Date of publication: April 24, 2025.

Biochemical Characterizations of Acacia mellifera Seed Extract and Evaluation of its Biological Applications

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ABSTRACT. Plant bioactive chemicals have numerous biological impacts that are vital to human health. In the present study, Acacia mellifera (*A. mellifera*) seeds were collected from Jazan in Saudi Arabia, where *A. mellifera* was extracted using 90% methanol. GC-Mass analysis of *A. mellifera* extract revealed the presence of seventeen various molecules, six of which were from different chemical classes and were the most prevalent compounds. The extract showed a significant antibacterial impact on *Klebsiella pneumonia* and *Salmonella typhi*; their minimal inhibitory levels and bactericidal impact were recoded. Testing 25, 50 and 75% of the Minimum Bactericidal Concentrations (MBC) of the extract illustrated anti-biofilm impact of *A. mellifera* extract, especially at 75% of MBC. The minimum inhibitory concentration (MIC) values of *A. mellifera* extract compared to different bacteria showed dramatic anti-hemolytic action, especially at 75% of MIC levels. *In vitro* wound scratch assay using HFB4 cells highlighted the useful role of *A. mellifera* extract in wound healing. Hence, Saudi *A. mellifera* seeds have many pharmaceutical applications to be applied on a large scale in future after verifications of these outcomes.

INDEX TERMS Acacia Mellifera, Antimicrobial, Anti-Biofilm, Anti-Hemolytic, GC-Mass, Wound Healing.

I. INTRODUCTION

Microbial infections are a major global health concern, mostly affecting individuals in the third world and affecting the digestive and respiratory systems [1]. However, several of the medications that are now being used have unfavorable adverse reactions [2,3,4]. It is vital to look for novel antibacterial compounds with little adverse effects [5,6]. Throughout the world, medicinal products are the preferred medicinal approach in rural regions and play a crucial part in conventional medicine. The plant utilized throughout conventional healthcare is among the most potential frontiers in the quest for novel biologically functional molecules [7,8,9,10].

In human and animal; healing a wound is significant but intricate, with several distinct stages controlled by interconnected, consecutive phases. A more effective therapy strategy and a growing variety of *in vitro* or *in vivo* investigations are available to support the maintenance of a controlled and coordinated inflammatory reaction during the wound healing process [11].

One significant attribute of infected wounds is the presence of biofilms [12]. In reaction to signaling substances generated by other species in the biofilm, bacteria living in a biofilm exhibit phenotypic changes that lead to modifications in the generation of virulence factors. They also have reduced rates of metabolism and more confined growth [13]. A developed biofilm provides a milieu of protection for the microbes, enhancing their resistance to traditional antibiotics and insulating them from the phagocytic action of neutrophils [14]. The difficulty in the healing of persistent ulcers may be attributed to the presence of biofilms [15].

Inflammation and discomfort are initiators of numerous degenerative illnesses. The synthetic medications employed for the treatment of these illnesses have adverse effects that are incredibly harmful. In an attempt to provide safe, inexpensive, and efficient medications, new medicinal plants are being introduced globally [16].

To discover novel remedies and create cutting-edge pharmaceuticals, ethnopharmacology conducts in-depth studies of plants [17,18]. Plants in the genus Acacia have some intriguing medicinal characteristics. The genus Acacia, which has about 1200 species and is found in nearly every type of ecosystem, is the second biggest in the Leguminosae family [19]. *Acacia mellifera* (*A. mellifera*) is one species of Acacia, a shrub or small tree that can reach a height of nine meters. With its wide-ranging root system, it

may survive in arid regions by absorbing vast amounts of dirt. Spread over arid and semi-arid areas of Africa and the Arabian Peninsula, *A. mellifera* is a drought-resistant plant. *A. mellifera* is a slow-growing, versatile tree that can be used for human respiratory infections, infertility, and stomach issues [20,21]. However, little research has been done on the potential biological uses of *A. mellifera*'s phytochemical compounds.

The present investigation designed to test various bioactive molecules in *A. mellifera* seeds grown in Saudi Arabia using GC-Mass and in vitro testing its antimicrobial, anti-biofilm, anti-hemolytic and wound healing activities of *A. mellifera* extract.

