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PHYTOCHEMICAL AND IN-VITRO ANTI-INFLAMMATORY ASSESMENT OF *ROSA DAMASCENA* EXTRACT

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ABSTRACT:

Rosa damascena, commonly known as Damask rose, is renowned for its therapeutic properties, particularly in traditional medicine. This study investigates the phytochemical composition and evaluates the in-vitro anti-inflammatory activity of *Rosa damascena extract*. Comprehensive phytochemical screening revealed the presence of flavonoids, tannins, saponins, glycosides, and phenolic compounds. The anti-inflammatory potential was assessed using the protein denaturation assay,. The results demonstrated that *Rosa damascena extract* exhibits significant anti-inflammatory activity, comparable to standard anti-inflammatory drugs. These findings suggest that *Rosa damascena could* be a promising natural source for developing anti-inflammatory therapeutics.

Keywords: *Rosa damascena*, Damask rose, phytochemicals, Anti-inflammatory, Medicinal plants.

1. INTRODUCTION

A promising source of natural antioxidants and antibacterial agents, medicinal and aromatic plants (MAPs) represent the largest bioresource of phenolic compounds. Thus, a thorough examination of the phenolic content of MAP extracts and an assessment of their antioxidative and antibacterial activity are necessary in order to find novel, valued products that may be used in the food, pharmaceutical, and cosmetic industries **(Trendafilova et al., 2023).**

Rosa damascena, commonly known as Damask rose, is a species of rose that has been renowned for its fragrance and medicinal properties for centuries. Native to the Middle East and extensively cultivated in countries like Iran, Bulgaria, and Turkey, *Rosa damascena is* a critical component in the production of rose oil, which is widely used in perfumery and aromatherapy. Beyond its olfactory appeal, this plant has been traditionally utilized in various folk medicines, suggesting a broad spectrum of therapeutic potentials **(Baydar et al., 2004).** Recent scientific investigations have increasingly focused on the phytochemical profile of *Rosa damascena,* revealing a complex array of bioactive compounds including flavonoids, anthocyanins, terpenes, and glycosides. These compounds are believed to contribute to the plant's pharmacological activities, particularly its anti-inflammatory properties **(Boskabady et al., 2011; Loghmani-Khouzani, 2007)**. According to traditional medicine, *R. damascena's* greatest beneficial benefits include relieving chest and abdominal pain, stimulating the heart **(Wood and Lawall, 1926),** treating menstrual bleeding and digestive issues **(Shatafkhandy, 1990)**, and reducing inflammation, particularly in the neck **(Buckle et al., 1993).** Native American tribes in North America employed a decoction of the root of the *R. damascena* plant to treat children's coughs **(Libster, 2002).** Additionally, this plant is utilized as a mild laxative **(Zargari, 1997).** Rose oil relieves tension, nervous tension, melancholy, and grief. It aids in the healing of old wounds, the alleviation of women's specific problems, the healing of cuts, and the maintenance of healthy skin. Rose oil vapour therapy is beneficial for some allergies, migraines, and headaches **(Zargari, 1997; Momeni and Shahrokhi, 1991).**

Inflammation is a biological response to harmful stimuli, and while it is a protective mechanism, chronic inflammation is associated with numerous pathological conditions such as arthritis, cardiovascular diseases, and cancer **(Mdzhitov, 2010).** The in-vitro assessment of the anti-inflammatory effects of *Rosa damascena extract* is essential to substantiate its traditional uses and to explore its potential as a natural therapeutic agent. In-vitro studies provide a controlled environment to evaluate the anti-inflammatory activities of phytochemicals by observing their effects on specific inflammatory markers and pathways. This study aims to investigate the phytochemical constituents of *Rosa damascena extract* and assess its anti-inflammatory properties through in-vitro experiment, thereby contributing to the scientific validation of its traditional medicinal uses and exploring its potential for novel therapeutic applications**.**

2. MATERIAL AND METHOD

2.1 Plant collection

The medicinal plant *Rosa damascena whole* plant was collected from Bhopal, M.P., and dried under shade for 3 days and then dried in an oven at 45°C. The dried parts were stored in air-tight containers to prevent contamination. A plant taxonomist authenticated the leaves to confirm their identity and purity.

