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Characterization of *Mirabilis Jalapa* Seed Extract and Its Effect on Human Erythrocytes in Vitro

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ABSTRACT

Mirabilis Jalapa seed extract (MJSE) has been traditionally used for its medicinal properties. The aim is to establish the biochemical composition and therapeutic potential of MJSE, including estimation of the extraction yield, analyzing protein, phenolic, and carotenoid contents, in addition to assessing the phenolic and lipid profiles, and evaluating the anti-inflammatory and antioxidant activities, as well as osmatic fragility and lipid peroxidation. The phenolic profile was identified using LC-MS/MS, and the lipid profile was analyzed using GC-FID. The ELISA technique was used to conduct the malondialdehyde assay. The other experiments were performed by spectrophotometric methods. The extraction yield of. Mirabilis Jalapa seed extract (MJSE) was .30.2%. The protein content was 0.85 ± 0.009 mg/mL, and the total phenolic content (TPC) of MJSE was 16.2 ± 0.011 mg GAE/g of dry extract. Carotenoid analysis revealed β-carotene and lycopene contents of 0.079 mg/kg and 0.225 mg/kg, respectively. LC-MS/MS analysis identified high levels of ascorbic acid (7.72 µg/mg) and catechin (22.8 µg/mg). The GC-FID analysis detected 17 fatty acid methyl esters with a total concentration of 4,032,367 ppm. The anti-inflammatory and antioxidant activity were 94.9± 1.52% and 31.01± 0.74 %, respectively. The lipid peroxidation in normal RBCs with MJSE was achieved at 0.13 ± 0.005 pg/mL; the osmatic fragility showed a potential anti-hemolytic effect of RBCs with the extract. In conclusion, MJSE gives high yields for extraction and includes abundant proteins, phenol, carotene, and other essential lipids. Combining the fact that MJSE exhibits significant antioxidant and anti-inflammatory properties, and also protects red blood cells from lipid peroxidation.

Keywords: *Phenolic compounds, Anti-inflammatory activity, Antioxidant activity, Osmotic fragility*

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INTRODUCTION

Plants, being a source of multiple bioactive molecules containing beneficial factors to human health, have an old history. Meanwhile, the organisms' phenolics and lipids function as key players in many physiological and biochemical processes. The chemical properties of these compounds, as well as their impact on human biochemistry, receive much attention and inquiry in this area of science (Hanani *et al*., 2017; Pavithra *et al*., 2023).

M. jalaba, an herbal plant also referred to as Four O'clock flower for its precious beauty, is a one-of-its-kind by virtue of its breath-taking charm. Also, its seeds demonstrate some phenolic compounds (Singh *et al*., 2010), which are the productive grounds for the research. M. jalaba (Night blooming Cereus) is a perennial plant indigenous to South America who have long gained the reputation of pride in gardens throughout the world. Boasting an unusual size and appearance, the Four o'clock flower is known for the shape of its trumpet flower and, at the same time, for its afternoon blooming schedule. The leaves of this plant are dark green, but there are also variegated ones. The botanical characteristic comprehending criterion is the basic requirement for both scientific and ornamental purposes.

Raspberry Pie (M. jalaba) is a novel plant that has been proposed as one of the sources of the phenolic compounds that are necessary for pharmaceutical uses, as it has been found to have many therapeutic properties. According to research by Liya *et al*. (2021), Perrine *et al*. (2023), the particular phenolic compounds- quercetin and kaempferol- that are flavonoids and able to act as antioxidants and anti-inflammatory agents are present in the M. jalaba research. Being an alkaloid-rich plant, it contains, among others, Saponins, phenolic compounds, flavonoids, and others. The major phytochemicals that the M. jalaba research has highlighted have attracted attention, based on their various health potentials and pharmacological effects (Hanani *et al*., 2017; Chidambaranathan & Culathur, 2022). Therefore, the pharmacological properties of M. jalaba phytochemicals indicate a variety of uses for its fractionation. These applications are found to be relevant in the fields of medical science, nutritional requirements, and plant cultivation. For instance, the pharmaceutical industry can use the extract or a bioactive compound of the plant in the formulation of new herbal medicinal product or a nutritional supplement. Similarly,

the flowering and adaptability of the ornamental flower to various climatic conditions make it the widespread choice in soft scape.