II. MATERIALS AND METHODS

A. Collection of plant specimen

A. mellifera seeds were assembled from Jazan, Saudi Arabia (16° 54' 34.8588" N and 42° 34' 4.4472" E) in August 2023. The seeds were authenticated at Jazan University Herbarium (JAZUH), Biology Department, College of Science. The gathered seeds were cleaned with flowing tap water and sterile water to remove any remaining dust. Seeds are dried in the shade for three days at room temperature and processed using an electric mixer,

B. Preparation of extract & Chemicals

The collected dried seeds (100 g) were homogenized and crushed into a fine powder using a motorized mixer. They were then macerated for eight days in a sealed bottle with two hundred milliliters of 90% methanol. The extract was then subjected to a 120-minute sonicator treatment at 45 °C for traditional extraction. To get 6.0 g of crude extract, this extract was passed through filters and then centrifuged using Rota vapor for 40 minutes at 45 °C. [22]. Investigations of its chemical composition and biological functions were conducted on the extract. Every chemical utilized in this project was bought from Sigma-Aldrich, Louis, USA.

C. GC-Mass analysis of A. mellifera extract

The GC examination was conducted using (Stabilwax-560 column (98.0 m × 0.26 mm × 0.23 µm; Stabilwax, US), auto-sampler (CHROMSERVIS, US), and flame-ionization detecting. The data was extracted from the FID using chromatography equipment (Claurs 20400, PerkinElmer, Germany). There are nine minutes in the pre-run. The equilibrium takes 0.8 minutes to attain. Two ramps were finished: ramp 1.0, which produced an end maximum temperature of 207°C at a rate of 6°C/min, and ramp 2, which produced an average temperature of 6°C/min. The temperature started at 44°C and increased to a maximum of 285°C. The tests were conducted using the systematized heat mode, which ran from 100 to 282 °C for 14.0 minutes until homogeneity was reached. A transfer flow of 1.3 ml/min was used [23].

D. Antimicrobial activity

The well agar diffusion assay was used to ascertain the in vitro anti-microbial activity of *A. mellifera* extract against a range of bacteria and fungi. For the bacteria, Mueller Hinton agar plates were employed (2.0 g. Beef infusion solids, 1.5 g. Starch, 17.5 g. Casein hydrolysate, 17.0 g. Agar, Final pH 7.3) in 1 liter of distilled water. For the fungi, malt extract media was employed (30.0 g. Malt extract, 5.0 g. Mycological peptone, 15.0 g. Agar, Final pH 5.4) in 1 liter of distilled water. A total volume of 100 µl of different suspended bacterial cells ($1.6X10^6$ colony forming units/ml) was used; for the bacteria. The assessed extract was then put into the medium-created well using a sanitized cork borer edge. Gentamicin (0.07 mg/ml) and fluconazole (0.24 µg/ml) were utilized as standards and DMSO as our control. After developing for 72 hours at 35°C for bacterial cells, 5-7 days, and 27°C for fungi, the area of inhibition was evaluated [24,25].

E. Minimal inhibitory level and minimal bactericidal action

The micro-dilution broth approach was performed to find the specimen's least inhibiting value (MIC) using the nutrient-rich broth for bacteria. The final levels of every specimen under analysis ranged from 0.98 to 1000 µg/mL and were determined by diluting the specimen twice. 200 µl of the specimen dilutions under examination in broth media have been added to prepare every hole in the 96-well micro-titrate plate. Fresh microbial cultures that met the turbidity criteria of the 1.0 McFarland standard were used to generate the inoculum. Each well received 2.0 µl of sterilized 0.9% NaCl to achieve a $3.0X\ 10^6$ CFU/ml level. Subsequently, the bacteria and fungi were cultivated at 36°C for 72 hours and 35°C for 5–7 days, respectively. To ascertain the lowest inhibiting levels (MICs) that happened when the reference strain's development was stopped entirely, Beckman DU-70 UV-Vis. was used to measure the optical density of the sample at a wavelength of 600 nanometers for each sample. On each microplate, there was a positive reference (an inoculum without the examined specimens) and a negative reference (tested specimens without an inoculum) [26, 27]. Besides, 100 milliliters of the culture of microbes from each well were sub-cultured onto plates containing media from a developmental control, the final positive specimen, and the medium with 100% growth suppression. This made it possible to calculate MBC. When it came to samples that failed to promote the development of microbes during the incubation time at the appropriate temperature, the MBC was found to be the lowest level [26, 27].

