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Antibacterial, Antifungal and Phytochemical Properties of *Alcea rosea* Leaves Extract and Evaluate its Contribution to Healing Wound that Infected by *Staphylococcus aureus*

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Abstract

Medicinal plants have been used to treat various infectious illnesses in humans, a significant component of traditional medicine. Medicinal plants are currently considered a promising alternative in treating diseases caused by antibiotic-resistant microorganisms, one of the most essential therapeutic problems. *Alcea rosea* is a medicinal plant used for multiple therapeutic properties. This study investigated the antibacterial, antifungal, antioxidant and phytochemical properties of *A. rosea* aqueous extract leaves. It evaluated its contribution to healing wounds Infected by *Staphylococcus aureus* to verify its ability to heal wounds scientifically. Aqueous extracts of the *A. rosea* leaves were tested using a rat dermal excision wound model. The antibacterial activity was assessed using the broth dilution test; the antioxidant activity was evaluated using the total antioxidant capacity, phenolic and flavonoid content, ferric reducing power, and DPPH free radical scavenging. Topical use of extract creams demonstrated significant wound healing. On the 10th day, the groups treated with conventional medication and blank cream, 10% stem bark and leaf creams, and 95.8%, 96.3%, and 73.9% total wound surface closure were noted, respectively. Significant collagen formation, sparse infiltrations of inflammatory cells and re-epithelialization were observed histopathologically in the sections of healed tissue. In contrast, isolated regions of dispersed inflammatory cells and an abscess were seen in the tissues from the blank cream treatment group. *S. aureus* was susceptible to the antibacterial properties of the methanol stem bark extract.

Keywords*: Alcea rosea* , antioxidant , phytochemical , antibacterial, antifungal , wound healing.

1- Introduction

Experiences gained from traditional uses have practically proven that plants contain substances with medicinal properties that can be used as raw materials for the manufacture of certain drugs that have commercial value, To prevent problems related to drugs, researchers have sought to replace chemical drugs with drugs made from natural plant ,This

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does not mean that medicinal plants do not also contain chemicals, as is the case with synthetic drugs(Jamshidi *et al*,2017). which have the potential to cause relatively severe harmful side effects, Investigating the effects of biologically active components in medicinal plants can help determine our use of these plants (Banaee *et al*,2011; Jamshidi *et al*,2017).

For more than 6,000 years, humans have used Hollyhock or Gulkhaira, is the common name for the plant Alcea rosea for pharmaceutical purposes. Studies in recent years have shown that parts of this plant from seeds, roots, flowers and leaves have antimicrobial, anti-inflammatory, analgesic, antioxidant, antitussive and immunomodulatory effects, it has also been used to treat fever, digestive problems and even skin burns (Banaee *et al*,2011; Choi *et al*,2012).

Phytochemical compounds phenol, flavonoids, fatty acids, proteins, and polysaccharides, and contain alkaloids, flavonoids, steroids, terpenoids, glycosides, and saponins as a secondary plant metabolites that acquired significance due to their various pharmacological properties, such as hypolipidemia, analgesia, anti-inflammatory and so forth. (Faujdar et al, 2016).

As a result of the public health concern over antimicrobial resistance and tolerance, alternative antimicrobial agents concept will inevitably be developed as a replacement For industrial pharmaceutical antibiotics that are designed to target both humans and animals within the same health category (Ersanli et al., 2023). *S. aureus* s a well-recognized bacterium commonly linked to wound infections along with other several types of infections bacteria these bacteria typically invade the wound's outermost layer (Serra et al, 2015).

Wound infections induced by S. aureus could be considered a possible Methicillin resistant *S. aureus* (MRSA) risk factor concern (Almeida et al, 2014). A Gram-positive bacterium known as *S. aureus* has been associated of certain diseases which acquired from both hospital and community settings, it is a very common opportunistic human pathogen that is associated to the morbidity and mortality in a high rate of (Abbasian et al , 2018).

Especially (MRSA) *S. aureus* is known as a biofilm when it adheres to living or inert surfaces. Biofilm is an extracellular polymeric material composed of polysaccharides, proteins, nucleic acids and water, which acts as a physical barrier preventing the permeability of the drug to the bacterial community, which helps the microbe to resist and reduce the effect of antibiotics on it. (Idrees et al , 2021). The scientific community is now very interested in creating herbal remedies with antimicrobial activity as a safer, more environmentally friendly substitute (Taiwo & Adebayo,2017).

Using medicinal plants to treated a variety of infectious diseases is a major component of traditional medicine. Herbs such as *A. rosea* have long been utilized as antibacterial agents and wound healers. (Nazir et al,2022).

Plant antioxidants are members of the class of free radical scavengers agents , which means that they will inevitably to control the damaging effects that these unstable species

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have on the human body and functions as a treatment for few disease complications (Haider, 2020).

The skin is the largest organ in the body, providing a protective barrier between the body and the external environment against pathogens, hazardous materials, mechanical and thermal damage, and accidents. On the other hand, an appropriate wound healing strategy must be chosen, especially when performing a specific surgery that inevitably leads to surgical wounds on the body, in order to reduce the risk of complications. The skin has developed effective and rapid mechanisms in a process that is a complex and dynamic process supported by a myriad of cellular events known as the wound healing response to efficiently repair damaged tissue. (Han, 2023;Wilkinson& Hardman 2020). Wound repair is classically simplified into four main phases: Hemostasis Phase Occur immediately, Blood vessels constrict to stop bleeding and finally of this phase is Blood clot form. Inflammation Phase Occurs within 4 days, Neutrophil & macrophage work. To remove debris, Infection prevention.