Phenolic compounds are a group of complex secondary metabolites in plants, and due to their wide potential biological activities, they are enjoying increasing interest. They are divided into several families: flavonoids, phenolic acids, tannins, and lignans, which have different structural features and biological properties (Tungmunnithum *et al*., 2018; Mutha *et al*., 2021; Canassa & Baldin, 2022; Wilhelmy *et al*., 2022). Polyphenols (or phenolics) are known for their antioxidant property and consequently are engaged in protecting the cells from oxidative stress, which leads to cell injury, especially due to ferric ions. They have also been identified to be associated with anti-inflammatory effects as well as anticancer and cardiovascular benefits (Haminiuk *et al*., 2012; Macrì *et al*., 2023).

Trans fatty acids are formed through a process known as lipid peroxidation, where free radicals derived from antioxidant depletion attack lipids, leading to cellular injury and inflammation (Niki, 2009). It is essential to note that lipid peroxidation is connected with numerous diseases, such as cardiovascular diseases and neurodegenerative diseases, as it leads to the breaking of cellular membranes and a decrease in their functions (Halliwell, 2006; Lobach *et al*., 2023). These detrimental effects can be offset by anti-inflammatory substances, which include some of the antioxidants that are likely to scavenge free radicals while at the same time modulating the inflammation that results from oxidative stress (Calder, 2006; Bugti *et al*., 2024).

The human erythrocytes serve as the soul of the cardiovascular system, which is committed to oxygen delivery to all the body parts. The comprehensive assessment of the influence of M. jalaba on the human RBCs in an in vitro circumstance is the crucial step on the path to their appraisal for human health. Changing the form or functionality of erythrocytes raises a lot of questions regarding oxygen transportation and health (Singh *et al*., 2010; Skeie & Klock, 2023).

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to detoxify them. ROS include superoxide anion, hydrogen peroxide, and hydroxyl radicals. ROS are generated during normal cellular metabolism, primarily in mitochondria. External factors, including smoking, pollution, and ionizing radiation, can also contribute to ROS production (Jomova *et al*., 2023). The body possesses natural antioxidant defense mechanisms, such as superoxide dismutase and glutathione, to neutralize ROS and maintain redox balance (Leuti *et al*., 2019).

One of the most commonly evaluated types of. Indirect biomarkers are oxidatively modified lipids (Marrocco *et al*., 2017). During oxidative stress, lipids in cell membranes undergo a process called lipid peroxidation, leading to the formation of a variety of end products (García-Quevedo *et al*., 2020). Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are aldehydes produced during the breakdown of polyunsaturated fatty acids. These compounds can form adducts with proteins and DNA, influencing their structure and function (López-Acosta *et al*., 2018). The levels of MDA and 4-HNE, or their adducts, provide a snapshot of the lipid peroxidation process and thus oxidative stress status (Milković *et al*., 2023; Son & Lee, 2024).

Hemolysis has detrimental consequences, hindering oxygen delivery and leading to complications like anemia. Research suggests that the anti-hemolytic potential of M. jalaba might be attributed to its antioxidant properties (Soliman *et al*., 2023). Free radicals, highly reactive molecules, can damage RBC membranes, contributing to hemolysis. The antioxidants in the extract may act as scavengers, neutralizing these free radicals and protecting the delicate RBC membrane. Some studies suggest the extract directly influences RBC membrane stability. By interacting with the membrane components, it might. enhance its resilience and make it less susceptible to rupture from external stressors (Baccarin *et al*., 2015). Antioxidants detected in MJCE may effectively chelate these free radicals to prevent them from. rupturing the membrane of the RBC.