F. Anti-biofilm action

The influence of the specimens on the biofilm-forming process was evaluated using 96-well polystyrene flat-bottom trays. A final level of 10^6 CFU/mL was determined by introducing 270 µL of newly seeded trypticase soy yeast broth (TSY) to each well of a microplate. The microplate

was then cultured in MBC at the previously determined sub-lethal concentrations of 75, 50, and 25%. Wells containing medium and those containing only alcohol without any samples were used as controls. Plates were stored at 38°C for 48 hours. The floating cells in every well were thoroughly rinsed with sterile distilled water after the supernatant was discarded. The plates were left to air dry for 30 minutes, and then the formed biofilm was colored for twenty minutes at ambient conditions using a water-based solution of 0.10% crystal violet. The excess color was removed again by rinsing thrice with sterile distilled water after incubation. Following a 20-minute incubation period and adding 250 µL of 96% ethanol to each well to eventually degrade the dye adhered to the cells, absorption was assessed at 580 nm utilizing a plate reader [28].

G. Anti-hemolytic activity

Using hemolytic and phospholipase C (PLC) protocol, the hemolysis reactions in specimens in sub-MIC (25% and 50% of MIC) manipulated by bacteria were measured [29]. Centrifuged at 21,000× g for 20 minutes, tested bacteria treated with 25%, 50%, and 75% of MIC (sub-MIC) or untreated cultures have been adjusted to an OD600 of 0.4. Freshly erythrocyte solution (2%) in 0.8 mL saline was mixed with supernatants (500 µL), incubated for 2 hours at 37 °C, and then spun at 11,000× g for 10 minutes at 4 °C. By adding 0.1% Sodium Dodecyl sulfate to an erythrocyte suspension, a positive control (full hemolysis) was created. A negative control (un-hemolyzed erythrocytes) was created by incubating erythrocytes in LB broth according to the same conditions.

The experiment was run in triplicate, and the hemoglobin discharge was measured by measuring absorbance at 540 nm. Drug-induced hemolysis in sub-MIC treated cultures was expressed as mean ± standard error of percentage reduction from untreated control cultures' hemolysis. After comparing the released hemoglobin with the positive and negative controls, the following formula was used to determine the percentage of hemolysis:

[(Specimen with bacterial culture − Negative control)/ (Positive control − Negative control)] × 100

H. In vitro wound healing assay

An extracellular matrix base containing 10 µg/mL fibronectin was applied to the plate. And then two hours of incubation at 37 °C. Following its release, the unattached extracellular matrix was cleaned with phosphate-buffered saline. Trypsin was used to separate the developing cells from a tissue culture-filled dish. The cells were grown on the assay plate with scratches wounds and then incubated to allow them to proliferate and form a confluent monolayer. A pipette tip scraped the monolayer cell, incorporating the confluent monolayer. After the cell monolayer has been scraped, gently wash it to remove any detached cells. Replace with a new medium that contains the tested extract. The plate was cultured in the cell culture unit for 48 hours at 37 °C. Phosphate-buffered saline was used to wash the cell monolayer following the incubation period. The cells were then treated with 3.7% paraformaldehyde for 15 minutes. For ten minutes, the cells were stained with crystal violet (1% in ethanol). A phase-contrast microscope was then used to inspect the cell culture [30].

I. Statistical evaluation

Each experiment was run three times, and the average ±SD of the findings is shown. The *t*-test and one-way ANOVA were utilized to distinguish across means utilizing the Graph Pad Prism V5 (San Diego, CA, USA) software. Findings indicating $p < 0.05$ were designated as noteworthy modifications.

