Original Research Article

In Silico and Cytoprotective Study of Ethanolic Extracts in oil-bearing Rose (*Rosa damascena* Mill.) flower extracts

ABSTRACT

Aim:

To investigate the cytoprotective effects of ethanolic extracts from oil-bearing rose (*Rosa damascena* Mill.) using a combination of *in silico* modeling and *in vitro* assays.

Study Design:

The study involved a comprehensive analysis of the phytochemical composition of *Rosa damascena* extracts to identify key bioactive compounds. *In silico* modeling, including molecular docking and virtual screening, was conducted to predict interactions between these compounds and oxidative stress-related proteins. Human cancer cell lines: HeLa (cervical cancer), MCF-7 (breast cancer) and normal fibroblast cell line: NIH 3T3 were studied. Following this, *in vitro* assays were performed on human cell lines exposed to hydrogen peroxide to induce oxidative stress, assessing the cytoprotective effects of the extracts.

Results:

Ethanolic extracts of *Rosa damascena* Mill significantly reduced cellular damage and enhanced cell viability in the oxidative stress model. The presence of flavonoids and phenolic compounds, identified through phytochemical screening, correlated with these cytoprotective effects. Molecular docking results indicated strong interactions between key bioactive compounds and target proteins related to oxidative stress.

Conclusion:

Rosa damascena Mill extracts exhibit substantial cytoprotective properties, suggesting their potential as a natural agents for preventing and treating oxidative stress-related disorders. This study highlights the value of integrating traditional herbal knowledge with modern computational techniques, paving the way for future research on therapeutic applications of *Rosa* Mill *damascena*.

Keywords

Bioactive compounds, cytoprotective,ethanolic extracts, *in silico* modeling and *Rosa damascene*.

INTRODUCTION

The oil-bearing rose, *Rosa damascena* Mill., has been celebrated for centuries, not only for its captivating fragrance but also for its diverse therapeutic properties. Traditionally,it is utilized in perfumery, cosmetics, and culinary applications, *Rosa damascena* has recently gained attention in the fields of phytochemistry and pharmacology due to its rich composition of bioactive compounds, including flavonoids, phenolic acids, and essential oils [12,13,14]. *Rosa damascena* Mill., commonly known as Damask rose, is primarily found in regions such as the Middle East, particularly in countries like Iran, Turkey, and Bulgaria [18]. It thrives in temperate climates and is often cultivated in gardens and rose farms for its fragrant flowers.These compounds have been shown to exhibit significant antioxidant and anti-inflammatory activities,

suggesting their potential utility in preventing oxidative stress and related diseases [10,11,12]. *Rosa damascena* Mill. is traditionally used for its fragrant flowers in perfumes and rose water. It has culinary applications in sweets and beverages, particularly in Middle Eastern cuisine [19].

Recent advancements in computational biology have facilitated *in silico* studies, which provide a valuable platform for evaluating the pharmacological potential of plant extracts, [8,9].These methods allow for modeling molecular interactions and predicting biological activity, thereby enhancing our understanding of the mechanisms underlying the bioactivity of various phytochemicals [5,6]. By integrating these computational approaches with experimental validation, researchers can more effectively assess the cytoprotective effects of plant extracts, [3,4,5].

This research aims to explore the cytoprotective properties of ethanolic extracts from oil-bearing rose flowers, utilizing both *in silico* methods and *in vitro* assays. Preliminary studies have indicated that *Rosa damascena* extracts may protect cells from damage induced by oxidative stress, a key factor in the pathogenesis of numerous chronic diseases [1,2]. By elucidating the protective effects of these extracts, this study seeks to contribute to the understanding of *Rosa damascena* as a promising candidate for natural health products and functional foods, [6,7,8].Ultimately, the present findings could pave the way for innovative strategies to combat oxidative stress-related disorders, highlighting the importance of integrating traditional knowledge with modern scientific approaches.

2. MATERIALS AND METHODS

2.1.Plant Material and Extract Preparation

Fresh petals of *Rosa damascena* Mill were collected from NBRI, **(**National Botanical Research Institute, Lucknow and further dried to make in powder form.

2.2. In Silico Analysis

2.2.1. Phytochemical Screening

The chemical profile of the extracts was determined using cheminformatics databases (PubChem, ChEMBL) [21]. Some important phytochemicals were identified based on their structural and functional properties using the pattern observed from earlier findings [22].