This study elucidates the phenolic and lipid profile of M. jalaba, particularly on its antioxidant activity and the in vitro anti-inflammatory activity. In detail, it is proposed to assess the level of lipid peroxidation in human erythrocytes and measure the effectiveness of M. jalaba seed extracts in the prevention of the hemolysis of these cells. Therefore, this study aims to identify and quantify the phenolic and lipidic compounds of M. jalaba and to assess the antioxidant and anti-inflammatory potential of the crude extract. Furthermore, to investigate the effect of different concentrations of MJSE on the protection of lipid peroxidation and osmotic fragility of human erythrocytes.

MATERIALS AND METHODS

Study design

The study's experimental design was a controlled research study to establish the effects of MJSE on the RBC cell membrane; the experiment was tested before and after being treated with different concentrations of MJSE.

M. jalaba were harvested from gardens in the region of Ajloun, Jordan. M. jalaba seed was collected and dried away from direct sunlight for a period of two weeks until completely dried. Approximately 1.2 kilograms of dried seed were obtained and finely ground, resulting in 635.5 grams of powdered seeds, and the powder was stored in a dry place for analysis.

Mirabilis Jalapa seed extraction

50 g of powdered M. jalaba seeds was dissolved in 100 mL of 80% methanol and incubated in a conical flask at 37°C and 100 rpm for 24 hours on a hot plate shaker. The extracted mixture was filtered using qualitative filter paper, and the filtrate was collected in a clean flask. Then the methanol was removed using a rotary evaporator in order to get the crude extract. The concentrated extract was effectively withdrawn from the rotary evaporator and kept in a refrigerator at 2-8 °C.

Extraction yield calculation

The extraction yield was calculated to determine the proportion of the extract obtained relative to the initial amount of raw plant material used. This calculation was performed after the extract had been dried. The extraction yield percentage was calculated using the following formula:

$$Extraction\ yield\ (\%) = \left(\frac{Weight\ of\ extract}{Weight\ of\ dry\ \ material}\right) \times 100 \quad (1)$$

Determination of total phenolic content

The total phenolic content of the MJSE was determined using the modified Folin-Ciocalteu technique (Seyedreihani *et al*., 2017). To make a stock solution, two milligrams of the dried MJSE extract were first reconstituted in one milliliter of ethanol. Next, one milliliter of distilled water was added to two milliliters of the stock solution to dilute it. For the Folin-Ciocalteu test, 0.1 mL of the diluted extract solution and 0.5 mL of Folin-Ciocalteu's reagent were mixed together and left to stand at room temperature for five minutes. Next, 2.5 mL of a 20% aqueous sodium carbonate (Na2CO3) solution was added to the mixture. The resulting solution's absorbance at 765 nm was measured using an ultraviolet-visible spectrophotometer. By measuring the absorbance of different doses (250–6.25 µg/mL) at 765 nm, gallic acid was utilized as a reference component to create a calibration curve. Plotting the concentration of gallic acid against its absorbance yielded a linear regression equation (y = 0.009x + 0.0736) with a coefficient of determination (R2) of 0.9941. The total phenolic content of the MJSE was determined using the gallic acid calibration curve equation and reported as milligrams of gallic acid equivalent per gram of the MJSE extract (mg GAE/g MJSE).

LC-MS/MS analysis of MJSE for phenolic profile

To analyze flavonoids and phenolic compounds, a SciEx UPLC (Exion-UPLC, USA) equipped with the LC-ESI-MS/MS-4500-QTRAP system (AB SciEx Instrument, Framingham, MA, USA) was utilized. The software Analyst 1.7 was used to analyze the data. A chromatographic separation was performed at 50±0.5 °C using a Ther-mo-Fisher ODS column (2.1 × 100 mm, 5 µm). A gradient elution consisted of an 80% mobile phase A (1% formic acid in water) and a 20% mobile phase B (methanol, 1% formic acid). The mobile phase B ratio (% B, min) that was employed during the gradient program was 0.00–1.00 min, 20–20% B, 1.00–12.00 min, 12.0–18.00 min, and 100–100%. 100-20% (18.0-19.00 minutes), 20-20% (19.0-22.0 minutes). 5 µL was the injection volume, and 0.35 mL/min was the solvent flow rate. MS/MS was used in negative ion mode for the analysis. Nitrogen gas was applied at 60 psi as a nebulizing and drying gas. The mass spectra were obtained in the range of 100–900 amu m/z.

