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Effect of *Mirabilis jalapa* Plant Fortified with Nano-Quercetine to Accelerate Wound Healing in Vivo

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Given the problem of incomplete wounds affecting the skin, which leads to tearing and damage to its parts in accidents, investigation and discovery of effective medicines for wounds affecting the skin has become an important aim. Therefore, it is necessary to continue studying and experimenting with new and effective medicines, including effective substances and the discovery of effective nanomaterials. To reach efficient treatments to treat such injuries. In this study, Nano-Quercetine was prepared by grinding. This material was examined by (FTIR), (XRD) and then (FE-SEM) was used, and the results obtained were on the nanoscale with sizes ranging between (13.40-44.66 nanometers). The effectiveness and effect of the materials prepared from nanoparticles and Mirabilis Jalapa plant were studied in the living body and at different concentrations (10, 50, 100) mg on wounds in the skin of laboratory mice. The results of the study showed the effectiveness and speed of healing of wounds that affect the skin of laboratory mice using a Nano-Quercetine preparation supported by Mirabilis Jalapa plant extract. The result of wound healing was significant ($p<0.05$) on the eighth day of the experiment, as the average and standard deviation of healing were (1.66±0.577) for the concentration of 10 mg, (0.00±0.000) for the concentration of 50 mg, (0.00±0.000) for the concentration of 100 mg, compared to the second day of the experiment, which was (28.00± 0.005, 26.96± 0.398, 25.07±0.887) for concentrations 10 mg, 50 mg, and 100 mg, respectively. The results of the study showed effective activity and rapid healing of wounds affecting the skin of laboratory mice using the nano-complex of the alcoholic extract of the Mirabilis Jalapa plant fortified with quercetin. It is one of the medicinal plants that have great therapeutic value and are important vital sources. When the active substances are loaded onto the surfaces of Nano-Quercetine, it leads to their reaching the target, as plant materials are natural compounds that have enormous potential as antioxidants and are of great importance in providing health benefits against many diseases. Each part of the plant has its medicinal properties that possess different types of secondary metabolites that play an important role in treating different types of diseases, including wounds that affect humans and animals.

Keywords: Mirabilis Jalapa, nano-quercetine, wound healing, nanoparticles, antioxidants, extracts, healing efficacy.

INTRODUCTION

Wounds are defined as a breakdown in the skin or in the Mucous membrane, which facilitates the entry of microbes, leading to infection (Falanga *et al.*, 2022). The injury causes suppuration of the damaged area, which in turn leads to the formation of wound abscesses, and exposed wounds are also susceptible to infection with various types of microscopic organisms (Eriksson *et al.*, 2022). Sometimes serious wound infections cause death or illness. Sometimes serious wound infections cause death or illness. Non-healing wounds are a major medical problem. To solve this problem, researchers around the world have tried to use numerous products and medicines to treat acute and chronic wounds (Al-Snafi, 2021). Many experimental and pre-clinical studies have described the beneficial effects of treating wounds with natural materials. Medicinal plants and herbs are considered all over the world. All over the world, valuable materials are used in treating various diseases, due to their ease of use, effectiveness, and low cost when compared to expensive chemical treatments (Adeyemi *et al.*, 2022). Phytochemicals are natural compounds that have enormous antioxidant potential and are of great importance in providing health benefits against many diseases. Each part of the plant has its medicinal properties that possess different types of secondary metabolites that play an important role in treating different