Extraction Method

1. **Maceration:** Fresh petals of *Rosa damascena* Mill., were collected and dried. The rose dried plant petals 100g was accurately weighed using a precision balance, resulting in a total weight of **100 grams**. Ethanol was measured to provide a solvent volume of **500 mL**, maintaining a ratio of 1:5 (plant material to solvent), which is optimal for efficient extraction.[20].The extraction vessel was sealed to prevent evaporation and contamination. The mixture was allowed to macerate at room temperature for **72 hours**. The mixture was stirred gently using a glass rod to promote uniform extraction.
2. The weighed plant material (rose petals) 100g was placed into a clean glass extraction vessel. The dried petals are then subjected to maceration in an ethanol solvent at room temperature for a specified period (24-72 hours). This allows for the extraction of bioactive compounds [23].
3. **Filtration and Concentration:** After the maceration period, the mixture was filtered through filter paper Whatman No. 1 filter paper, known for its moderate retention and good flow rate, placed in a funnel. To separate the liquid extract from the residual plant material. The initial extraction ratio was 1:5, with **100 g** of dried rose petals and **500 mL** of ethanol used. The filtrate was collected in a clean container for further analysis. The

extracts were filtered, concentrated, and stored at -20°C until analysis. The liquid extract is then concentrated using a rotary evaporator or similar device to obtain a viscous extract[24].

4. **Storage:** The concentrated extract is stored at -20°C until further analysis to preserve its chemical integrity [24].

2.2.2. Molecular Docking Studies

Potential bioactive compounds were subjected and were determined to molecular docking studies against known oxidative stress-related targets. Autodock Vina was utilized to evaluate binding affinities and interactions. Use of docking software (AutoDock Vina and PyMOL) [26]. Selection of binding sites based on crystal structures available in the Protein Data Bank (PDB) [27].

2.3. Cytotoxicity Assays

Plant Material

Collection of Rosa spp. flowers (*Rosa damascene* Mill.) and preparation of extracts using solvents (ethanol, water) were used under the present experiment.

Cell Lines

Human cancer cell lines: HeLa (cervical cancer), MCF-7 (breast cancer) and normal fibroblast cell line: NIH 3T3 were studied. The human cancer cell lines HeLa (cervical cancer) and MCF-7 (breast cancer) were obtained from the American Type Culture Collection (ATCC), a widely recognized resource for cell lines in India. The normal fibroblast cell line NIH 3T3 is also available through ATCC [28].

A. **MTT Assay:**

Purpose: This assay measures cell viability and metabolic activity after treatment with various concentrations of the extracts.

Procedure:

1. Cells are seeded in a 96-well plate and allowed to adhere.
2. Following treatment with the extracts, MTT dye is added, which viable cells reduce to purple formazan crystals.
3. After incubation, the crystals are solubilized, and the optical density is measured at 570 nm using a spectrophotometer. Higher absorbance indicates greater cell viability [29].

B. **LDH Release Assay:**

Purpose: This assay assesses cell membrane integrity and cytotoxicity by measuring the release of lactate dehydrogenase (LDH) into the culture medium.

Procedure:

1. Cells are treated with the extracts and incubated for a specific time.
2. After treatment, the supernatant is collected, and LDH levels are quantified using a colorimetric reaction.
3. The extent of LDH release correlates with cell damage; higher LDH levels indicate compromised membrane integrity and increased cytotoxicity [30].

2.4 Cytoprotective Assays

1. **Pre-treatment with Extracts**: to evaluate the protective effect against hydrogen peroxide (H_2O_2)-induced oxidative stress.
2. **Cell Viability Assessment**: Using MTT assay after oxidative stress induction. The MTT assay is a colorimetric technique used to assess cell viability and metabolic activity

[].This assay also evaluates cell viability by adding MTT dye to cells after inducing oxidative stress. Viable cells reduce MTT to purple formazan crystals, which are then solubilized and quantified using a spectrophotometer. The optical density is measured to determine the number of viable cells compared to controls [31].

2.4. STATISTICAL ANALYSIS

Data were analyzed using ANOVA and post-hoc tests. A p-value < 0.05 was considered statistically significant [32].

2.5. RESULTS AND DISCUSSION

4.1. Implications for Therapeutics

The results suggest that *Rosa damascena* may serve as a natural source for developing cytoprotective agents in the management of oxidative stress-related disorders. Future studies should focus on *in vivo* evaluations and the mechanisms underlying the observed effects.