Lipid profile analysis

To conduct the lipid profile study of the MJSE, fatty acid methyl esters (FAMEs) were analyzed using gas chromatography (GC), more precisely a Shimadzu Model 2010 equipped with a DB-23 capillary column. The ideal operating conditions for the gas chromatograph were an initial oven temperature of 70 °C maintained for two minutes, followed by a temperature ramp to 200 °C at a rate of 4 °C/min and a hold time of fifteen minutes at 200 °C. Helium gas was used as the carrier gas, maintaining a linear velocity of 35 mL/min, and a sample injection volume of 1 µL, with a split ratio of 1:20 was employed. The injector port and detectors were maintained at 240 °C. The signals were obtained during the analysis using GC-solution software (version 1.25), which is based on Windows 7. The various FAMEs found in the seed oil were identified by contrasting the results with standard FAMEs, which included a full set of 37 methyl esters ranging from C4 to C24 fatty acids. This method allowed for a detailed characterization of the fatty acid content of the MJSE.

Determining total protein content

The MJSE's total protein content was ascertained using the Biuret method (Zhou & Regenstein, 2006). Several standard solutions of bovine serum albumin (BSA) with concentrations ranging from 0.1 to 1.2 mg/mL were made in order to create a calibration standard curve. The extract's absorbance at 540 nm was measured using a UV-visible spectrophotometer (Biotech Engineering Management Co. Ltd., UK). The total protein concentration of the sample was then ascertained by comparing the absorbance results with the calibration curve.

Carotenoid content estimation

A method for extracting carotenoids from the MJSE to measure its carotenoid content (lycopene and β-carotene) was described by Hunter *et al*. (2021). Prior to performing MJSE, a 2 mL solution was made by diluting the MJSE with distilled water to a 50% (w/v) concentration. Next, the 2 mL MJSE solution was mixed with 10 mL of a 6:4 (v/v) hexane-acetone mixture. The resulting mixture was vigorously shaken for ten minutes to facilitate the extraction of carotenoids. After shaking the mixture, the organic layer containing the recovered carotenoids was filtered out. The absorbance of the filtered organic layer was measured at three distinct wavelengths (663 nm, 505 nm, and 453 nm) using a spectrophotometer. The reference was deionized water (DI). The concentrations of β-carotene and lycopene in the extract were then calculated using the following formulas:

$$\begin{aligned}\beta - \text{carotene (mg)} = {} & 0.216 \times \text{Abs.}\lambda 663 \\ & - 0.304 \times \text{Abs.}\lambda 505 \\ & + 0.452 \times \text{Abs.}\lambda 453\end{aligned} \quad (2)$$

$$\begin{aligned}\text{Lycopene (mg)} = {} & 0.0458 \times \text{Abs.}\lambda 663 \\ & + 0.372 \times \text{Abs.}\lambda 505 \\ & + 0.452 \times \text{Abs.}\lambda 453\end{aligned} \quad (3)$$

Where. A represents the absorbance at the specified wavelength.

DPPH radicals-scavenging assay

The Matusiewicz *et al*. (2018) methodology was used to measure the DPPH• scavenging activity, with a few minor adjustments. This strategy is predicated on antioxidants' ability to decrease the DPPH radical by providing an electron or a hydrogen atom, which causes the absorbance of the solution to change proportionately to the non-radical form DPPH. A DPPH• methanolic solution was prepared at a concentration of 0.2 mM. The DPPH solution was then carefully mixed with varying MJSE concentrations added in a 1:1 (v/v) ratio. The mixture was left in the dark for 30 minutes in order to accelerate the interaction between the extracts and the DPPH• radical. The antioxidant activity of the extracts was evaluated by measuring the absorbance of the solution at 517 nm after the incubation period using a spectrophotometer. The percentage of DPPH scavenging was calculated using the following formula:

$$\text{Inhibition activity (\%)} = \frac{(\text{Ac} - \text{As}) \times 100}{\text{Ac}} \quad (4)$$

where Ac is the absorbance of the control (DPPH solution without the extract) and As is the absorbance of the sample solution.