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types of diseases (Liya *et al.*, 2021). Natural products contribute to the development of important therapeutic drugs currently used in modern medicine and are a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Zang *et al.*, 2021). The Mirabilis Jalapa plant is one of the medicinal herbs that has a therapeutic effect on many diseases that humans face in stages. His different lives (Peiris *et al.*, 2022). It is used in the treatment of many diseases as a medicinal substance and has a therapeutic effect on many conditions such as digestive system disorders as a laxative. It is also used in skin diseases as an antidote for itching, pimples, skin ulcers, wounds, and scratches. It is used by many women against many fungi and microbes present in the uterine area. The reproductive organs and the Mirabilis Jalapa herb have an effective effect against intestinal worms of all shapes and types that appear in the majority of children (Teja *et al.*, 2022). In its purified form, Quercetine is characterized by its crystalline hardness and yellow color at room temperature, with weak solubility in water, but its solubility increases in alkaline aqueous solutions and alcohols. Quercetin is widely found in many vegetables and fruits, including pears, spinach, soybeans, Coffee, tea, oats, onions, garlic, and broccoli (Forni *et al.*, 2022). Quercetin is a plant pigment and secondary metabolite, and it is one of the effective plant flavonoids that has antioxidant activity (Mahdi *et al.*, 2019). Quercetin is a natural compound that has many pharmacological properties, such as anti-inflammatory and diverse biological activities, including antiviral, anti-nervous, cardiovascular, anti-diabetic, liver disease prevention, reproductive system protection, anti-obesity agent, anti-malarial agent and is used in the synthesis of hybrid molecules. Which carries the drug (Mahdi *et al.*, 2019). There is a wide range of nanoparticles synthesized with unique properties used in a wide range of biological applications (Beus *et al.*, 2023). Various physical and chemical properties of nanomaterials such as size, shape, composition, surface charge, and aggregation affect their biomedical applications and the size of the nanomaterial is mainly dominant while other properties are controlled because reducing the size of the nanomaterial provides an opportunity to improve the absorption and interaction potential with biological tissues to a greater extent (Abbasi *et al.*, 2023). Hybrid nanomaterials are being developed to regulate sensing functions in the field of nanomedicine and the pharmaceutical industry. The mechanical strength, chemical stability (Abdul Latif and Mahdi Alzubaidy, 2023; Alzubaidy and Hussain 2022) durability, and flexibility of hybrid nanomaterials make them suitable for the development of healthy human life and important applications such as drug delivery, antimicrobial effects, nutrition, orthopedics, dentistry, and the innovation of antibiotics, antifungals, and antifungals. oxidation and improved wound healing (Vig *et al.*, 2022). The following are the aims of the study:

1. Preparation of Nano-Quercetine carried the plant extract of *Mirabilis Jalapa*.
2. Investigating the effect of Nano-Quercetine on wound healing and conducting in vivo assessments.

MATERIALS AND METHODS

Investigating the effect of Mirabilis Jalapa extract and Nano-Quercetine on wound healing in vivo. Mirabilis Jalapa was collected from the gardens of Diyala Governorate, and the species was confirmed by classifying it by the College of Science/University of Baghdad based on Flora of Iraq.

Preparation of hot alcoholic extract: Mirabilis jalapa leaves were extracted using the Saxolite system and ethanol as a solvent according to the method of (Hanani *et al.*, 2017). where 100 grams of the leaf powder were placed in a funnel-shaped filter paper and tightly closed to avoid the release of the plant powder. After that, it was placed in the Saxolite device and placed on it. 500 ml of solvent for 24 hours, then the solution was concentrated using a rotary evaporator device, and the solvent was separated, resulting in a thick liquid of a dark greenish-brown color. The filtered extract was stored in the refrigerator at a temperature of 4°C for later use.

Detection of plant active compounds: The active compounds in the leaves of the Mirabilis Jalapa plant were detected by using (GC-MS) for the alcoholic extract of the leaves.

GC-MASS Analysis of Essential Oils: The essential oils were analyzed in the laboratories of the Basrah Oil Company by gas chromatography (GC) using a flame ionization detector (FID) and (GC - MS) using Agilent GC - mass detection system (MSD) with the use of helium as a carrier gas, the separation conditions in the device and the volume of the extract injected into it were adjusted, as in Table 1.

Table 1. Separation conditions of gas chromatography combined with GC-Mass mass spectrometry of the alcoholic extract of leaves Mirabilis Jalapa.

No.	Separation conditions	Information about separation conditions
1	Starting column temperature	40C°
2	Final shaft temperature	300 C°
3	Temperature rise rate	10 C° /min
4	Ionization detector temperature	290 C°
5	Carrier helium gas flow rate	10 Cm²/min
6	Total flow	19 ml/min
7	Column flow	1 ml/min
8	Disinfection flow	3 ml/min
9	Column type	HP-5MS 5 % Phenyl methyl siloxane
10	Shaft Dimensions: Length X Inner Diameter	30 m x 0.25 x 0.25 mm
11	Injection volume	1µL
12	Pressure	7.0699 Psi

Preparation of Nano-Quercetine: Nano-Quercetine was prepared in the laboratory of the College of Science, University of Diyala / Department of Physics, by grinding method and at room temperature. An electric mortar was used, as in Figure 1, and Quercetine was placed in it and ground for 30 minutes. After that, the aforementioned material was emptied into a box. After that, an ivory steel mortar sterilized with ethanol and dry heat was used, and in the process of re-grinding Quercetine in a small amount of 1-2 grams in the mortar, it was Grinding again, then repeating the same process for the rest of the amount of Quercetine until its grinding was complete, and it was filled in an opaque glass box to protect it from the effects of light. After that, a sample of the ground material was sent for examination to ensure that the Nano-Quercetine material was obtained.