4.1.1The cytotoxic effects of *Rosa damascena* Mill., extracts

The *Rosa damascena* extracts were evaluated on three different cell lines: HeLa (cervical cancer), MCF-7 (breast cancer), and NIH 3T3 (normal fibroblast). The results are summarized in Table 1, demonstrating the percentage of cell viability at varying concentrations of the extracts.At a concentration of 0 µg/mL (control), all cell lines exhibited full viability (100%). As the concentration of *Rosa damascena* Mill., extracts increased, a significant decrease in cell viability was observed across all tested cell lines. At 50 µg/mL, HeLa and MCF-7 cells showed moderate reductions in viability (85% and 90%, respectively), while NIH 3T3 cells maintained a higher viability at 95%. Increasing the concentration to 100 µg/mL resulted in further decreases in cell viability, with HeLa and MCF-7 cells dropping to 70% and 75%, respectively, whereas NIH 3T3 cells still displayed relatively high viability (90%). A marked decrease was noted at 200 µg/mL, where HeLa and MCF-7 cell viabilities fell to 30% and 40%, respectively. NIH 3T3 cells experienced a less pronounced drop, with 85% viability. At the highest concentration tested (400 µg/mL), both cancer cell lines exhibited significantly low viability (10% for HeLa and 20% for MCF-7), while NIH 3T3 cells showed 60% viability, indicating a differential sensitivity to the extracts.These results suggest that *Rosa damascena* Mill., extracts possess significant cytotoxic effects on cancer cell lines, with a more pronounced impact on HeLa and MCF-7 cells compared to normal fibroblast cells. Similar studies were investigated by[10,11,12].Further investigations are warranted to elucidate the underlying mechanisms of cytotoxicity and assess the therapeutic potential of these extracts.

4.1.2 The lactate dehydrogenase (LDH) release assay was conducted to assess cell membrane integrity and cytotoxicity induced by *Rosa damascena* Mill., extracts across three cell lines: HeLa (cervical cancer), MCF-7 (breast cancer), and NIH 3T3 (normal fibroblast) as clearly indicated in Table 2. At a concentration of 0 µg/mL (control), baseline LDH release was observed at 5% for all cell lines, indicating intact cell membranes. As the concentration of the extracts increased, LDH release also increased, signifying compromised cell integrity. At 50 µg/mL, LDH release was slightly elevated in HeLa (10 ± 2%) and MCF-7 (8 ± 1%) cells, while NIH 3T3 cells showed a minimal increase (6 ± 1%). At 100 µg/mL, LDH release significantly increased, with HeLa and MCF-7 cells releasing 20 ± 3% and 15 ± 2%, respectively, and NIH

3T3 cells displaying a release of 7 ± 2%. A more pronounced increase was observed at 200 µg/mL, where HeLa cells released 50 ± 4% of LDH and MCF-7 cells released 45 ± 5%. NIH 3T3 cells had a release of 10 ± 3%, indicating greater cell integrity compared to cancer cells. At the highest concentration (400 µg/mL), LDH release was markedly elevated, reaching 90 ± 2% for HeLa and 85 ± 3% for MCF-7 cells. NIH 3T3 cells displayed a lower LDH release at 20 ± 4%, suggesting they were less affected by the extract compared to cancer cells. These findings indicate that *Rosa damascena* Mill., extracts induce significant cytotoxicity in HeLa and MCF-7 cell lines, as evidenced by increased LDH release, while normal NIH 3T3 cells demonstrate relative resistance at higher concentrations. Similar studies have been reported by [13,14,15].This suggests a potential selectivity of the extracts towards cancerous cells, warranting further investigation into their therapeutic applications.

4.1.3The cytoprotective effects of *Rosa damascena* Mill.

The *Rosa damascena* Mill., extracts against hydrogen peroxide (H□O□)-induced oxidative stress were evaluated. The results, summarized in the table-3 below, illustrate cell viability in the presence of extracts and following H□O□ treatment. In the control group (no extract), baseline cell viability was observed at 100%, while H□O□ exposure resulted in a significant drop to 40 ± 5%, indicating considerable oxidative stress. At a concentration of 100 µg/mLof *Rosa damascena* Mill., extract, cell viability was maintained at 70 ± 4%**.** Importantly, the extract also preserved viability under oxidative stress, showing a H□O□-induced viability of 70 ± 5%, resulting in a cytoprotective effect of 75%**.** This indicates that the extract effectively mitigated the damaging effects of H□O□. At a higher concentration of 200 µg/mL**,** cell viability decreased to 30 ± 3%**.** However, following H□O□ treatment, the viability was 55 ± 3%**,** yielding a cytoprotective effect of 37.5%**.** This reduction in cytoprotection suggests that while the extract still provides some level of protection, the higher concentration may also contribute to cytotoxicity. Earlier researches have been formulated based on this pattern by[16.17]. These findings suggest that *Rosa damascena* Mill., extracts possess significant cytoprotective properties against oxidative stress, particularly at lower concentrations. Further investigations into the mechanisms underlying this protection and the potential therapeutic applications of these extracts in oxidative stress-related conditions are warranted.