The half-maximal inhibitory concentration (IC50) is the concentration at which half of the DPPH radicals are scavenged. By examining the concentration-response curves, the IC50 was determined.

Determining anti-inflammatory activity

According to Hemashree and Thangavelu (2018), the extract's anti-inflammatory activity was evaluated. A test solution was made by mixing 0.45 mL of bovine serum albumin (BSA) (42 mg/mL) with 0.05 mL of the extract. After creating a control tube with 0.05 mL of the sample and 0.45 mL of distilled water, 1.0 mg/mL of the diclofenac standard stock solution was added. After 20 minutes at 37 °C, the mixtures were incubated for 10 minutes at 70 °C. After incubation, 2.5 mL of phosphate buffer (PH=7) was added, and the absorbance at 660 nm was measured. The percentage inhibition of the anti-inflammatory activity was calculated using the following formula:

$$\text{Anti} - \text{inflammatory activity } (\%) = 100 - (\text{AT} - (\frac{\text{AP}}{\text{AC}})) \times 100 \quad (5)$$

Where. AT is the test solution; AP is the product control, and AC is the test control.

Osmotic fragility test (OFT)

To evaluate the effect of. MJSE on the hemolysis of normal red blood cells, the Osmotic. Fragility. Test (OFT) was performed (Sundaram *et al*., 2011).

The red blood cell pellet is washed three times with a normal saline solution to remove any remaining plasma proteins or. EDTA. Subsequently, 1 ml of the washed red blood cells is suspended in 9 ml of .0.9%. NS is to achieve the desired cell concentration for the hemolysis experiment.

Osmotic fragility of RBCs was measured according to the ability of RBCs to resist lysis in different concentrations of NaCl solutions (0.3%, 0.45%, 0.6%, 0.9% w/v). In order to set up a positive control, a solution of distilled water (DW) was used instead of NaCl. For sampling, 500 µL of washed RBCs was mixed with 500 µL of MJSE and 1500 µL of the corresponding salt solution in a test tube. The mixtures were incubated at room temperature for 30 minutes, then further incubated at 37 °C for another 30 minutes. The samples were later centrifuged for 10 minutes at a rpm of 4000-5000 after incubation. The remaining liquid was carefully put into another cuvette with a micropipette, and the absorbance of the supernatant was measured at 540 nm using a spectrophotometer. The percentage of hemolysis for each NaCl concentration was determined using the following formula:

$$\text{Hemolysis } (\%) = \frac{\text{As} - \text{A } 0.9\%}{(\text{ADW} - \text{A } 0.9\%)} 100\% \quad (6)$$

where: *As* is the absorbance of the sample, *ADW* is the absorbance of the positive control, and *A*0.9 % is the absorbance of the 0.9% NaCl solution.

Lipid peroxidation assay

The. Human. MDA (Malondialdehyde). ELISA Kit. (ELK Biotechnology CO., LTD., USA) was used for the estimation of lipid peroxidation. This assay targets the MDA marker of lipid peroxidation explicitly—increased levels of. MDA indicates higher levels of oxidative stress and potential cell damage. The blood samples and the extract were incubated for 3 hours at 37°C in the microtiter plate provided. Human. MDA ELISA Kit. Samples or standard solutions containing known. MDA concentrations were loaded into wells. An antibody specific to MDA, linked to Biotin, was then added to bind any MDA present. After washing to remove unbound molecules, a Streptavidin molecule conjugated to the enzyme Horseradish Peroxidase (HRP) was introduced. This. Streptavidin binds to the. Biotin on the antibody-MDA complex effectively links HRP to wells with MDA. Following another wash, a substrate 3,3′,5,5′-. Tetramethylbenzidine. (TMB) solution was added to each well, and HRP reacts with it, generating a colored product. The stop reagent was added to the wells; the liquid turned yellow upon the addition of the stop reagent. The intensity of this color, measured at 450 nm, is proportional to. MDA concentration. A standard curve constructed with known MDA concentrations was used to determine the MDA content in the test samples by comparing their absorbance readings.