III. RESULTS

A. Detection of bioactive molecules using GC-Mass

Testing of *A. mellifera* seeds extract revealed the existence of 17 various compounds from different classes, and it could be seen that six different significant compounds present in conductive order in the extract as follows 1,2,3-Benzenetriol (35%), Hexadecane (21.53%), Isobutyl gallate (11.81%), 12-methoxy-19-n orpodocarpa-4(18),8,11,13-tetraen3-one (6.76%), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (4.54%) and 4H-1-benzopyran -4-one, 2-(3,4-dimethoxyp henyl)-3,5-dihydr oxy-7-methoxy (4.49%) as shown in (Fig. 1 , Table 1)

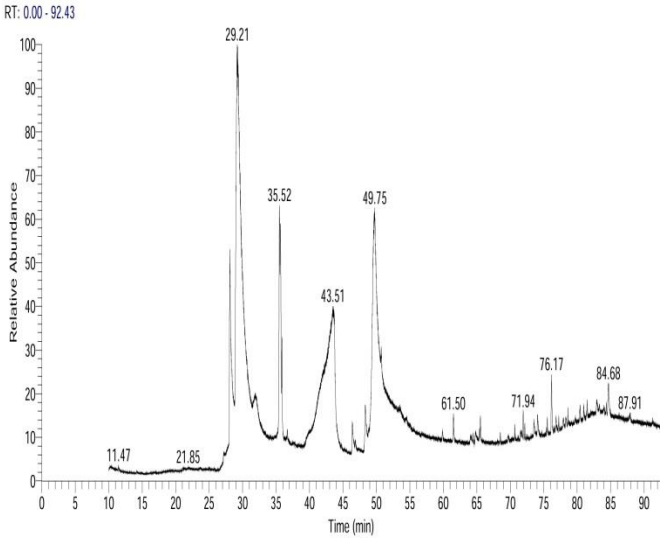

FIGURE 1. **GC-Mass testing of various compounds in *A. mellifera* extract.**

TABLE I
DIFFERENT COMPOUNDS AND THEIR PEAK AREAS AS WELL AS THEIR CLASSES IN *A. mellifera* SEEDS EXTRACT

RT Retention Time	Compound Name	Class	Peak area %	Molecular Formula	Molecular Weight
28.09	12-methoxy-19-norpodocarpa-4(18),8,11,13-tetraen3-one	Di-terpenoid	6.76	$C_{17}H_{20}O_2$	256
29.16	1,2,3-Benzenetriol	5-unsubstituted pyrrogallols	35	$C_6H_6O_3$	126
35.51	Hexadecane	Alkanes	21.53	$C_{16}H_{34}$	226
36.69	Prednisone	Corticosteroids	0.4	$C_{21}H_{26}O_5$	358
43.66	3-Methylmannoside	Oligosaccharide glycans	4.57	$C_7H_{14}O_6$	194
46.40	Dinaphtho[1,2-b:2',3'-d]furan-7,12-dio ne, 5-methoxy	Heterocyclic Phenol	1.09	$C_{21}H_{12}O_4$	328
48.32	Hexadecanoic acid, 1-(hydroxymethy l)-1,2-ethanediyl ester	Fatty acid ester	1.67	$C_{35}H_{68}O_5$	568
49.75	Isobutyl gallate	Galloyl esters	11.81	$C_{11}H_{14}O_5$	226
50.73	3,4,5-Triethoxybenzoic acid	Methoxybenzenes	1.71	$C_{13}H_{18}O_5$	254
61.51	Phenol, 2,2'-methylenebis[6-(1 ,1-dimethylethyl)-4-methyl	Dihydric phenol	1.39	$C_{23}H_{32}O_2$	340
64.81	i-Propyl 9-tetradecenoate	Unsaturated Fatty Acids	0.62	$C_{17}H_{32}O_2$	268
65.39	Allogibberic acid	Fluorenes	0.23	$C_{18}H_{20}O_3$	284
65.54	1,2-Benzenedicarboxylic acid	Dicarboxylic acid	0.77	$C_{24}H_{38}O_4$	390
71.94	4H-1-benzopyran -4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydr oxy-7-methoxy	Coumarins	4.49	$C_{18}H_{16}O_7$	344
74.08	à-Tocospiro A	Alpha-tocopheroids	1.33	$C_{29}H_{50}O_4$	462
76 .17	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Alkanes	4.54	$C_{26}H_{54}$	366
84.68	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)-	Saturated hydrocarbon	1.48	$C_{27}H_{40}O_4$	428
87.91	Lupeol	Triterpenoids.	0.62	$C_{30}H_{50}O$	426

B. Determination of Antimicrobial Activity

It could be noted that *A. mellifera* extract has inhibition zones towers *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 6538) of 20±0.1 and 21±0.2 mm which was lower relative to standard drug. While, *A. mellifera* extract showed higher inhibition zone than standard drug. The zones were measured as 24±0.2 and 23±0.2 mm towards *K. pneumoniae* (ATCC13883) and *S. typhi* (ATCC 6539), consecutively. Furthermore, *A. mellifera* extract showed no impact towards *P. glabrum* (OP694171) as well as 22±0.2 mm inhibition zone versus *C. albicans* (ATCC 10221) as shown in (Table 2 & Fig. 2).