Figure 1. (A & B) the electric grinder, (C) the ceramic mortar used in biosynthesis Nano-Quercetine.

Preparation of compounds used in wound treatment in vivo: The compound used in the in vivo experiments was prepared by mixing Nano-Quercetine and Mirabilis Jalapa extract with local butter at concentrations of 10 mg, 50 mg, and 100 mg. Each concentration was placed in boxes as shown in Figure 2.

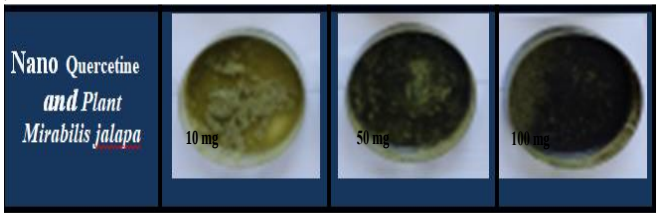

Figure 2. Shows preparation of Nano-Quercetine and Mirabilis Jalapa extract with municipal butter at a concentration of 10mg, 50mg, 100mg.

Statistical analysis: The data obtained were consistently analyzed using the F-test by ANOVA. The values were presented as (mean ± std) of the three repeaters with a significant difference at the significance level > 0.05 (Abdulhameed *et al.*, 2023).

RESULTS AND DISCUSSION

The individual components of the leaf extract were identified by matching data to commercial mass spectral libraries, the Wiley GC/MS Library, the 3 Mass Finder Library, and the Baser Library of internal essential oil components, which includes over 3,200 authentic compounds with mass spectra and retention data from the pure ones. It is effective in the extract, as shown in Figure 3. Table 1 contains the names of these compounds and their composition, and the results were identical to those reached by (Shehabeldine *et al.*, 2022).

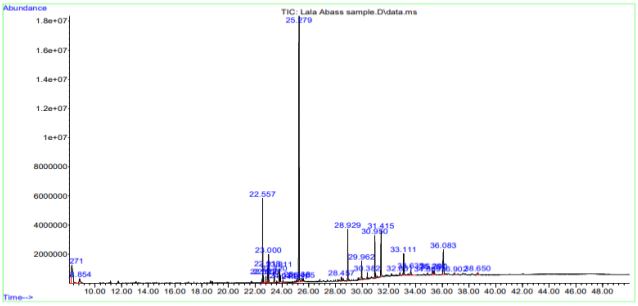

Figure 3. Shows the results of GC-Mass analysis of Mirabilis Jalapa leaf extract.

Results of (FTIR) of Quercetine: The acquisition of quercetine was confirmed using instant infrared spectroscopy, as it was found that there was a band with a wavelength of (3407.04 cm-1) as shown in Fig (4), which indicates the hydroxyl group OH that follows the water molecule and the presence of an absorption band. It is (399.193 cm-1), and these results are consistent with the findings of (Ababutain *et al.*, 2021).

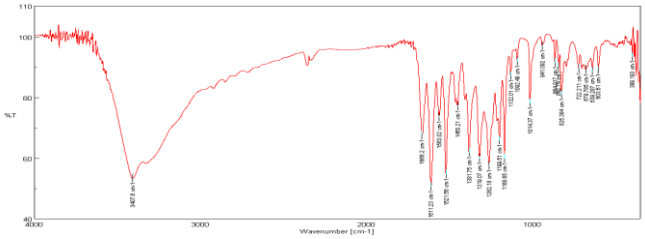

Figure 4. Measurement of infrared spectrometry (FTIR) of Quercetine.

X-ray Diffraction examination: The results of the tests of X-ray diffraction patterns of quercetine showed by analyzing these patterns and knowing the locations of the peaks as in Fig (5), the polycrystalline membrane shows that there are preferable peaks for the growth of crystalline grains in the directions (440),(140),(330),(220) and (200) (Athiyah *et al.*, 2019).

Scanning Electron Microscope (SEM) examinations: The nanostructures of quercetine were characterized using a scanning electron microscope (SEM) as in Figure 5. The image had a magnification of (150 KX). It is noted that most of the quercetine is dense and has irregular spherical shapes, with sizes ranging between (13.40-44.66). nanometer, as these results were consistent with the findings of (Jiang *et al.*, 2022).