2.2 Physiochemical evaluation

Physiochemical parameters evaluated for dried *Rosa damascena* whole plant such as total ash, acid insoluble ash, water-soluble ash, alcohol extractive value, water extractive value, los on drying, of *Rosa species* were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plants Material **(Fathima and Murthy, 2019; Mukhi et al., 2016).**

2.2.1 Loss on drying

The crude drug was weighed immediately after collection and recorded as ‘wet weight of sample’. Using a hot air oven the wet sample was dried at a temperature not exceeding 115 ^{o}C. The sample was set aside to be cooled and weighed again after 1 and 2 hr and recorded as

the 'dry weight of sample', sequentially. The amount of loss on drying was calculated using the formula given below **(Mandal et al., 2017).**

$$\% \text{ Loss on drying} = \frac{(Weight\ of\ sample - Weight\ of\ dried\ sample)}{Weight\ of\ sample} \times 100$$

2.2.2 Sample preparation for ash content

Crude drug of plant undergoes determination for water-soluble, acid insoluble, and total ash by following the protocol of Indian Pharmacopoeia, 2007. For ash estimation of crude drug, a platinum crucible was heated to turn red for 10 min, kept in desiccators allowed to be cooled and to be weighed. Samples for different ash parameters were prepared as follows (**Mandal et al., 2017).**

- **Determination of Ash value**

Ash content in the investigated plants of *Rosa damascena was* calculated by the methods given below:

Total ash

When the powdered drug is ignited at 7000ºC white-colored ash is obtained. It contains inorganic salts in oxide form **(Mishra et al., 2011)**.

Determination of total ash

Five grams of the crushed plant material were taken in a silica crucible previously ignited and weighed. A fine even layer of the crushed plant material was then spread in on the bottom of the crucible. Then the material is incinerated in a muffle furnace by gradually increasing the heat not exceeding dull red heat until free from carbon and then cooled and weighed. If we could not obtain carbon-free ash in this way, the overdone mass was exhausted with hot water. The residue was collected on an ash less filter paper which was then incinerated. The ash percentage was calculated with reference to the air-dried material.

$$\% \text{ Total ash value} = \frac{Weight\ of\ ash}{Weight\ of\ drug} \times 100$$

2.2.3 Water-soluble ash

Water-soluble ash contains inorganic salts which are soluble in water.

Determination of Water-Soluble Ash

100 mg of ash was boiled for five minutes with 10 ml of distilled water. The insoluble matter was collected in a silica crucible or on ashless filter paper. It was washed with hot water and then ignited to constant weight at low temperatures. The weight of the insoluble matter was subtracted from the weight of the ash. The percentage of water-soluble ash was calculated with reference to the amount of ash taken.

2.2.4 Acid Insoluble ash

It contains inorganic salts which are not soluble in water as well as in 10% hydrochloric acid example, salts of silicate, etc.

Determination of Acid Insoluble Ash

The total ash was boiled for five minutes with 25 ml of 10% HCl. The insoluble ash was collected in a silica crucible or ashless filter paper. It was washed with hot water and then ignited and weighed. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight represents the acid-insoluble ash. The percentage of acid insoluble ash was calculated with reference to the amount of ash taken.

2.2.5 Determination of extractive value with different solvents

Estimation of extractive value was done according to method **(Mishra et al., 2011).** A known quantity of the powdered drug was taken. Extraction was made in soxhlet apparatus with different solvents i.e. ethyl acetate, and methanol. The extract was filtered and the solvent was evaporated, the accurate weight of the extract was taken. The percentage (%) was calculated with reference to air dried drug **(Grover et al., 2014)**.

$$\% \text{ extracting value} = \frac{(Weight\ of\ flask\ with\ extract - Weight\ of\ empty\ flask)}{Weight\ of\ sample} \times 100$$

2.3 Preparation of extracts

The plant's materials were dried under shade at 25 ± 2 ºC & then pulverized by a mechanical grinder & sieved. The extraction was done with a suitable solvent having potent activity. Ethyl acetate and methanol as a solvent were used for the extraction of plant material. Extraction was done by the Soxhlet apparatus. After completion of the extraction process, the sample was filtered with filter paper and the solvent was evaporated using a rotary evaporator under 40-45 ºC for 30 min resulting in semisolid crude extract and weighed **(Khan et al., 2018)**.

2.4 Preliminary phytochemical study

The stock solution was prepared from the methanol extract, and ethyl acetate, extracts of *Rosa damascena* . The obtained stock solutions of extract were then subjected to qualitative screening for identification of plant constituents such as tests for alkaloids, tannins, steroids glycoside, flavonoids, saponins, carbohydrates, terpenoids, and proteins.