TABLE 2
ANTIMICROBIAL IMPACT (MM) OF *A. mellifera* EXTRACT VERSUS DIFFERENT TEST MICROORGANISMS (RESULTS WERE TABULATED AS MEANS ± SD).

Specimen / Test organisms	*A. mellifera* extract	Standard
Bacillus subtilis (ATCC 6633)	20±0.1	29±0.2
Staphylococcus aureus (ATCC 6538)	21±0.2	25±0.2
Klebsiella. peregrine (ATCC13883)	24±0.2	21±0.1
Salmonella typhi (ATCC 6539)	23±0.2	20±0.2
Candida albicans (ATCC 10221)	22±0.2	30±0.2
Penicillium glabrum (OP694171)	NA	25±0.1

C. Evaluation of MIC and MBCs towards test microorganisms

It could be noticed that *A. mellifera* seeds extract had similar MIC and MBC levels for *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 6538) which were 31.25±0.1 and 62.5±0.1 µg/ml respectively. Besides, *A. mellifera* seeds extract have similar MICs and MBCs versus *K. peregrine* (ATCC13883) and *S. typhi* (ATCC 6539) which were 15.62±0.2 and 31.25±0.2 µg/ml, respectively. Finally, *A. mellifera* seeds extract had values of 15.62±0.1 and 62.5±0.1 for MIC and MBC 15.62±0.1 towards *C. albicans* (ATCC 10221) as illustrated in (Table 3).

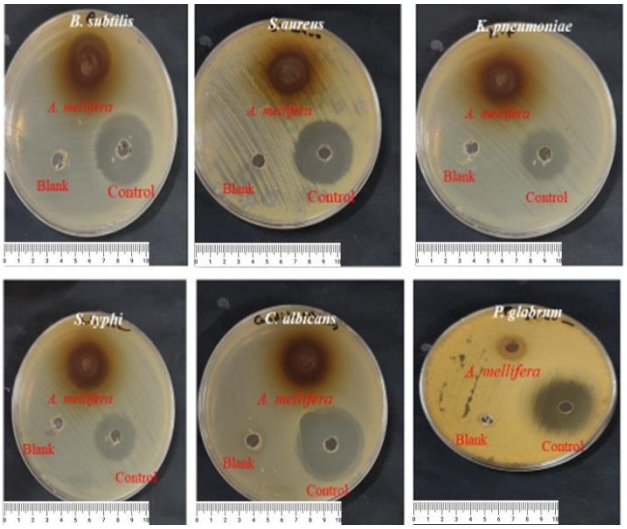

FIGURE 2. **Antimicrobial impact (mm) of *A. mellifera* seeds extract compared to standard drugs and blank in several Plates containing various test microorganisms (Results were tabulated as means ± SD).**

TABLE 3
DETERMINATION OF MIC AND MBC (µG/ML) OF *A. mellifera* SEEDS EXTRACT VERSUS DIFFERENT TEST MICROORGANISMS (RESULTS WERE TABULATED AS MEANS ± SD)

Test organisms	MIC	MBC
Bacillus subtilis (ATCC 6633)	31.25±0.1	62.5±0.1
Staphylococcus aureus (ATCC 6538)	31.25±0.2	62.5±0.2
Klebsiella. peregrine (ATCC13883)	15.62±0.2	31.25±0.2
Salmonella typhi (ATCC 6539)	15.62±0.1	31.25±0.2
Candida albicans (ATCC 10221)	15.62±0.1	62.5±0.1

D. Anti-biofilm impact of A. mellifera extract

Anti-biofilm impact (%) of *A. mellifera* extract versus *S. aureus*, *K. pneumonia*, *B. subtilis* and upon applying (25, 50 and 75%) of MBC of the extract. There is a gradual elevation of anti-biofilm impact (%) of the extract towards various tested bacteria and reaches its maximal level upon using 75% of MBC of the extract as depicted in (Fig. 3 and 4).