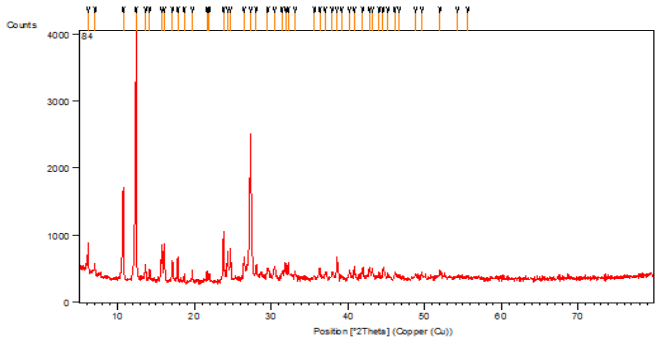

Figure 5. Shows X-ray quercetine diffraction.

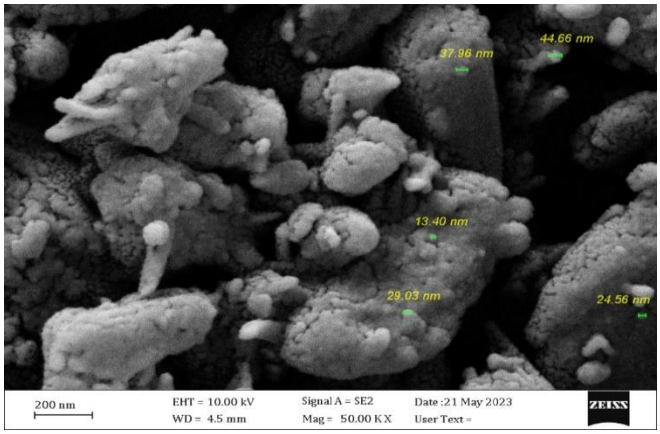

Figure 6. Scanning Electron Microscope (SEM) examinations.

Transmission electron microscope (TEM) tests: Figure 7 represents TEM images of Nano-Quercetine mixed with local butter. Note the shape and size of Nano-Quercetine, which range in size from (4-6; l) (Huang *et al.*, 2023).

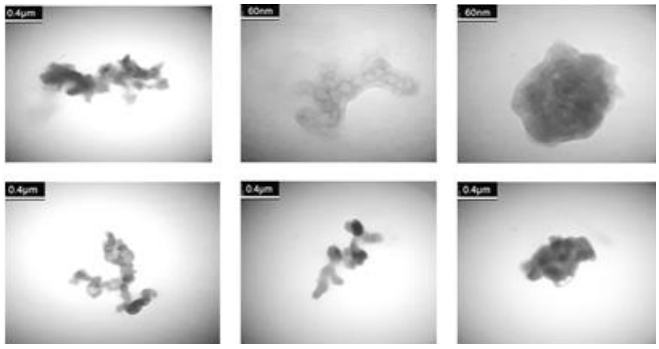

Figure 7. Images (TEM) of Nano-Quercetine mixed with municipal butter.

Results of the effect of Nano-Quercetine mixed with Mirabilis Jalapa extract on wounds in Vivo that were exposed to the following concentrations: The results of the current study show in Table 2 that there are statistically significant differences ($p<0.05$) between the three concentrations used. It was found that there was an increase in the rate of healing of wounds in experimental animals after applying the mixture of Nano-Quercetine mixed with Mirabilis Jalapa extract, as the results appeared from the second day of the study, reaching (28.00± 0.005, 26.96± 0.398, 25.07± 0.887) compared to the eighth day (1.66± 0.577, 0.00± 0.000, 0.00± 0.000). In concentrations of 10 mg, 50 mg, and 100 mg, respectively. The high rate of healing in animals is due to quercetin and its derivatives enhancing the healing of skin wounds in animals, by inhibiting the inflammatory response and accelerating blood vessel formation, fibroblast proliferation, and collagen deposition (Zulkefli *et al.*, 2023). This is because flavonoids possess wound-healing properties due to their anti-inflammatory effects, angiogenesis, re-epithelialization, and their antioxidant effects (Cheng *et al.*, 2023).

Table 2. Effect Nano-Quercetine mixed with Mirabilis Jalapa extract on wounds in Vivo.