Statistical analysis

The data were displayed using mean ± standard deviation (SD). The Mann-Whitney U test was utilized to determine significant differences between groups by analyzing the mean difference. P-values were considered statistically significant if they were less than 0.05. The statistical analysis was performed using Graph Pad Prism 8.0.3 software.

RESULTS AND DISCUSSION

Extraction yield calculation

The extraction yield percentage was calculated by dividing the weight of MJSE (15.1 g) by the weight of the dried peels (50.0 g), then multiplying the result by 100%. This yield percentage was 30.2%.

Total proteins

BSA served as the reference, and the protein content was determined using the Biuret method. The findings suggest that MJSE contains a specific quantity of protein. The protein content of the MJSE was 0.85 ± 0.009 mg/mL, as shown in **Table 1**.

Total phenolic content

The Gallic acid equivalent method was used to measure the total phenolic content in MJSE at a wavelength of 750 nm. The regression equation (y=0.009x+0.0736) (R2 = 0.9941) with a coefficient of determination 0.9997 was prepared from the calibration curve of gallic acid. Thus, as indicated in **Table 1**, the total phenolic content (TPC) in MJSE was found to be 16.2 ± 0.011 mg GAE/g of dry extract.

Carotenoid content estimation

The concentrations of. β-carotene and lycopene were calculated based on the absorbance values obtained. The results of the calculations indicated that. β-Carotene. Content was 0.079

mg/kg, which corresponds to 0.0032% of the sample, and Lycopene content was 0.225 mg/kg, which corresponds to 0.073% of the sample **(Table 1)**.

Table 1. The content of total protein, total phenols and carotenoids in MJSE.

	Total protein (mg/mL)	Total phenol (mg/g)	β. Carotenoid (mg/kg)	Lycopene (mg/kg)
MJSE	0.85 ± 0.009	16.2 ± 0.011	0.0798± 0.013	0.225± 0.045

Mean ±SD, n=3.

LC-MS/MS analysis of MJSE for phenolic profile

The LC-MS/MS analysis of MJSE revealed the presence of various phenolic compounds. MJSE showed high levels of ascorbic acid. (7.720 µg/mg) and. catechin. (22.800 µg/mg), as well as gallic acid. (0.188 µg/mg), Carnosic acid. (0.852 µg/mg), and. apigenin. (0.0722 µg/mg). The extract also contained notable quantities of other phenolic compounds **(Table 2)**.

Table 2. LC-MS/MS Analysis of MJSE for Phenolic Profile

Sample Name	Result in µg/mg
Ascorbic acid	7.720
Gallic acid	0.188
Catechin	22.800
Carnosic acid-03	0.852
Apigenin	0.0722
Vanillic acid	5.360
Apigenin-7-glucoside	0.0514
Quercetin	0.85
3-O-methyl quercetin	0.0874
Ferulic acid	2.460
Syringic acid	0.526
Hesperidin	0.0672
Luteolin	0.001926
Resveratrol	0.082

Lipid profile of MJSE

Table 3 and **Figure 1** present the lipidomic profile of MJSE. The GC-FID analysis of the FAME revealed that the sample contained 17 distinct fatty acid methyl esters. These esters' concentrations were expressed in parts per million (ppm). The most prevalent FAMEs were methyl esters of Octanoic acid (C8:0) at 328,758 ppm, Decanoic acid (C10:0) at 435,383 ppm, and undecanoic acid (C11:0) at 1,590,401 ppm. The analysis also identified a number of other saturated and unsaturated fatty acid methyl esters.

Table 3. Lipid profile of MJSE.