E. Anti-hemolytic action of A. mellifera extract

The lysed hemoglobin was evaluated upon applying 25, 50, and 75% of MICs of *A. mellifera* treated for various bacteria relative to the positive control. It could be noticed that there is a dramatic evaluation of anti-hemolytic impact of the extract, and it reached its maximal level upon using 75% of MIC. Furthermore, there is a dramatic difference in anti-hemolytic action upon using 25% of MIC of the extract relative to 75% of MIC of the extract, as shown in (Fig. 5 and 6).

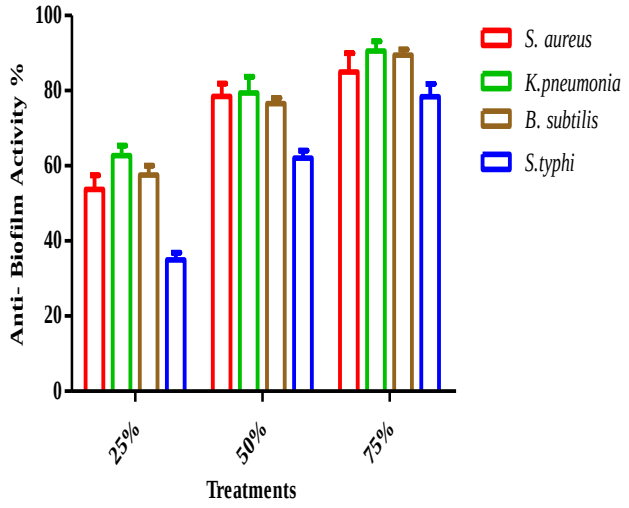

FIGURE 3. **Statistical analysis for anti-biofilm action (%) of *A. mellifera* extract versus various bacteria upon using 25, 50 and 75% of MBC (Data are drawn as means ± SD).**

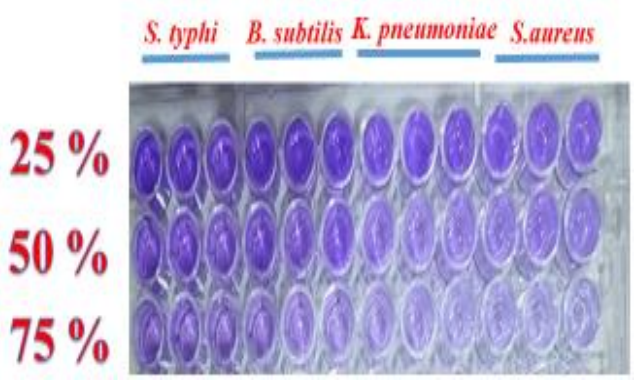

FIGURE 4. **Various in color intensities in Elisa plate upon using the tested levels for detection of anti-biofilm of the extract.**

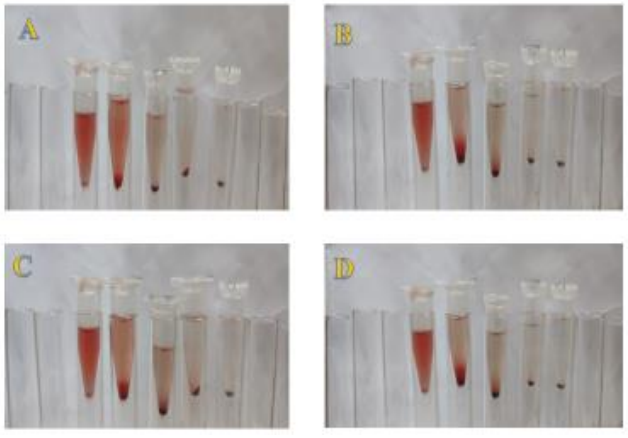

FIGURE 5. **A: Anti-hemolytic action upon using MICs for (A) *B. subtilis*, (B) *K. peregrine*, (C) *S. aureus*, and (D) *S. typhi*.**