2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content was determined by the spectrophotometric method **(Saeed et al., 2012).** In brief, a 0.5 ml of sample (1 mg/ml) was mixed with 2.5 ml of Folin-Ciocalteu's phenol reagent (prediluted 10-fold with distilled water). After 5 min, 2 ml of a 7.5% Na_2CO_3 solution was added to the mixture. Immediately, the volume of reaction mixture was made to 7 mL with distilled water. The mixture was kept in the dark for 120 min at room temperature, after which the absorbance was read at 760 nm. The TPC values were calculated using gallic acid calibration curve within range 20 -100 µg/ml. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

2.6 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined following a method by **Park *et al.*, 2008**. In a test tube, 0.2 ml of extracts, 0.15 ml of $NaNO_2$ (5%) and 0.15 ml of $AlCl_3.6H_2O$ (10%) were mixed. After 5 min, 2 ml of NaOH (4%) was added. The solution was mixed well and volume of reaction mixture was made to 5 mL with distilled water. The absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoids was made using rutin standard solution (20 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried fraction.

2.7 In vitro anti-inflammatory activity

2.7.1 Inhibition of Denaturation of Albumin

The ability of the plant extracts to inhibit the denaturation of albumin was investigated **(Esho et al., 2021).** Varying concentration of the extracts (50 µg-300 µg) was prepared and the volumes were made up to 2.5 ml with 0.85% NaCl. This was followed by the addition of 0.5 ml albumin (1.5 mg/ml). The mixture was incubated at 37 °C for 20 minutes and further incubated at 57 °C for 20 minutes. The tubes were cooled and 2.5 ml of 0.5 M sodium phosphate buffer (pH 6.3) was added. The turbidity was measured spectrophotometrically at 660 nm. The experiment was carried out in triplicates and aspirin was used in place of the extract as the standard drug**.**

$$\%\ Inhibitiobn = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ drug}{Absorbance\ of\ control} \times 100$$

3. RESULTS

3.1 Pharmacognostical evaluation of plant sample

Table 1Pharmacognostical evaluation of plant sample

Parameters	*Rosa damascene*
Loss on Drying %	14.32
Total ash	3.34
Water soluble ash	2.48
Acid insoluble ash	1.51
Water extractive value	13.16
Alcohol extractive value	11.24

3.2 Plant Extraction

Table 1 Percentage yield

Plant name	Solvent	Theoretical yield (in gm)	Actual Yield (in gm)	Percentage Yield (%)
Rosa damascena	Ethyl acetate	150	7.44	4.96
Rosa damascena	Methanolic	142	14.924	10.51

3.3 Solubility Determination: -

Table 2 Solubility Determination of Ethyl acetate extracts

Solvent	Ethylacetate extract	Methanolic extract
Water	Insoluble	Slightly soluble
Methanol	Slightly Soluble	Soluble
Ethyl Acetate	Soluble	Soluble
DMSO	Soluble	Soluble
Petroleum Ether	Soluble	Insoluble

3.4 Phytochemical Analysis:

Table 4 Qualitative Phytochemical Analysis of extracts

S. No.	Experiment	ethyl acetate extract of *Rosa damascena*	methanolic extract of *Rosa damascena*
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	+	+
2.	Hager's Test	+	+
3.	Wagner's Test	+	+
4.	Dragendroff's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	-	-
2.	Libermann-Burchard's	-	-

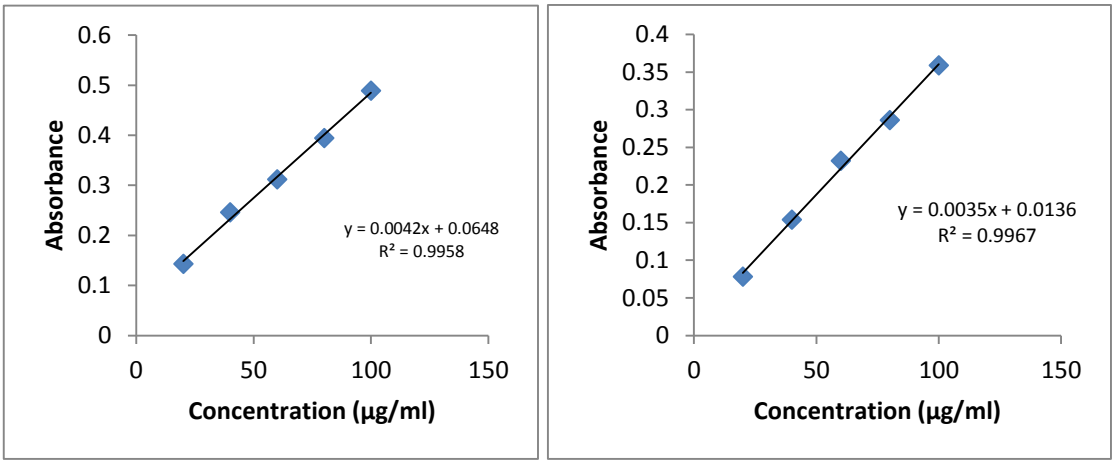
	Test		
Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	+	+
3.	Shinoda Test	+	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	-	-
Test for Protein and Amino acids			
1.	Ninhydrin Test	+	-
2.	Biuret's Test	+	-
3.	Million's Test	+	-
Test for Glycosides			
1.	Legal's Test	+	+
2.	Keller Killani Test	+	+
3.	Borntrager's Test	+	+