Proliferation Phase Occurs within 2 weeks, wound rebuilds connective tissue for protection, Granulation of the skin promoted, Tissue repair. Remodeling Phase Occurs anywhere from 24 days to a year , New epithelial tissue forms (new healthy skin)(Palta *et al*,2014**)**.

2- Materials and Methods

2.1. Collection, identification, and preparation of Alcea rosea materials

Kia pink papers were completely collected from Karbala governorate, Iraq, during the month of December 2023, and the sample was documented and deposited at the College of Applied medical sciences, Department of Environmental health of Kerala University, to be referenced in the future. Leaves were washed, cleaned of dust with water, and left to dry under sun light. The dried leaves were ground to a coarse powder using the mill and they were stored for later use.

2.2.Methods of Extraction

In preparing the aqueous extract of A.rosea, the soaking method and the digestion method were used at the same time in an overlapping manner. 10 grams of A.rosea leaf powder, which had been prepared previously, were weighed and soaked in a beaker containing 200 ml of water for one day with shaking Every now and then using a shaker at room temperature. The extract was placed on a magnetic stirrer at 30 °C for 1 hour, and the extract was filtered using Whatman

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No. 1. Finally, the waste was reprocessed using a centrifuge to get rid of fine impurities for 10 minutes, at a rate of 2500 rpm, and then the samples were stored in the refrigerator until later use(Khoshnamv *et al*,2019).

2.2.1 GC mass- spectroscopy of A. *rosea*

The aqueous solution was cured to investigate the volatile compounds that could be present in the aqueous extract of *A. rosea*.

2.2.2 Antioxidant study

Used The 1,1-diphenyl-2-picrylhydrazyl (DPPH) to emulate the antioxidant activity of aqueous extract of A. rosea. The free radical DPPH is stable. DPPH is reduced by antioxidants to 2, 2-diphenyl 1-picryl hydrazine, which has a wavelength of 517 nm. The most standard kind of antioxidant is ascorbic acid. The test is carried out using the Williams et al. brand technique. The reaction mixture employed to assess this activity comprises a 3.9 mL methanolic solution of DPPH and 0.1 mL methanolic solution containing various extracts (12.5, 25, 50, 100, and 200 µg/mL, similar concentrations were used for the ascorbic acid, which was used as a standard.

Moreover, the methanol was used as a blank. The combination was allowed to incubate exactlly for half an hour at room temperature when it's darkness. Awavelength of 517 nm was used to measure the absorbance. All extracts' percentage of DPPH radical scavenging activity was measured and compared with ascorbic acid acts as a standerd. (Mali et al., 2023).

2.3.Antibacterial assay for aqueous in vitro

Antibacterial testing was performed by agar well diffusion method for A. rosea aqueous extracts as described by (Nazir et al. 2022) with some modifications. Using a sterile cotton swab soaked with a pathogenic bacterial suspension solution (0.5 Mc Farland), Staphylococcus aureus was spread on plates containing Mueller-Hinton agar where the Staphylococcus aureus strain was obtained from the specialized Al-Rasoul Laboratory accredited by the Iraqi Ministry of Health - Holy Karbala and diagnosed by VITEK system (Johnson, J. R et al 2012). One well (5 mm in diameter) was drilled on the surface of M.H. Agar. The well was filled with 50 µl of *Alcea rosea* leaf extract after leaving it for thirty minutes at room temperature to allow the medicinal *Alcea rosea* extracts to diffuse into the plate agar before bacteria began to grow. Distilled water and some types of antibiotics such (1: Cephalexin (CL 5 µg) 2: Vancomycin (VA 5 µg) and 3: Gentamicin GEN 10 mcg as a negative and positive control . At 37°C for 24 hours, the plates were incubated, after which the antibacterial activity was determined using a standard micrometer and measuring the diameters of the growth inhibition zone in millimeters (mm) (Hussain *et al*. 2014).

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2.4Antifungal Activity Assay

The antifungal test was performed using the well-in-agar method. After pouring Sabouraud dextrose agar (SDA) medium into Petri dishes and allowing it to solidify, a sterile cork punch was used to create a 5 mm well in the medium used. After that, (85 ml) of the aqueous extract was transferred to different wells. At a temperature ranging from 28 to 31 °C for 48 h, the dishes were incubated, and then the inhibition zone was measured after 48 h. For each extract in the well, three duplicate were made from it . (Muzafar *et al* , 2012) .

2.5.In vivo Antibacterial and wound healing study

2.5.1.Experimental animal

Twenty mature rats were brought from 180 - 200 grams from the animal house at Karbala University, College of Pharmacy. It was transferred to a private room at an appropriate temperature and humidity . Female mice are placed in plastic cages with dimensions of 30 x 50 cm. When an appropriate amount of food and water is placed in the cage every day to complete the experiment. The sawdust is changed to cages every week. To adapt, the mice remained in the house of animals for ten days before the start of the experiment (Leng *et al*, 2020).

2.5.2.Experimental design

The experiment involved the used female Sprague-Dawley rats (S.D.R.) which ten-week-old & weighing (250–270 g) in cages in the animal house and fed a commercial diet and water. The experiments involved 24 (S.D.R.) divided into 4 groups (n = 6). Each animal was anesthetized with 70% chloroform. Using a scalpel, toothed forceps and scissors, full-thickness circular excision wounds measuring approximately (1 × 1 cm^2) were made along the marks using techniques similar to those in the excision wound model. Animals in groups (2, 3 and 4)were inoculated with a (50 µl) suspension containing (1 × 106 CFU/ml) of bioluminescent *S. aureus* with a pipette tip over each marked area containing the wounds three minutes after the wound, while animals in group (1) were used as a control group and those infected in this group no received any treatment. The wounds of the animals in groups 1&2 included clean wounds that were not infected with S. aureus and which treated for 16 days with A. rosea leaf extract. The wounds of the animals in groups3&4 contained infected wounds with S. aureus and treated with Alcea rosea leaf extract for 16 days too. measuredThe size of the injury and photographed on days 2^{ed} , 8^{ed}, and 16^{ed} after treatment

2.6. Statistical analysis

The IBM/USA software statistics package for social sciences (SPSS) version 24 was used for this study's statistical analysis, where the data were expressed as a mean standard error of the

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mean, and independent-sample t-tests with 95% confidence intervals were used to find differences in means between two groups in the same categorical category, $P \leq 0.05$ was considered statistically significant, the significance level was specified as * between the groups. The probability level was stated as * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$,**** $P \leq 0.0001$ and Ns
:Nonsignificant.