Fatty Acid Methyl Ester	Concentration (ppm)	Percentage
Undecanoic acid (C11:0)	1,590,401	39.4%
Decanoic acid (C10:0)	435,383	10.8%
Octanoic acid (C8:0)	328,758	8.2%
Butanoic acid (C4:0)	193,475	4.8%
Hexanoic acid (C6:0)	256,718	6.4%
Dodecanoic acid (C12:0)	255,393	6.3%
Tetradecanoic acid (C14:0)	152,017	3.8%
cis-10-Pentadecenoic acid (C15:1)	106,392	2.6%
Octadecatrienoic acid (C18:3, n-6)	110,736	2.7%
Total	4,032,367	100%

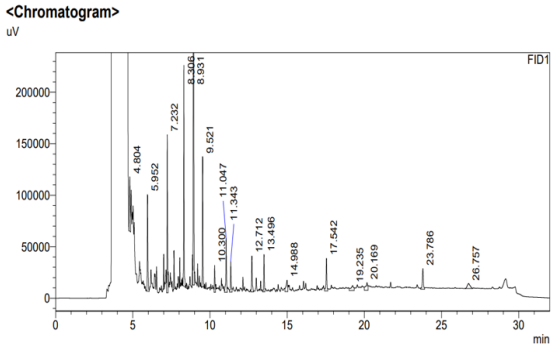

Figure 1. The Chromatogram of the lipid profile of MJPE

Anti-inflammatory activity

The extract's ability to reduce inflammation was assessed using a BSA denaturation experiment. The results demonstrated that the extract inhibited protein denaturation in a concentration-dependent manner. Potential activity (%) for the extract was 94.9, 94.5, 93.1, 91.3, and 88.5 at 1000, 500, 250, 125, and 62.5 µg/ml, respectively. These results imply that the extract has significant anti-inflammatory potential, with the highest activity observed at the highest tested concentration **(Table 4)**.

Antioxidant activity

The DPPH radical scavenging method was used to determine the ability of the MJSE to eliminate free radicals, and gallic acid was used as a standard. The MJSE has antioxidant activity of 31.01± 0.74 % with an IC50 value of 1.7124µg/ml **(Table 4)**.

Table 4. Percentage of antioxidant and anti-inflammatory activity of MJSE with IC50 values

Activity Assay	Inhibition %	IC50 (µg/mL)
Antioxidant	31.01 ± 0.74	1.7124
Anti-inflammatory	94.9± 1.52	2.065

Mean± SD, n=3.

Osmotic fragility test

The hemolysis experiment with and without MJSE demonstrated varying levels of hemolysis **(Figure 2)**. Samples with MJSE consistently showed less hemolysis than those without, across the different salt levels. This protective effect seemed to be influenced by the amount of MJSE used. The salt concentrations with MJSE resulted in even less hemolysis, suggesting that the extract has an anti-hemolytic effect on RBC cells.

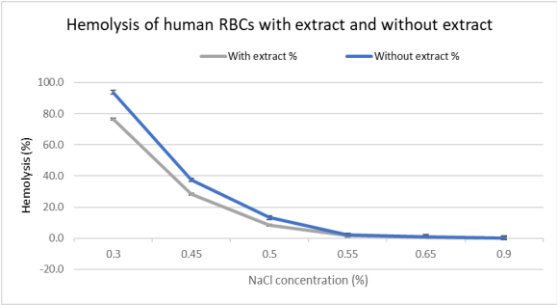

Figure 2. Osmotic Fragility of RBCs (%).

Lipid peroxidation of MJSE

The impact of MJSE on lipid peroxidation in the blood of individuals was assessed by measuring MDA levels. The mean MDA concentration was notably high in blood samples (0.19 ±0.008 pg/mL). Further analysis showed that the presence of Vitamin C and Gallic acid had a significant effect on MDA levels in samples, as shown in **Table 5**. The One-sample t-test results confirmed the significance of these findings, with all comparisons showing statistical significance ($p < 0.05$), which means there was a highly significant effect of MJSE on lipid peroxidation.

Table 5. The concentration of MDA (pg/mL).

Sample	MDA Concentration (pg/mL)
Normal RBCs	0.19 ± 0.01
NRBCs with Vitamin C	0.098 ± 0.008
NRBCs with Gallic Acid	0.0882 ± 0.009
RBCs with MJSE	0.13 ± 0.005
RBCs with MJSE + Vit C	0.07 ± 0.007
RBCs with MJSE + Gallic acid	0.055 ± 0.014

NRBCs: Normal RBCs; Vit C: Vitamin C

The extraction yield of the. MJSE was calculated to be. 30.2%, suggesting efficient extraction. method was employed. Previous studies on plant-based extractions typically report yields in the range of. 20-35% for aqueous extractions (Ramesh *et al*., 2007). The yield demonstrates efficient extraction, which could be attributed to optimized extraction parameters or inherent properties of the sample. M. jalaba seeds.

