physical Parameter	Inference
Appearance	Crystalline Solid
Color	Reddish-brown
Odor	Strong and pungent
Solubility	Alcohol
pH	4.0 - 6.0
Loss on Drying	2.7 ±0.3 % w/w

3.2. Qualitative and quantitative phytoconstituents analysis of hydroalcoholic extract of R.damascena:

Qualitative phytochemical analysis of the petals extract revealed a rich abundance of glycosides, saponins, terpenoids, flavonoids, and phenolic compounds (Table 2). However, cardiac glycosides were not detected using the Keller-Killiani test. Quantitative analysis further demonstrated a significant concentration of total phenols (11.25 ± 0.48 mg gallic acid equivalent/g dried extract) and total flavonoids (7.98 ± 1.75 mg rutin equivalent/g dried extract) in the R.damascena petals extract.

Table 2: Preliminary phytochemical analysis of the flower petals of *R. damascena.*

Phytochemical	Name of test to be performed	Inference
Alkaloid	Dragondroff ‘s test	Absent
	Mayer’test	Absent
	Wagner’test	Absent
Glycosides	Liebermann’s test	Present
Tannin and phenolic compounds	Ferric chloride test	Present
	Acetic acid test	Present
Saponin	Frothing test	Absent
Terpenoids	Salkowski ‘s test	Present
Flavonoids	Lead acetate test	Present
Cardiac glycoside	Keller-kelliani test	Absent

3.3. GC-MS analysis of hydroalcoholic extract of R.damascena:

GC-MS analysis revealed a variety of chemical compounds in the hydroalcoholic extract of R. damascena, as detailed in Table 3 and Figure 1. The most abundant compounds were aromatic terpenes, notably citronellol (7.11% at RT 3.45) and geraniol (6.41% at RT 9.84). Other noteworthy terpenes that were identified included caryophyllene (4.78% at RT 11.95), D-limonene (3.71% at RT 5.19), and α-Pinene (0.71% at RT 4.74). Nevertheless, the high abundance of terpenes in extract not only contributes to the characteristic rose aroma, but making the extract valuable for fragrance applications. Furthermore, these compounds are also recognized for their potential biological activities, such as antimicrobial and insect-repellent effects. Alcohols also formed a significant portion of the extract, with n-pentadecanol (4.92% at RT 8.66) and 2-hexadecanol (3.82% at RT 12.24) present in considerable amounts. Additionally, hydrocarbons like 3-hexadecene (6.64% at RT 9.30) and 10-heneicosene (3.95% at RT 9.91) were found in substantial quantities. These key compounds collectively shape the chemical fingerprint of the R. damascena hydroalcoholic extract

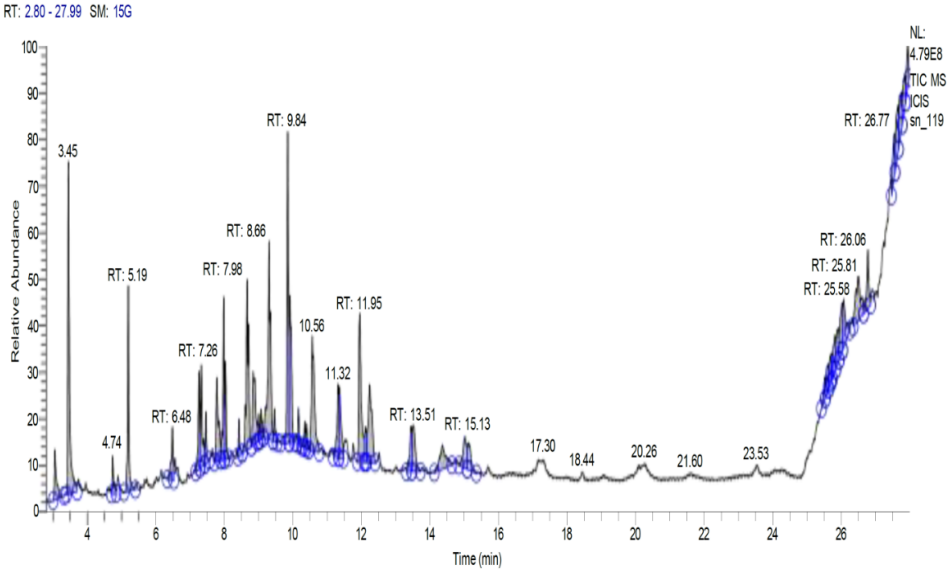

Figure 1: GC-MS spectrum of hydroalcoholic extract of *R.damascena*

Table 3: List of major phytochemicals identified in the hydroalcoholic extract of *R.damascena*