3- Results

The obtained 69 compounds were drawn using ChemDraw 15 software, as shown in Figure 1. The highest percentage was mentioned in the preceding section of the compounds was 4(1H) Quinolinone, 2-(methylthio)-1-phenyl- 31.70%. By using hot extraction method with methanol, ethyl acetate and hexane, their phytochemical screening showed to present tannins, saponins, flavonoids, terpenoids, and cardiac glycosides**,** The photochemistry of the aqueous extract of A. rosea was cured using GC-mass spectroscopy to identify volatile chemical compounds, the results showed sixty-nine compounds were obtained from the leaves. as shows in table 1 Six compounds were observed in highest percentage including 2-(Cinnamyloxy)-3-methyl-1-nitrobu tane, 8.90% Hexanoic acid, tetradecyl ester 8.90%, 2-(4-Nitrophenyl)cyclopropyl phenyl ketone14.08%, Ethanone, 1-(2,3-dihydro-5-benzofuryl)-, oxime-, o-benzyl- 14.08%, N-Benzyl-2-aminocinnamate, methyl ester 7.18% and 4(1H) Quinolinone, 2-(methylthio)-1-phenyl- 31.70%, while three compounds were identify with low percentage including Naphtho[2,1-b]furan, dodecahydro-6 ,9a-dimethyl-, [3aS-(3a.alpha.,5a. alpha.,6.beta.,9a.beta.,9b.alpha.)]- 0.13%2-Biphenylenecarboxylic acid, 3-[(acetyloxy)methyl]-, methyl ester 0.13%, and (6aS,8R,8aR,9aS,11aR)-8-chloro-1-[(1R)-1,5-dimethylhexyl]-9a,11a-dimethyloctadecahydro-7H-cyclobuta[h] 0.08%.

Table 1: Chemical compounds that were identified in GC-mass spectroscopy

NO	Compound name	Composition %	R. Time	Chemical formula
1.	1,3-Pentanediol, 2,2,4-trimethyl-	0.40	5.133	$C_8H_{18}O_2$
2.	N-ISOPROPYL-5-METHYL-1,3,4-THIADIA ZOL-2-AMINE	0.40	5.133	$C_5H_9N_3S$
3.	Heneicosanoic acid, isobutyl ester	1.51	5.156	$C_{25}H_{50}O_2$
4.	Toluene	0.64	7.334	C_7H_8
5.	(1R)-2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene	0.47	13.192	$C_{10}H_{16}$
6.	2-Pinene	0.47	13.192	$C_{10}H_{16}$
7.	Oxirane, 2,2-dimethyl-3-propyl-	1.28	13.712	C7H14O
8.	3-Hexanol	1.28	13.712	C6H14O
9.	2-Butene, 2,3-dimethyl-	0.86	14.123	C6H12
10.	2-Pentene, 4-methyl-, (Z)-	0.86	14.123	C6H12
11.	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.64	25.868	C10H16

12.	BETA. FENCHYL ALCOHOL	0.64	25.868	C10H18O
13.	2-spiro[Cyclopropano]-3-methylcyclohex-3-en-1-one	0.64	25.868	C7H10O
14.	Decanal	0.81	26.103	C10H20O
15.	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6, 6-trimethyl-, acetate	1.03	28.566	C12H18O2
16.	BENZENE, (1,1-DIMETHYLETHYL	1.03	28.566	C10H14
17.	3-Oxabicyclo[3.3.0]octan-2-one, 7- methylene-	0.43	34.476	C8H10O2
18.	8-Oxabicyclo[4.3.0]non-3-ene-7-one	0.43	34.476	C8H10O2
19.	1-Methylcyclohexa-2,4-diene	0.43	34.476	C7H10
20.	Nonadecane	1.84	54.011	C19H40
21.	Hexadecanoic acid	0.28	56.205	C16H32O2
22.	Docosanoic acid	0.28	56.205	C22H44O2
23.	Thiosulfuric acid (H2S2O3), S-(2-a] minoethyl) ester	0.32	56.222	C2H7NO3S2
24.	9-Octadecenoic acid (Z)-	0.32	56.222	C18H34O2
25.	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	2.17	58.571	C19H36O3
26.	cis-10-Heptadecenoic acid, methyl ester	2.17	58.571	C18H34O2
27.	Heneicosane	1.23	60.429	C21H44
28.	Piperazine, 1-(2-furanylcarbonyl)-	1.54	61.600	C9H12N2O2
29.	2-Decanol	1.54	61.600	C10H22O
30.	1-(2,2,c-3,c-6-tetramethyl-r-1-cyclohexyl)-3-hexanol	0.23	61.749	C15H30O
31.	Cyclopropane carboxamide, 2-cyclop ropyl-2-methyl-N-(1 cyclopropylethyl)-	0.23	61.749	C13H21NO
32.	esquisabinene hydrate	0.38	61.795	C10H18O
33.	Pyrimidine, 2-(4-nitro-2-thienyl)-	0.38	61.795	C8H5N3O2S
34.	trans-1-methylene-2-hydroxy-8-meth oxycarbonyl-5,9-(2',2'-dimethyleth ylene)indane	0.23	64.247	C21H26N2O4
35.	(6aS,8R,8aR,9aS,11aR)-8-chloro-1-[(1R)-1,5-dimethylhexyl]-9a,11a-dim ethyloctadecahydro-7H-cyclobuta[h]	0.08	64.281	C23H25ClO5
36.	2-(3-Hydroxy-2-nitro-1-phenylbutyl)cyclohexanone	1.28	64.430	C16H21NO4
37.	1,1,2,2-Tetramethyl-5-methylidene-octahydrocyclopropa[e]azulene	1.28	64.430	C15H24
38.	1,3-Dithiane, 2-(5-bromopentyl)-2- (phenylmethyl)-	1.28	64.430	C16H23BrS2