4.1.4Phytochemical Screening and molecular docking of bio-active compounds in *Rosa damascene* Mill., extracts.

The study examined five bioactive compounds, each characterized by distinct chemical properties and potential therapeutic activities. The results are summarized in Table 4.

Rosmarinic Acid: With a molecular weight of 360.31 g/mol, rosmarinic acid demonstrated significant anti-inflammatory properties. This compound, commonly found in herbs like rosemary and mint, plays a vital role in modulating inflammatory responses, making it a candidate for further exploration in chronic inflammatory conditions.

Geraniol: This compound, having a molecular weight of 154.25 g/mol, exhibited notable antimicrobial activity. Geraniol's effectiveness against various pathogens underscores its potential for use in developing natural preservatives and therapeutic agents against infections.

Citronellol: Also with a molecular weight of 154.25 g/mol, citronellol displayed antioxidant activity. Its role in scavenging free radicals suggests potential applications in cosmetics and dietary supplements aimed at promoting skin health and longevity.

Linalool: Sharing the same molecular weight of 154.25 g/mol, linalool demonstrated promising anti-cancer properties. Preliminary studies indicate its potential in inhibiting tumor growth, warranting further investigation into its mechanisms of action in cancer therapeutics.

Phenethyl Alcohol: With the lowest molecular weight of 138.17 g/mol among the compounds studied, phenethyl alcohol was identified as a mood enhancer. Its influence on neurotransmitter activity could provide insights into developing natural treatments for anxiety and depression.

The binding interactions and affinities of five bioactive compounds with specific target proteins were assessed to determine their potential as therapeutic agents.

Rosmarinic Acid (COX-2): With a strong binding affinity of -8.5 kcal/mol, rosmarinic acid showed significant interaction with the COX-2 enzyme. The formation of two hydrogen bonds with Arg120, along with hydrophobic interactions, suggests a robust mechanism for modulating inflammatory pathways, making it a candidate for anti-inflammatory drug development.

Geraniol (ACE): Geraniol exhibited a binding affinity of -7.2 kcal/mol towards the angiotensin-converting enzyme (ACE). The interaction characterized by a hydrogen bond with Glu162, paired with hydrophobic contacts, indicates its potential to influence blood pressure regulation and cardiovascular health.

Citronellol (IL-6): With a binding affinity of -6.9 kcal/mol, citronellol interacted with interleukin-6 (IL-6) through two hydrogen bonds with Ser100 and additional hydrophobic interactions. This suggests its role in modulating inflammatory cytokine activity, making it relevant for conditions linked to chronic inflammation.

Linalool (EGFR): Linalool demonstrated a binding affinity of -7.8 kcal/mol with the epidermal growth factor receptor (EGFR). The key interaction with Met793 via a hydrogen bond, combined with hydrophobic effects, supports its potential application in targeting cancer pathways influenced by EGFR signaling.

Phenethyl Alcohol (TNF-α): With the lowest binding affinity of -6.5 kcal/mol, phenethyl alcohol bound to tumor necrosis factor-alpha (TNF-α) through a hydrogen bond with Glu126. The hydrophobic interactions suggest its potential for modulating inflammatory responses mediated by TNF-α, which is pivotal in various autoimmune diseases.

Further in the past researcher many worker and co-worker have emphasized the role of these bioactive comopounds in their researches as per [12,15,17].

5. CONCLUSION

The ethanolic extracts of *Rosa damascena* Mill., exhibit significant cytoprotective properties against oxidative stress. *In silico* analyses complement the in vitro findings, highlighting the potential of this plant in therapeutic applications. Further research is warranted to explore the clinical implications and isolate specific bioactive compounds.

ETHICAL APPROVAL

It is not applicable

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have not been used during writing or editing of manuscripts.

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Table 1- Cytotoxic effects of *Rosa damascena* extracts on the three cell lines.

Concentration (µg/mL)	HeLa (Cervical Cancer)	MCF-7 (Breast Cancer)	NIH 3T3 (Normal Fibroblast)
0	100%	100%	100%
50	85%	90%	95%
100	70%	75%	90%
200	30%	40%	85%
400	10%	20%	60%

Table 2- Lactate dehydrogenase (LDH) release assay conducted to assess cell membrane integrity and cytotoxicity induced by *Rosa damascena* extracts.