3.5 Quantitative Phytochemical analysis

3.5.1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Estimation:

Table 5 Standard table for Gallic acid

Concentration (µg/ml)	Absorbance (Gallic acid)	Absorbance (Rutin)
20	0.143	0.078
40	0.246	0.154
60	0.312	0.232
80	0.394	0.286
100	0.489	0.359



Graph 1 Graph represent standard curve of Gallic acid and Rutin

Table 6 Total Phenolic Content in extracts

Extracts	**Total phenolic content (mg/gm equivalent to Gallic acid)**	
	Absorbance (Mean±SD)	TPC
Ethyl acetate extract of Rosa damascene	0.319±0.006	63.75
Methanolic extract of Rosa damascene	0.369±0.012	76.25

Upon analysis, it is evident that the methanolic extracts consistently exhibited higher total phenolic content with an absorbance value of 0.369±0.012, equivalent to 76.25 mg/gm Gallic acid.

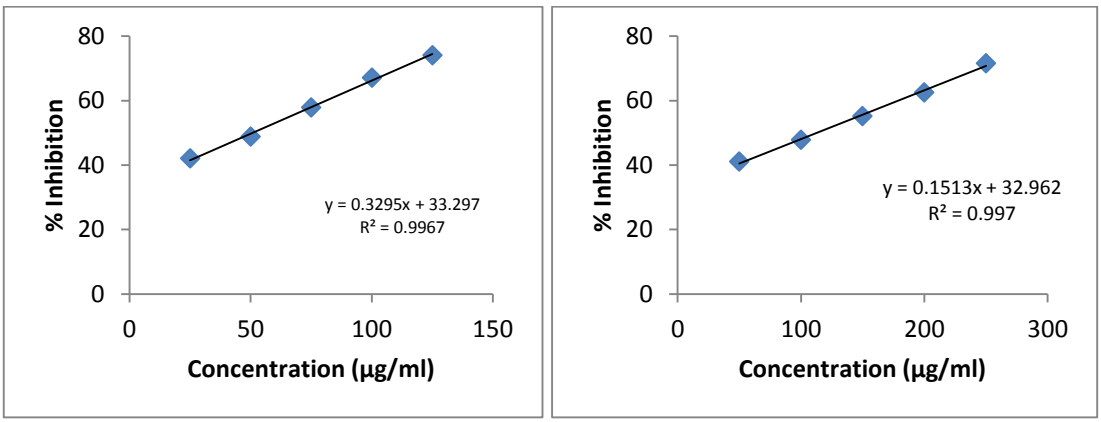
Table 7 Total Flavonoid Content in extracts

Extracts	**Total flavonoid content (mg/gm equivalent to rutin)**	
	Absorbance (Mean±SD)	TFC
Ethyl acetate extract of Rosa damascena	0.189±0.017	58.666
Methanolic extract of Rosa damascena	0.234±0.009	73.666

3.6 *Invitro* anti inflammatory activity by Protein denaturation assay

Table 8 Anti inflammatory activity of Diclofenac

Concentration (µg/ml)	**Absorbance**	**% Inhibition**
25	0.483	42.086
50	0.427	48.800
75	0.351	57.913
100	0.274	67.146
125	0.216	74.100
Control	0.834	
IC50	50.79	



Graph 2 Graphs represent anti inflammatory activity of Diclofenac and *Rosa damescena*

Table 9 Anti inflammatory activity of *Rosa damescena*

Concentration (µg/ml)	Absorbance	% Inhibition
50	0.491	41.127
100	0.435	47.841
150	0.374	55.155
200	0.312	62.589
250	0.237	71.582
Control	0.834	
IC50	112.847	