39.	Neryl acetal	0.34	64.458	C12H20O2
40.	PERFLUORO-2-METHYLPENTENE-2	0.34	64.458	C6F12
41.	1-{2-[Ethyl(trifluoromethyl)amino] pyridin-5-yl}-trans-4-(4-propylcyc lohexyl)cyclohexene	0.34	64.458	C8H8F3N3O2
42.	Butanedioic acid, 2,3-dimethoxy-, diethyl ester	2.59	64.584	C10H18O6
43.	Iron, tricarbonyl[(2,3,4,5-.eta.)-tetrahydroxy-2,4-cyclopent adien-1-one]-	2.59	64.584	C8H4FeO8
44.	2,2-Dibromocholestanone	2.59	64.584	C27H44Br2O
45.	2-(Cinnamyloxy)-3-methyl-1-nitrobu tane	8.90	64.772	C14H19NO3
46.	Hexanoic acid, tetradecyl ester	8.90	64.772	C20H40O2
47.	Benzenesulfonyl chloride, 2,4,6-tr is(1-methylethyl)-	5.69	64.807	C15H23ClO2S
48.	N-BZ-2AMINOCINNAMATE	5.69	64.807	C17H17NO2
49.	(8R,12S)-8,12-Epoxy-15-hydroxy-labd-13E-ene	0.36	65.738	C20H34O2
50.	sesquisabinene hydrate	0.36	65.738	C15H26O
51.	Tricyclo[9.3.1.1(4,8)]hexadeca-1(1 5),4,6,8(16),11,13-hexaene-5,14-di methanol	0.82	65.801	C18H20O2
52.	n-Hexyltrichlorosilane	0.40	65.841	C6H13Cl3Si
53.	4,6,10,10-Tetramethyl-5-oxatricyclo [4.4.0.0(1,4)]dec-2-en-7-ol	0.40	65.841	C13H20O2
54.	2-CARENE, 4-.ALPHA.-ISOPROPENYL-	0.40	65.841	C13H20
55.	2-[2-[2-(nonyl-phenoxy) ethoxy]ethoxy]- ethanol	5.89	66.024	C21H36O4
56.	4(1H)-Quinolinone, 2-(methylthio)- 1-phenyl-	5.89	66.024	C16H13NOS
57.	2-(4-Nitrophenyl)cyclopropyl phenyl ketone	14.08	66.121	C16H13NO3
58.	Ethanone, 1-(2,3-dihydro-5-benzofuryl)-, oxime-, o-benzyl-	14.08	66.121	C17H15NO3
59.	N-Benzyl-2-aminocinnamate, methyl ester	7.18	66.253	C17H17NO2
60.	4(1H)-Quinolinone, 2-(methylthio)-1-phenyl-	31.70	66.327	C16H13NOS
61.	Benzene, 1-(1-formylethyl)-4-(1-buten-3-yl)-	1.53	66.710	C13H16O
62.	1-Methyl-6-hydroxyisoquinoline	1.53	66.710	C10H9NO
63.	1H-Imidazole, 4,5-dihydro-2-(phenylmethyl)-	1.53	66.710	C10H12N2
64.	Tetracosane	0.44	69.293	C24H50
65.	Ethanone, 1,1'-[3,3'-biisoxazole]-5,5'-diylbis-	1.24	70.333	C10H8N2O4