S. No.	RT	Peak Area (%)	Compound Identified
1.	3.06	1.44	2-Pentene, 2,4-dimethyl-
2.	3.45	7.11	Citronellol
3.	4.74	0.71	á-Pinene
4.	5.19	3.71	D-Limonene
5.	6.48	1.40	N-Ethyl-3-methyl-3-octanamine
6.	7.26	1.78	1-Undecanol
7.	7.33	2.39	6,10,14-Trimethyl-pentadecan-2-ol
8.	7.46	0.82	1-Decanol, 2-methyl-
9.	7.78	2.59	5-(2-Thienyl)pentanoic acid
10.	7.98	2.61	1-Hexadecanol
11.	8.03	1.46	Pentadecane
12.	8.43	0.57	Cycloheptasiloxane, tetradecamethyl-
13.	8.66	4.92	n-Pentadecanol
14.	8.84	2.96	3-Eicosene, (E)-
15.	9.01	0.46	1-Hexadecanol, 2-methyl-
16.	9.07	0.61	Dodecanoic acid, 3-hydroxy-
17.	9.30	6.64	3-Hexadecene, (Z)-
18.	9.46	0.61	Cyclooctasiloxane, hexadecamethyl-
19.	9.84	6.41	Geraniol
20.	9.91	3.95	10-Heneicosene (c,t)
21.	10.16	0.68	1-Heneicosanol
22.	10.34	0.40	1-Dodecanol, 2-octyl-
23.	10.40	0.43	5-Octadecenal
24.	10.56	4.59	9-Eicosene, (E)-
25.	11.32	1.74	n-Nonadecanol-1
26.	11.95	4.78	Caryophyllene
27.	12.12	0.75	n-Hexadecanoic acid
28.	12.24	3.82	2-Hexadecanol
29.	13.44	1.27	9-Hexacosene
30.	13.51	1.95	Tetradecane, 2,6,10-trimethyl-
31.	14.35	1.87	trans-13-Octadecenoic acid
32.	15.02	1.48	n-Tetracosanol-1
33.	15.13	1.24	1-Dodecanol, 2-octyl-

34.	25.51	0.57	17-Pentatriacontene
35.	25.58	0.95	Nonahexacontanoic acid
36.	25.66	0.65	Tetrapentacontane, 1,54-dibromo-
37.	25.71	0.38	Octatriacontyl pentafluoropropionate
38.	25.81	1.19	Oleic acid, 3-(octadecyloxy)propyl ester
39.	25.87	1.20	Oleic acid, eicosyl ester
40.	26.02	1.61	17-Pentatriacontene 26.02
41.	26.06	1.82	Oleic acid, eicosyl ester
42.	26.49	2.84	9-Octadecene, 1,1'-[1,2-ethanediylbis(oxy)]bis-, 26.49 C38H74O2 17367-13-4 (Z,Z)-
43.	26.77	1.74	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-
44.	27.54	1.33	Hexatriacontyl pentafluoropropionate
45.	27.64	1.71	Octatriacontyl pentafluoropropionate
46.	27.70	1.55	Tetracosyl heptafluorobutyrate
47.	27.81	1.14	Ethanol, 2-(octadecyloxy)-
48.	27.93	1.20	17-Pentatriacontene

3.4. HPTLC fingerprinting profile of hydroalcoholic petals extract of R.damascena:

The hydroalcoholic extract of R.damascena was subjected to HPTLC analysis on silica gel 60 F254 plates using a mobile phase of toluene: ethyl acetate in 9:1 (v/v) to develop a fingerprint profile for the significant phytoconstituent present in the extract. Calibration curves were established for the geraniol all of which yielded a strong linearity with R^2 values of 0.9953 respectively, as depicted in Figure 2. The HPTLC densitograms demonstrated retention factor (Rf) values of 0.88 for geraniol, as illustrated in Figure 3. The developed plates were visualized under 254nm, 366 nm, and white light, before and after derivatization using anisaldehyde, as illustrated in Figures 4. The final concentration of geraniol in hydroalcoholic petal extract of R.damascena was found to be 3.54±0.23 w/w, as shown in Table 4