Days	Healing Concentrations						P. Value
	10mg		50mg		100mg		
	Mean	± Std	Mean	± Std	Mean	± Std	
T_1	30.00	0.00	30.00	0.000	30.00	0.000	<0.05
T_2	28.00	0.005	26.96	0.398	25.07	0.887	<0.05
T_3	25.85	0.755	22.17	1.055	20.89	0.770	<0.05
T_4	23.43	0.591	21.15	1.065	18.44	0.680	<0.05
T_5	19.74	0.652	17.45	0.577	14.61	0.476	<0.05
T_6	9.66	0.487	7.81	0.323	5.40	0.527	<0.05
T_7	5.33	0.949	2.78	0.383	1.33	0.577	<0.05
T_8	1.66	0.577	0.00	0.000	0.00	0.000	<0.05

1. mg10 concentration: The mice were exposed to Nano-Quercetine mixed with Mirabilis Jalapa extract at a concentration of 10mg. The average length of the wounds on the first day was 30.00 mm, the second day was 28.00 mm, the third day was 25.85 mm, the fourth day was 23.43 mm, the fifth day was 19.74 mm, the sixth day was 09.66 mm, and the seventh day was 05.33 mm. The eighth is 01.66 mm, as shown in Figure 8.

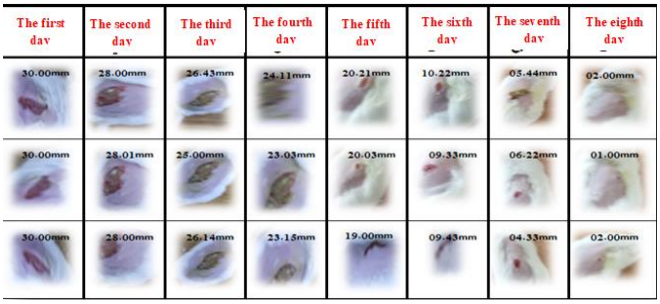

Figure 8. Shows the effect of exposure of mice to Nano-Quercetine mixed with plant extract Mirabilis Jalapa at a concentration of 10mg.

2. mg50 concentration: The mice were exposed to Nano-Quercetine mixed with Mirabilis Jalapa plant extract at a concentration of 50mg. The average length of the wounds on the first day was 30.00 mm, the second day was 26.96 mm, the third day was 22.17 mm, the fourth day was 21.21 mm, the fifth day was 17.45 mm, the sixth day was 07.81 mm, the seventh day was 02.78 mm, and the eighth day was 000 mm. .00mm, as shown in Figure 9.

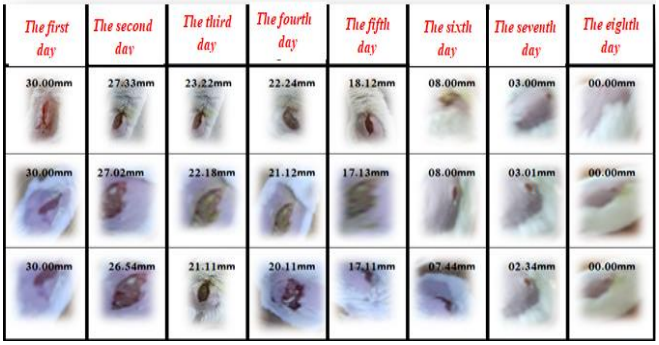

Figure 9. Shows the effect of exposure of mice to Nano-Quercetine mixed with plant extract Mirabilis Jalapa at a concentration of 50 mg.

3. mg100 concentration: Nano-Quercetine mixed with Mirabilis Jalapa extract at a concentration of 100mg was tested on mice. The average length of the wounds on the first day was 30.00 mm, the second day was 25.07 mm, the third day was 20.89 mm, the fourth day was 18.44 mm, the fifth day was 14.61 mm, the sixth day was 05.40 mm, and the seventh day was 01.33 mm. The eighth day is 00.00 mm, as shown in Figure 10.

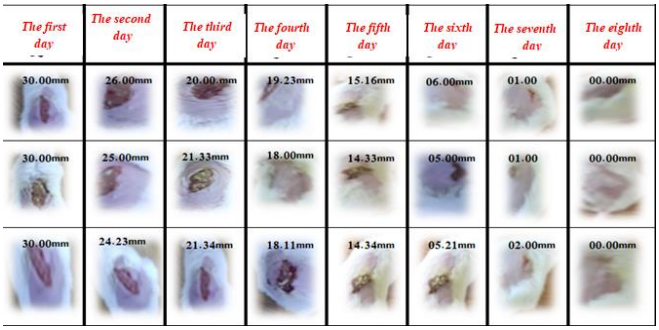

Figure 10. Shows the effect of exposure of mice to Nano-Quercetine mixed with plant extract Mirabilis Jalapa at a concentration of 100 mg.