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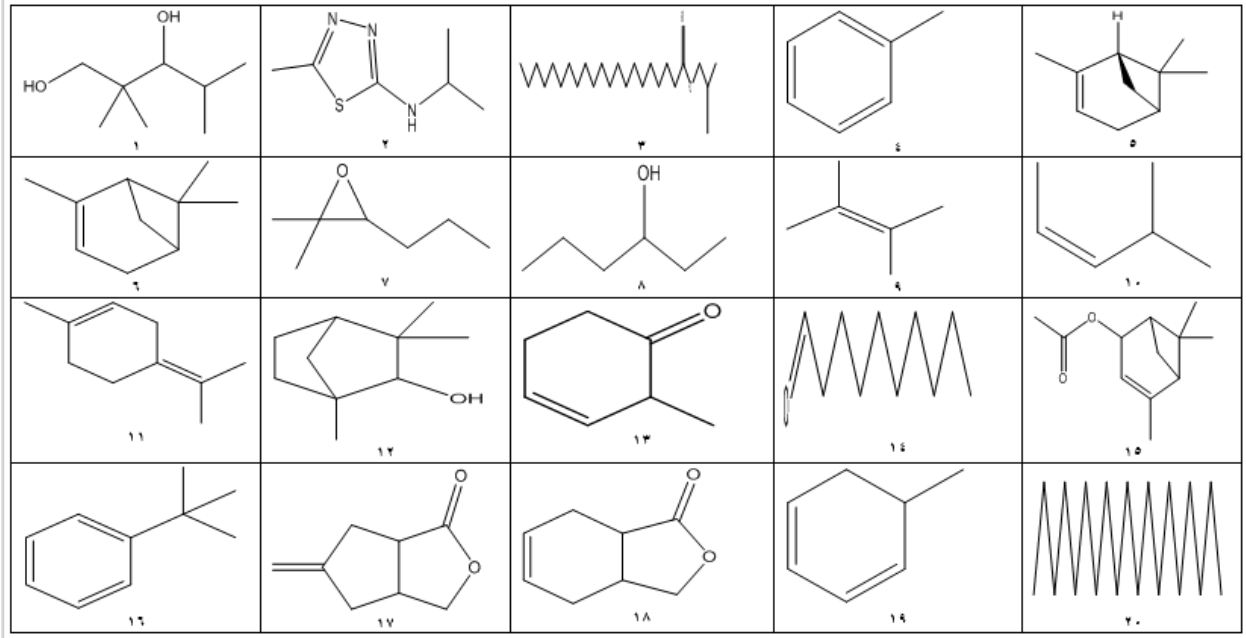
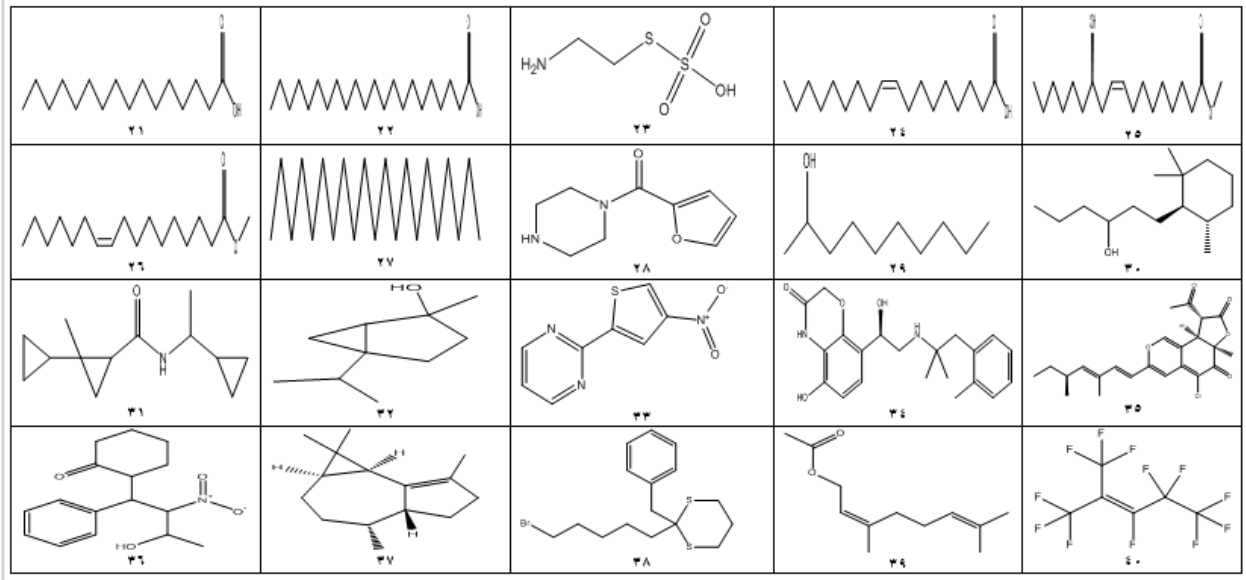
66.	1,3-Dioxolane, 4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-, trans-	1.24	70.333	C15H24F6O2
67.	O(2)-(Methoxymethyl) 1-[N-isopropy l-N-(methoxymethyl)amino]diazen-1- ium-1,2-diolate	1.24	70.333	C15H25NO3
68.	Naphtho[2,1-b]furan, dodecahydro-6 ,9a-dimethyl-, [3aS-(3a.alpha.,5a. alpha.,6.beta.,9a.beta.,9b.alpha.)]-	0.13	71.436	C16H28O2
69.	2-Biphenylenecarboxylic acid, 3-[(acetyloxy)methyl]-, methyl ester	0.13	71.436	C17H14O4