Table 10 Comparative table of Anti inflammatory activity of Rosa species

Sample name	IC 50 (µg/ml)
Diclofenac	50.79
Rosa damascena	112.847

4. DISCUSSION

In this study, we used the genus Rosa, known to have many medicinal properties. Thus, the purpose of our study was to investigate the anti-inflammatory effect of *Rosa damascena.*
Physico-chemical parameters such as the percentage of loss on drying (LOD), total ash, acid insoluble ash, water soluble ash were determined as per the Indian Pharmacopoeia.
Table 2 revealed extractive potency of the *Rosa damascena* in Ethylacetate giving yield of 4.96 and in methanol yield of 10.51 which was not close to the recommended value of between 20% and 30%, **(Njuguna, 2010).**
Percentage total ash, insoluble ash and soluble ash obtained were 3.34, 2.48 and 1.51% in *Rosa damascena respectively*, these parameters detect amount of organic and inorganic material in the plant sample. Recommended level should not exceed 12% **(Njuguna, 2010).** Percentage loss on drying for *Rosa damascena was* found to be 14.32 %.
The preliminary phytochemical screening of the ethyl acetate extract of R. damascena identified alkaloids, tannins, flavonoids, phenols, protein, and glycosides, while the methanolic extract showcased the presence of carbohydrates, alkaloids, tannins, flavonoids, phenols, protein, and glycosides. Currently, the most common method used to measure the TPC of all types of herbal samples is the Folin–Ciocalteu method. This particular method is based on the reduction of the phosphomolybdate heteropoly acids Mo(VI) center in the heteropoly complex to Mo(V), producing a blue coloration which is measured at around 750 nm **(Bobo-García et al., 2015)**. On the other hand, the TFC in plant extracts is widely measured using an aluminum chloride colorimetric assay. The method is based on the chelate formation of Al(III)-flavonoids. The numerous oxo and hydroxyl groups contained in this group's compounds, contribute a great affinity of flavonoids to bind metal ions such as Al(III), predominantly in a 1:1 ratio, depending on experimental conditions including pH value **(Kasprzak et al., 2015, Shraim et al., 2021). Table 5** summarizes that total phenolic compounds in extracts varied widely, ranging from 20 to 80 mg/g expressed as gallic acid equivalents (GAE).
Upon analysis, it is evident that the methanolic extract consistently exhibited higher total phenolic content compared to the ethyl acetate extract with an absorbance value of 0.369±0.012, equivalent to 76.25 mg/gm Gallic acid. This indicates that *Rosa damascena,* particularly when extracted using methanol, could be a valuable source of phenolic compounds. Moving on to flavonoid content, methanolic extract generally exhibited higher total flavonoid content compared to the ethyl acetate extract. Among the Rosa species

studied, *Rosa damascena's* methanolic extract showed the highest total flavonoid content with an absorbance value of 0.234±0.009, equivalent to 73.666 mg/gm rutin.

The variations in phenolic and flavonoid content between ethyl acetate and methanolic extracts can be attributed to the solvents' different capabilities in extracting these compounds. Methanol, being a strong solvent for polar compounds, efficiently extracts phenolics and flavonoids, resulting in higher content compared to ethyl acetate extraction.

The anti-inflammatory property of *Rosa damescena* extract was assessed in terms of their ability to inhibit protein denaturation (BSA). **Table 10** presents the IC50 values (half-maximal inhibitory concentration) of diclofenac (a commonly used anti-inflammatory drug) and extract of *Rosa damascena.* The IC50 value represents the concentration of a substance required to inhibit 50% of the inflammatory response in vitro. Comparing the IC50 values, diclofenac demonstrates the lowest value, indicating its potent anti-inflammatory activity with an IC50 of 50.79 µg/ml. *Rosa damascena exhibit* the most potent anti-inflammatory activity with an IC50 value of 112.847 µg/ml. This suggests that extracts from *Rosa damascena possess* considerable anti-inflammatory properties, albeit not as potent as diclofenac. These findings highlight the potential of Rosa species as natural anti-inflammatory agents.

5. CONCLUSION

In conclusion, the study on the phytochemical composition and in-vitro anti-inflammatory properties of *Rosa damascena extract* highlights its significant potential as a natural therapeutic agent. The comprehensive phytochemical analysis revealed the presence of key bioactive compounds, including flavonoids, tannins, and phenolic acids, which are known for their anti-inflammatory effects. The in-vitro assays demonstrated that *Rosa damascena extract* effectively inhibits inflammatory mediators, thereby validating its traditional use in treating inflammatory conditions. These findings suggest that *Rosa damascena extract* could be further developed and incorporated into pharmaceutical formulations aimed at managing inflammation-related disorders. However, additional in-vivo studies and clinical trials are necessary to fully understand the efficacy, safety, and mechanisms of action of this promising natural extract.

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