Through the synthesis of Nano-Quercetine loaded using Mirabilis Jalapa leaf extract, biological characterization reveals that both Nano-Quercetine and phenolic and flavonoid properties, which may be due to the presence of different functional groups on the surface of the particles, showed effective antibacterial properties (up to an inhibition zone of up to 0.00 mm). Nanoparticles have greater potential than non-nanoparticles against bacteria. Moreover, increasing the concentration of the Nano-Quercetine preparation mixed with the Mirabilis Jalapa plant extract used led to wound healing at the required speed, faster than Mebo ointment used to treat wounds in mice quickly. As shown in Figure 11, these results were better than what was achieved by (Nadeem *et al.*, 2019).

Results of effect Mebo Ointment on the wounds of white mice: White mice with wounds were exposed to the application of Mebo Ointment, and the average length of the wounds on the first day was 30.00 mm, the second day was 29.16 mm, the third day was 27.57 mm, the fourth day was 26.47 mm, the fifth day was 25.35 mm, the sixth day was 24.83 mm, the seventh day was 21.68 mm, and the eighth day was 17.34 mm, as shown in Figure 11. The results of the current study show that there is a relative decrease in wound contraction, and we find that wound healing in the skin of white mice through the histological results of treatment with Mebo Ointment, an analgesic and anti-inflammatory, is less active than the preparation used in this study when applied to wounds, perhaps because the preparation used under study is more effective and faster in healing wounds than Mebo Ointment, or because there are bacteria or other microorganisms resistant to Mebo Ointment, and this was consistent with what was reached by (Wang, 2022).

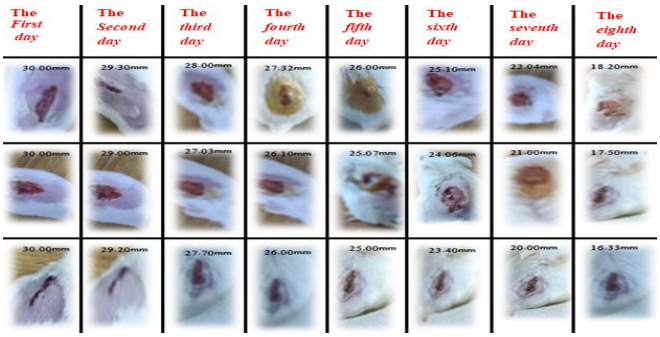

Figure 11. Shows the effect of Mebo Ointment on the wounds of white mice.

Conclusion: The preparation of Nano-Quercetine by grinding (from top to bottom) resulted in the production of a nanomaterial with high specifications and unique sizes ranging between (13.40-44.66 nm). Which was loaded with the active ingredient of Mirabilis Jalapa plant as a compound that proved its effectiveness and effect on skin wounds at different concentrations (10, 50, 100 µg/mg). It was used in live experiments and proved the speed of wound healing and the effectiveness of the preparation. The manufactured Nano-Quercetine loaded with the alcoholic extract of Mirabilis Jalapa plant had a faster effect on wounds in laboratory white mice and was more effective and faster than the effect of Mebo ointment. Histological evaluation showed that the regeneration of the injured skin was completed eight days

after the wound, and the formation of blood vessels and fibroblasts during the wound healing process was described in the skin of mice whose skin wounds were completely healed as a result of the use of the Nano-Quercetine compound mixed with the alcoholic extract of the M. Jalapa plant, which remodeled collagen fibers and regenerated hair follicles, skin, and muscles. This is because the preparation used contains the active substance of flavonoids loaded with Nano-Quercetine, which became a substance that quickly reached a positive effect on the speed of wound healing.

***Author's contributions statement*:** Rasha Ali Salman designed, and completed the experiments; Mohanad W. Mahdi Alzubaidy prepared the draft; Amer Talib Tawfeeq. reviewed and finalized the draft.

***Conflict of interest*:** The authors declare no conflict of interest.

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Availability of data and material: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

Code availability: Not applicable.

Consent to participate: All authors participated in this research study.

Consent for publication: All authors submitted consent to publish this research. article in JGIAS.

***SDG's Addressed*:** Good Health and Well-being, Responsible Consumption and Production, Life on Land, Industry, Innovation, and Infrastructure.

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