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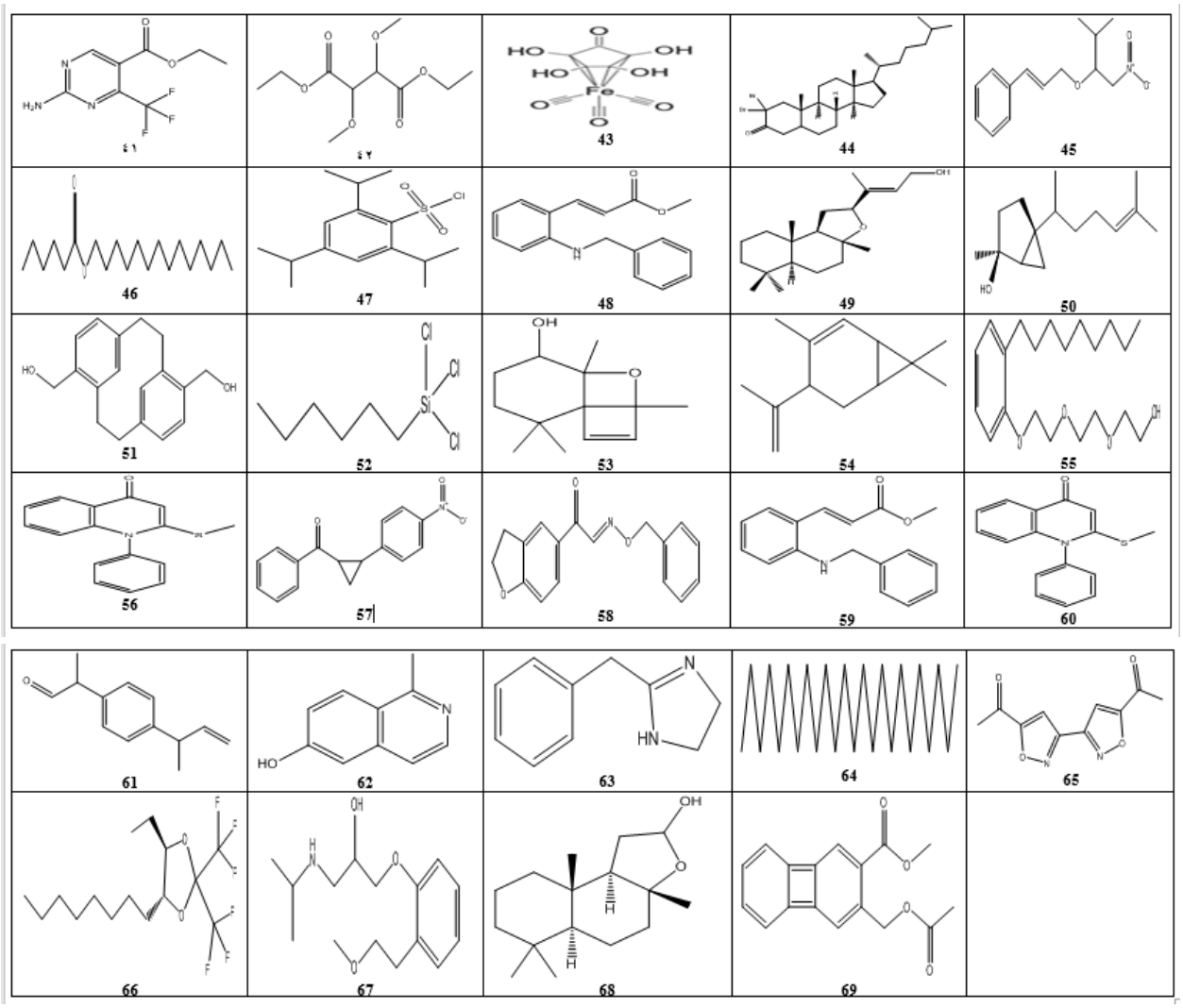

Figures 1 : The schematic structure of the compounds that have been reported to exist in *A. rose*

The antioxidant activity of A. rosea has already been reported (200, 100, 50, 25, 12.5). The aqueous extract had the most significant antioxidant activity in the DPPH method. Antioxidant activity was directly related to the total phenolic content. so the distilled water extracts found the highest total phenolic content.

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Table 2 Containing various extracts (12.5, 25, 50, 100, and 200 µg/mLof A. rosea, similar concentrations were used for the ascorbic acid, which was used as a standard.

Conc.	Vit. C		*A. rosea*	
	mean	**SD**	**mean**	**SD**
200	86.03333	4.02782	40.27767	2.391892
100	74.06667	1.006645	29.93867	1.816458
50	57.6	2.206808	20.062	1.571414
25	39	1.732051	17.39967	1.909718
12.5	22.9	1.835756	11.14967	2.588623

Data on the zone of inhibition of the aqueous extract to *A. rosea* used for measured antimicrobial activity and three antibiotics on *S. aureus* **.** As shown in figure (1) we can see the inhibition zone for antibiotics Cephalexin, Vancomycin, Gentamicin: negative, 7 mm, 8 mm respectively zone of inhibition around the well in Mueller-Hinton Agar plates. Aqueous extract of leaves plant showed zone of inhibition as 15 mm. the data showed that zone of inhibition formed surrounding the extract of *A. rosea* leaves it was very much comparable to the zone of inhibition formed around the antibiotics discs.

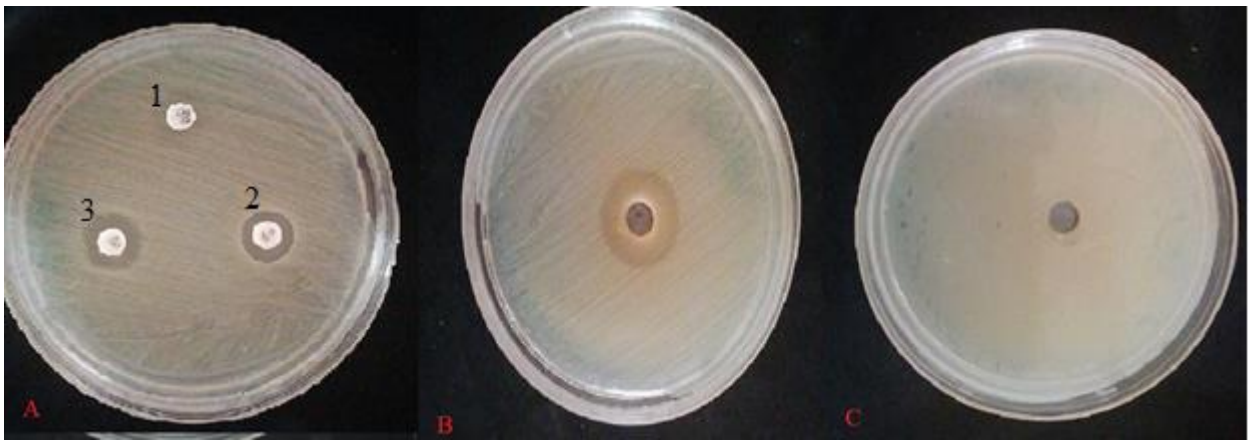

Figure 2: Comparison of the inhibition volume A: some types of antibiotics as control (1: Cephalexin (CL 5 µg) 2: Vancomycin (VA 5 µg) and 3: Gentamicin GEN 10 mcg/disk B: Aqueous *Alcea rosea* extracts against *Staphylococcus aureus* bacteria C: Distilled water as a negative control .