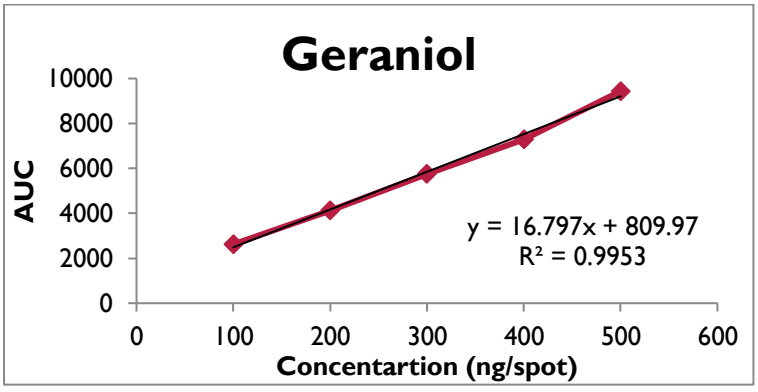

Figure 2: Calibration curve of Geraniol

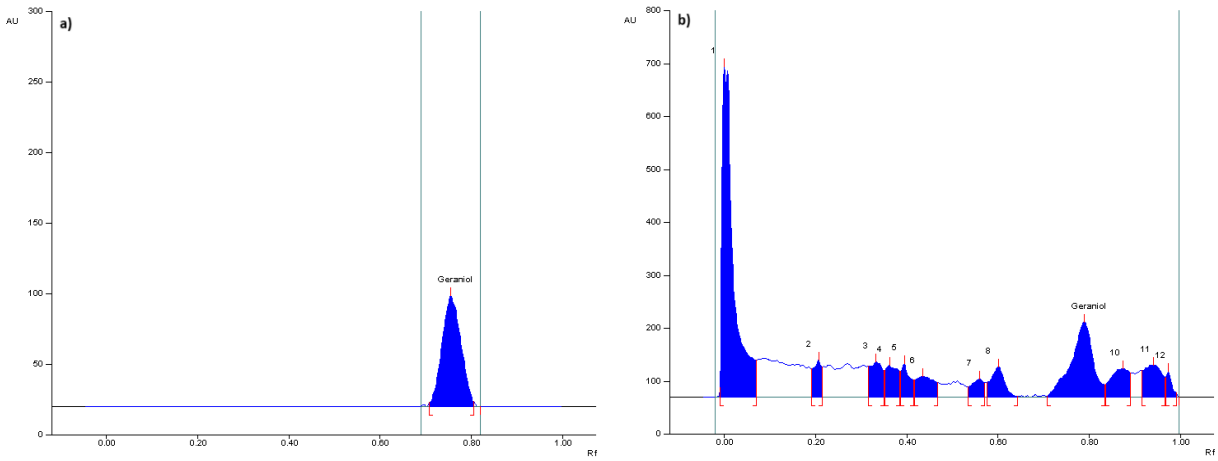

Figure 3: HPTLC densitogram of a) standard geraniol and b) and hydroalcoholic extract of *R.damascena* at 254 nm

Table 4*:* Quantitative analysis of geraniol in hydroalcoholic extract of *R.damascena*

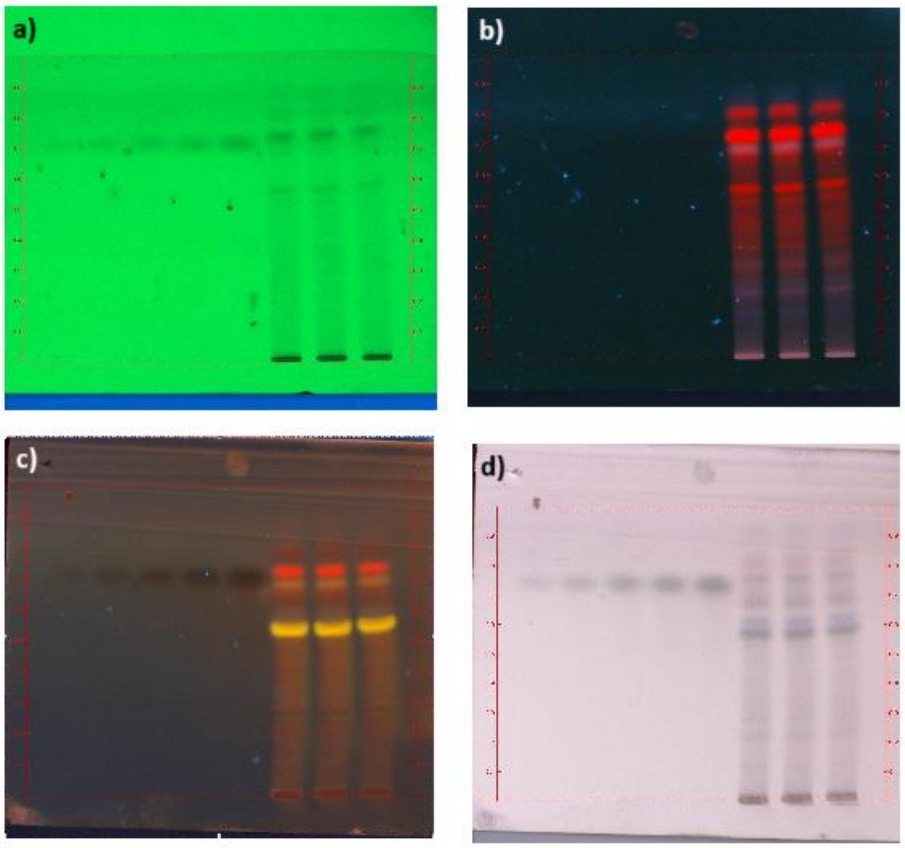

Figure 4: HPTLC fingerprinting profile of standard Geraniol and plant extract at a) 254 nm, b) 366 nm, c) at 366 nm after derivatization and d) at white light after derivatization

4. CONCLUSION:

In conclusion, the comprehensive analysis of R.damascena petals, utilizing phytochemical screening, and advanced analytical techniques such as HPTLC fingerprinting and GC-MS for major flavonoid profiling, underscores the significance of this botanical resource. It not only contributes to the standardization and quality control of R. damascena but also provides valuable insights into its complex chemical composition, particularly the flavonoid content. The detailed characterization facilitates the accurate identification and authentication of this important medicinal plant, ensuring its proper use in various

applications. Furthermore, the identification of key flavonoids may help elucidate the pharmacological basis for the traditional uses of R. damascena, paving the way for future research and the development of novel therapeutic agents.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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