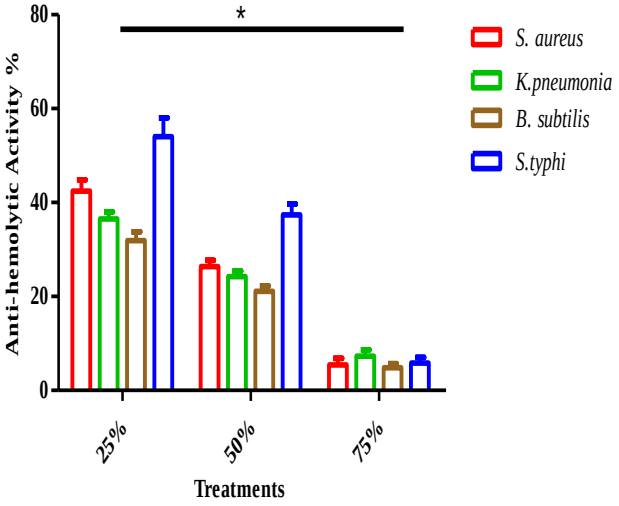

FIGURE 6. **Statistical analysis for anti-hemolytic activities of various MICs (Data are presented as means ± SD, * $P \leq 0.05$).**

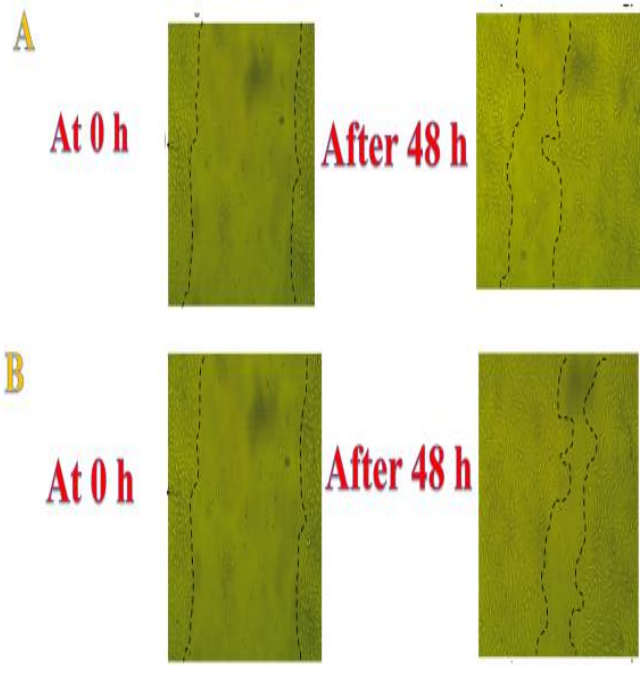

FIGURE 7. **Testing the effect of *A. mellifera* seeds extract on wound healing at 0 time and after 48 hours.**

TABLE 4
EVALUATION OF THE HEALING CHARACTERISTICS OF *A. mellifera* EXTRACT ON HFB4 CELLS (* REFER TO DRAMATIC DIFFERENCE P≤0.05).

Item	at 0 h		at 48 h	
	width	Area	width	Area
A. mellifera extract (125 µg/ml)	708.10	538187.30	246.01	186976.40
	670.03	509248.70	104.00	79044.37
	760.17	579298.20	128.06	97591.70
	684.00	519849.80	242.01	183928.70
	732.00	556322.30	244.01	185446.10
	806.00	6109480	266.12	201719.00
Mean	726.72	552309.00	205.03	155784.40
RM µm	10.87			
Wound closure % µm^2	71.79*			
Area difference	396.52*			
Control cells HFB4	708.10	538187.30	316.03	240192.30
	670.03	509248.70	238.03	180915.80
	760.17	579298.20	208.24	158692.60
	684.00	519849.80	340.01	258408.30
	732.00	556322.30	304.16	231164.60
	806.00	610948.00	318.02	241063.00
Mean	726.72	552309.00	287.42	218406.10
RM µm	9.15			
Wound closure % µm^2	60.46*			
Area difference	333.90*			

F. Determination of wound healing capabilities of A. mellifera extract

The efficacy of treatment was evaluated based on wound contraction, The simple ruler method consists of multiplying the greatest length and width of the wound to determine the surface area, *A. mellifera* seeds extract shown encouraging healing qualities for HFB4 cells; in contrast to control (cells not receiving treatment), *A. mellifera* seed extract consistently promotes healing (Table 4 and Fig. 7). The gap closing rate, which establishes the speed at which the cells move collectively, is the result of the evaluation of wound healing. Using *A. mellifera* seeds extract, the rate of migration (RM), wound closure percentage, and area difference percentage were 10.87 um, 71.79 µm^2, and 396.52%, consequently, with substantial improvement over control cells' corresponding values of 9.15 µm, 60.46 µm^2, and 333.90% (Table 4).