Data on Antimicrobial activity measure as a zone inhibition of aqueous extract of *A. rosea* on ***Candid albican*** As shown in figure (3) **12 mm** it was very much

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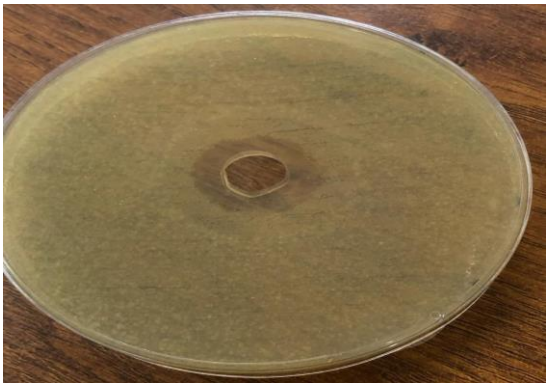

Figure 3: *Candid albican* zone inhibition diameters 12 mm

In vivo wound healing study

The Images were taken as shown in Figure (4) to monitor the degrees of change in the areas of superficial skin wounds. Although the animals in the control group were contaminated with S. aureus wounds at the start of the treatment, some of their secretions were still visible On day 2^{ed} of relevated wound surfacing. in day 8^{ed}, some secretions were still visible on the animals' wounds in the control and and the groups which treated with aqueous extract of *A. rosea* . Day 16: All 4th groups' superficial skin wound sites started to exhibit symptoms of healing in different rates.

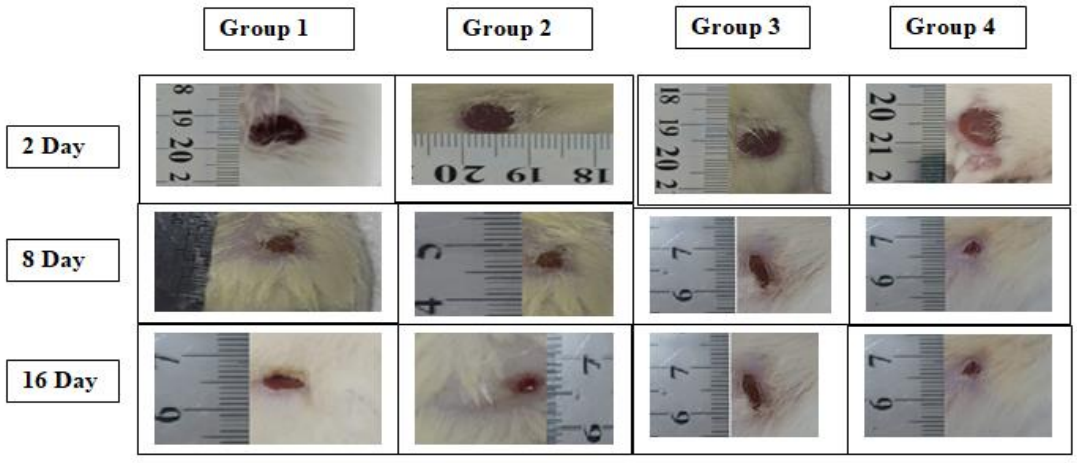

Figure 4: The Images of wound surface healing over time in each group

Wounds in Group 1 & 2in figure (3) healed better than those in the other two groups3&4 in figure (4). During the observation period, the control group showed slower wound healing

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compared to the other three groups. The study measured the wound areas of the selected rats and calculated the wound healing rate. Figure (3 and 4) showed the average wound healing rate on the 16th day after surgery, which was 78%,90% for Group 1 and Group 2 without contained by *S. aureus* & 55%,85% for Group 3 and Group 4 with contained by *S. aureus*.

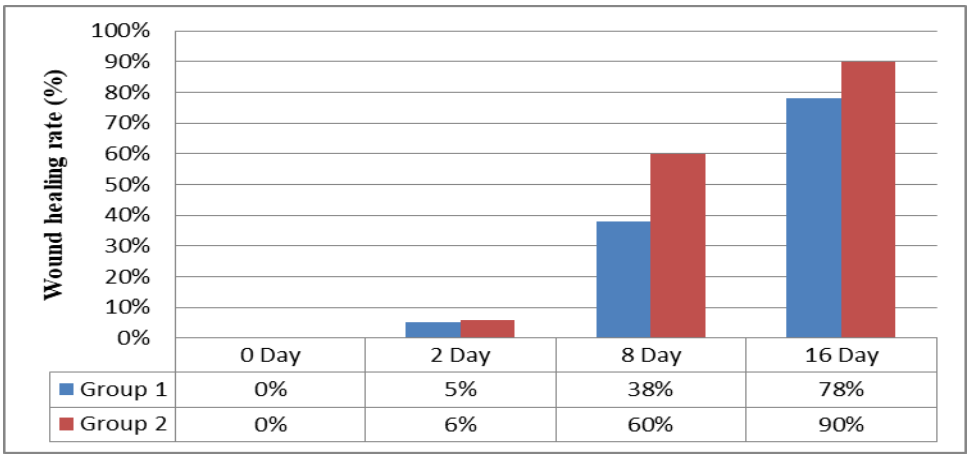

Figure 5: Wound healing rate for Group 1 and Group 2 without contained by S. aureus , the level of relevance was given as * between the groups. The level of probability was indicated as *** P ≤ 0.001 and as ** P ≤ 0.01 .Ns :Nonsignificant.