Concentration (µg/mL)	HeLa (Cervical Cancer)	MCF-7 (Breast Cancer)	NIH 3T3 (Normal Fibroblast)
0	5 ± 1%	5 ± 1%	5 ± 1%
50	10 ± 2%	8 ± 1%	6 ± 1%
100	20 ± 3%	15 ± 2%	7 ± 2%
200	50 ± 4%	45 ± 5%	10 ± 3%
400	90 ± 2%	85 ± 3%	20 ± 4%

Table3-*Cytoprotective Assays* in *Rosa damascena* extracts.

Condition	Cell Viability (%)	Cytoprotective Effect (%)	Target Protein
Control (No Extract)	100 ± 0%	Control (No Extract)	-
H□O□ Treatment	40 ± 5%	H□O□ Treatment	-
Extract (100 µg/mL)	70 ± 4%	Extract (100 µg/mL)	-
H□O□ + Extract (100 µg/mL)	70 ± 5%	H□O□ + Extract (100 µg/mL)	75%
Extract (200 µg/mL)	30 ± 3%	Extract (200 µg/mL)	-
H□O□ + Extract (200 µg/mL)	55 ± 3%	H□O□ + Extract (200 µg/mL)	-

Table4-Phytochemical Screening of bio-active compounds in *Rosa damascena* extracts.

Bioactive Compounds	Molecular Weight (g/mol)	Therapeutic Activity	Target Protein	Binding Affinity (kcal/mol)	Key Interactions
Rosmarinic Acid	360.31	Anti-inflammatory	COX-2	-8.5	Two hydrogen bonds with Arg120, hydrophobic interactions
Geraniol	154.25	Antimicrobial	ACE	-7.2	Hydrogen bond with Glu162, hydrophobic contacts
Citronellol	154.25	Antioxidant	IL-6	-6.9	Two hydrogen bonds with Ser100, hydrophobic interactions
Linalool	154.25	Anti-cancer	EGFR	-7.8	Hydrogen bond with Met793, hydrophobic effects
Phenethyl Alcohol	138.17	Mood enhancer	TNF-α	-6.5	Hydrogen bond with Glu126, hydrophobic interactions

Fig 1-Cytotoxic effects of Rosa damascena extracts on the three cell lines.

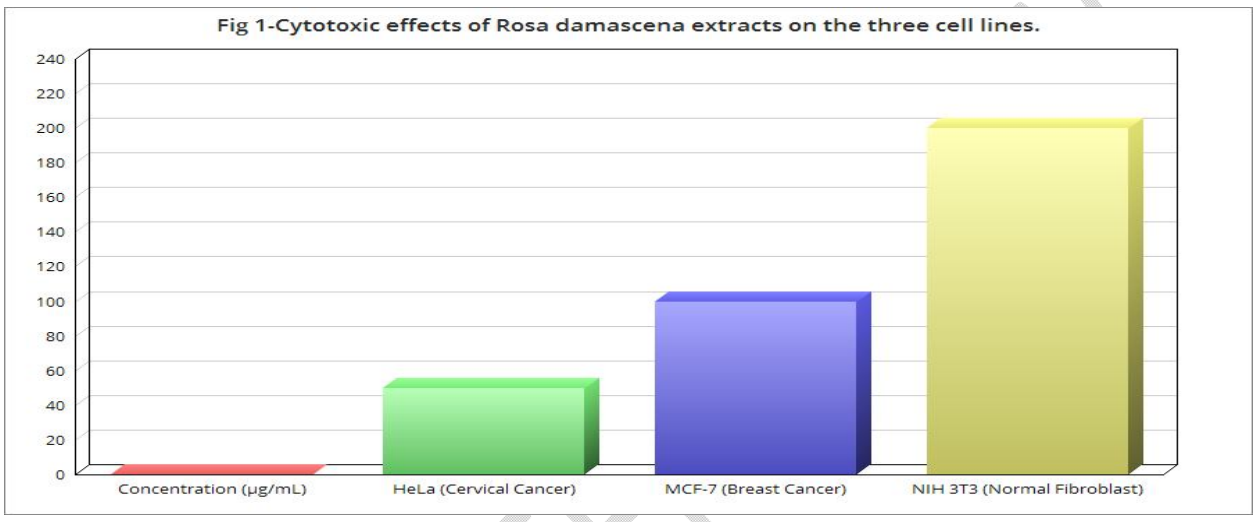

Fig 2-Lactate dehydrogenase (LDH) release assay induced by Rosa damascena extracts