IV. DISCUSSION

Plants with vast distribution are exposed to ecological variations, which causes variations in the bioactive compounds that these plants generate [28, 31]. Both grassy and woody plant species accumulated triterpenoids due to soil drying [32, 33]. According to earlier research, there is an important distinction in the manufacturing of plant phytochemicals with geographic location [34, 35].

Genus *Acacia* contains many species with many therapeutic applications, including flowers, stems, and leaves [36]. In the present work, *A. mellifera* seeds collected from Jazan in Saudi Arabia were extracted, and tested by GC-Mass. *A. mellifera* contained various molecules where seven compounds were the most common. These compounds were from different chemical classes 5-unsubstituted pyrrogallols, alkanes, galloyl esters, di-terpenoids, oligosaccharide glycans, and coumarins. Australia's *A. mellifera* seeds have been found to contain vernolic acid [37]. Kenyan *A. mellifera* bark's tannin level is relatively greater [38]. Furthermore, the extraction of African *A. mellifera* using various solvents yields various bioactive fractions with multiple activities [39].

The present work illustrated that *A. mellifera* extract has antibacterial action towards *K. peregrine* and *S. typhi* nd an anti-yeast impact on *C. albicans.* In the same line, *A. ataxacantha* had antimicrobial action toward many test microbes, including *C. albicans* [5]. Also, isolation of flavanocoumarin from *Plicosepalus acacia* with antimicrobial action [40]. The antimicrobial impact of aerial parts of various species of Acacia grown in Saudi Arabia was reported [41]. Furthermore, it characterized Phenolics from *A. dealbata* with antimicrobial impacts [42].

Biofilms are intricate cellular formations made up of numerous bacterial cells encased in layers of materials the bacteria manufacture. This creates a barrier that prevents the organisms from being eliminated [43, 44]. Bacteria become resistant to antibiotics quickly, no drug can effectively treat infections linked to biofilms. The nature and form of the biofilm, the supply of oxygen and food for bacterial cells, and innate and developed resistance to antibiotics are additional factors that contribute to tolerance [45, 46]. The present work showed that A. mellifera extract showed a promising anti-biofilm towards S. aureus, K. pneumonia, B. subtilis especially upon using 75% of MBC. Following this, he anti-biofilm activity of different plant extracts was reported [47].

The cells that carry out medicine transmission the most regularly are the erythrocytes found in human tissue [48]. Hemoglobin and fatty acids, oxidizing active transporter components and erythrocyte-targeting intermediaries, are the main factors that induce triggered oxygen species in the body. Hemolysis is a disorder caused by oxidative destruction of the lipids and proteins composing the erythrocyte surface. Various variables, such as inflammatory drugs, excessive transitional metals, and deficiencies in erythrocyte antioxidant interaction, could be involved [49, 50]. In the present work, various levels of Sub-MIC values ranged from 25 to 75 % of the MIC of A. mellifera extract, which showed a promising anti-hemolytic impact, especially at 75 % of the MIC of the extract.

Free radical scavenging enzymes may disable and eliminate reactive oxygen species, which are detrimental to

wound repair due to their detrimental impact on tissue. Using molecules that can neutralize free radicals may prevent tissues from oxidative harm and increase the rate of wound healing [51]. In this study using A. mellifera extract showed a promising healing impact on HFB4 cells. In accordance with [52], who reported the role of Acaia gum in wound healing. Furthermore, [53] illustrated the healing impact of A. catechu in diabetic animals.

V. CONCLUSION

A. mellifera seeds collected from Jazan in Saudi Arabia and extracted by methanol showed promising antimicrobial, anti-biofilm, anti-hemolytic action as well as *in vitro* wound scratch assay using HFB4 cells highlighted the useful role of *A. mellifera* extract in wound healing. Hence, Saudi *A. mellifera* seeds have many pharmaceutical applications to be applied on a large scale in future after verifications of these outcomes.

ACKNOWLEDGMENT

The author thanks the Biology Department, College of Science, at Jazan University, Jazan, Saudi Arabia.

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