The protein content of. MJSE was measured using the Biuret method, yielding a concentration of 0.85 ± 0.009 mg/mL. This value is considerably higher when compared to the protein content reported by Atrooz *et al*. (2024) found that the seeds contain 0.7118 ± 0.035 mg/mL on a dry weight basis.

The total phenolic content (TPC) of MJSE was 16.2 ± 0.0811 mg GAE/g of dry extract. This is rather minor when compared to the findings of Sarray *et al*. (2020). The highest concentrations of phenolic chemicals were found in the flowers of Mirabilis Jalapa (2977.41 ± 59.55 µg/mg), followed by the herb (304.25 ± 6.08 µg/mg), fruits (67.92 ± 1.36 µg/mg), and roots (12.44 ± 0.25 µg/mg) (Sarray *et al*., 2020).

The concentrations of β-carotene and lycopene were 0.079 and 0.225 mg/kg, respectively. Compared to other studies, such as the one by Bing *et al*. (2022), which demonstrated that M. jalaba had more carotenoids. However, the presence of these carotenoids in MJSE increases the likelihood of additional health benefits, like antioxidant activity. However, the addition of carotenoids improves MJSE's overall nutritional profile.

The. LC-MS/MS analysis. revealed the presence of various phenolic compounds, including high levels of ascorbic acid and catechin, along with notable quantities of gallic acid, vanillic acid, and quercetin derivatives. The concentrations of these compounds are comparable to or higher than those found.in other antioxidant-rich plants such as green tea and berries (Carlsen *et al*., 2010). This diverse phenolic profile underscores the potential health benefits of MJSE, particularly in terms of antioxidant and anti-inflammatory activities.

The GC-FID analysis identified 17 fatty acid methyl esters in MJSE, with high concentrations of undecanoic, Decanoic, and Octanoic acid methyl esters. The total concentrations of all identified FAMEs were 4,032,367 ppm. These values suggest that MJSE has a complex lipid profile, similar to other seeds known for their health benefits, like chia and flax seeds (Saphier *et al*., 2017).

MJSE exhibited significant. anti-inflammatory activity, with concentration-dependent inhibition of protein denaturation. At a concentration of 1000 µg/ml, the extract showed 94.9± 1.52% inhibition. This is comparable to the standard. anti-inflammatory agents such as diclofenac, which show similar inhibition percentages. The results highlight MJSE's potential as a natural anti-inflammatory agent.

The DPPH radical scavenging assay indicated that MJSE possesses antioxidant activity. 31.01 ± 0.74%, with an. IC50 value of 1.7124 µg/mL, which is notably lower than that of ascorbic acid (89.5 µg/mL). This suggests that. MJSE is a more potent antioxidant compared to ascorbic acid, aligning with studies that have reported strong antioxidant properties in extracts from. Mirabilis Jalapa (Harun-Or-Rashid *et al*., 2023).

The lipid peroxidation assay revealed a mean. MDA concentration of. 0.19 ± 0.008 pg/mL in normal. RBCs, indicating oxidative stress. The presence of. Vitamin. C and gallic acid were significantly reduced. MDA levels. The protective effect of these antioxidants in conjunction with. MJSE demonstrated a synergetic effect. These findings are corroborated by previous research indicating that antioxidants can mitigate lipid peroxidation and oxidative damage (Chen *et al*., 2023).

CONCLUSION

The study on. Mirabilis Jalapa seed extract highlights its substantial extraction yield, protein content, phenolic compounds, and diverse phenolic and lipid profiles. MJSE exhibits strong antioxidant, anti-inflammatory, and anti-hemolytic properties, which are corroborated by the high phenolic content and significant inhibition of lipid peroxidation. The presence of. various beneficial phenolic compounds and fatty acids further underscore its potential health benefits.

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