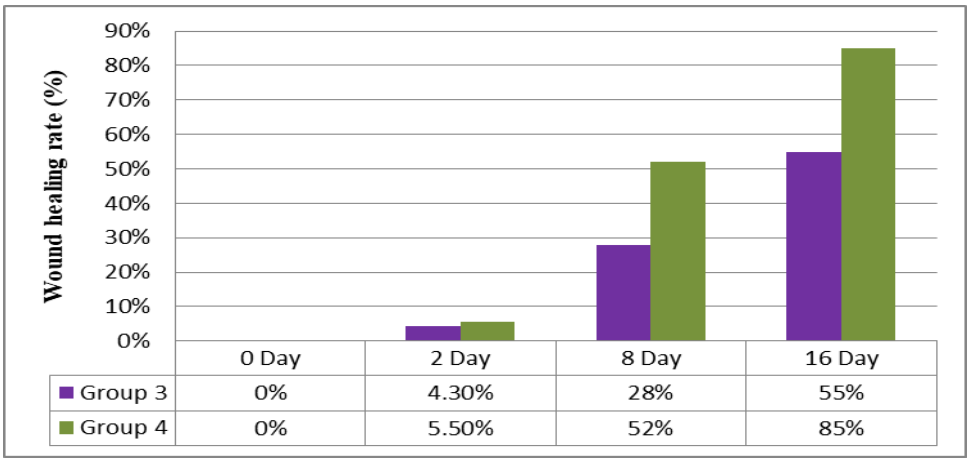

Figure 6: Wound healing rate for Group 3 and Group 4 with contained by S. aureus , the level of relevance was given as * between the groups. The level of probability was indicated as *** P ≤ 0.001,**** P ≤ 0.0001 . Ns :Nonsignificant.

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4- **Discussion:**

A number of traditional medical and phytotherapy researches have indicated that plants have great potential for wound management and treatment. The mechanisms of herbal extracts and plant medicines include their effective inhibition of microbial growth, stopping bleeding from fresh wounds, and accelerating wound healing (Vitale et al, 2022). A large number of plant extracts have been used to treat wounds and burns worldwide. These plant medicines stimulate healing and regeneration of lost tissues through multiple mechanisms. (Xie et al, 2015).

Alcea rosea, a traditional medicinal plant with multiple therapeutic properties (Nazir et al, 2022).

The biological properties of *A. rosea* were investigated and showed significant activities such as antioxidant, analgesic (Ahmad et al., 2023) and anti-inflammatory (Saha, 2019) These activities were due to its wide range of chemically active compounds present in the plant parts (Tepsongkroh et al., 2023) In this study, the chemical examination of the plant showed the presence of tannins, saponins, flavonoids, terpenoids and cardiac glycosides which are evidence that this plant has great potential for use in biomedical applications and by measuring the GCMass where the significant potential of this plant matched previous studies of this study (Nawaz et al., 2022) .

S. aureus is the most common type of bacteria in hospitals, as the wound area is the ideal environment for the proliferation of these bacteria and thus the infection occurs, so antibiotic treatment is one of the most important methods of wound care (Vivas et al, 2019; Wang et al, 2023)

According to the current study, the tested aqueous extract of the plant showed antibacterial activity according to the diameter of the recorded inhibition zones of (15 mm), where the activity is resistant if the inhibition zone is less than (8.00 mm), and moderate if the inhibition zone is greater than (11.00 mm). According to this study and according to Bauer, *S. aureus* can be considered sensitive to the aqueous extract of A. rosea. This study can also help in achieving the goal of reducing or destroying the targeted pathogens in a healthy and safe way. The aqueous extract of this plant showed strong anti-staphylococcal properties, which can be used in developing an antibacterial treatment for skin and soft tissue infections. (Morguette, *et al* 2023).

In this study, it was found that this extract has a higher antibacterial effect than some standard antibiotics used against certain types of bacteria. The extraction method and the type of solvent used can affect the effectiveness of this plant extract against bacteria, and as a result of the individual or synergistic effects of the biologically active molecules of the different components of this plant and their antimicrobial properties, wound healing can be faster, and thus these properties give the plant extract a specialized effect for wound healing. (Rezaei *et al*,2015).

The aqueous extract of A. rosea in this study also showed its maximum inhibitory effect on the growth of Candida albicans as it was observed that the inhibition zone around this fungus was significantly high around the well on the Petri dishes filled with bacteria. (Muzafar *et al* , 2012) .

Improving post-operative skin function and wound healing is crucial, Herbs have been used throughout the ages and nations to treat various diseases (Oda *et al*,2022) . The ongoing interactions between cells and between cells and matrix go through different interconnected stages and processes including inflammation, wound contraction, re-epithelialization, tissue remodeling, and granulation tissue formation with angiogenesis that allow the wound healing process to proceed more rapidly and lead to wound healing. (Herman & Herman, 2020).

One of the most important clinical barriers to wound healing is exposure of wounds to various microbial agents and wound infection as a result of immunosuppression. Wound infection may be due to pathogens including *S. aureus* which is the main cause of delayed wound healing. Infection in acute wounds is also one of the most common causes of poor wound healing. Therefore, treatment with topical antimicrobials is one of the most important methods of wound care. (Falcone et al, 2021).

The use of herbal products for wound treatment provides opportunities for direct destruction of pathogens which is an ideal factor for preventing and controlling wound infections as well as reducing local inflammation and tissue destruction. (Negut *et al* ,2018).

In the present study, A. rosea leaf extract showed anti-Gram-positive bacteria activity and wound healing efficacy in a mouse model of excision wound creation and also showed wound repair efficacy.(Rezaei *et al*,2015).

Conclusions

A. rosea has very potent secondary metabolites that have been previously reported to have wound healing potential and antioxidant properties. Therefore, we recommend that further scientific work should be done to characterize, identify and purify the plant components and uncover the molecular mechanism of wound healing potential and the mechanism responsible for its effects.

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