INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–1040/2022/27–2–154–164
DOI: 10.17957/IJAB/15.1912
http://www.fspublishers.org

Full Length Article

Oak Gall Extract: Molecular Docking of Wound Healing and Control of the Skin Pathogens *Staphylococcus aureus* and *Candida albicans*

Shymaa A Elbuckley[1], Noha M Sorour[1*], Ahmed A Tayel[2] and Rateb N Abbas[3]
[1]*Department of Industrial Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt*
[2]*Department of Food Biotechnology, Faculty of Aquatic and Fisheries Sciences Engineering, Kafr Elsheikh University, Egypt*
[3]*Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt*
[*]For correspondence: noha.sorour@mail.mcgill.ca
Received 15 December 2021; Accepted 20 January 2022; Published 28 February 2022

Abstract

Staphylococcus aureus and *Candida albicans* are common pathogens causing some health problems, however, the increase of their resistance to variety of medications increases the demand of searching for new antimicrobial agents. In this regard, the crude extract of *Quercus infectoria* (QIE) was verified as active antimicrobial with MBC of 1 mg/mL and MFC 10 mg/mL against *S. aureus* and *C. albicans*, respectively. SEM imaging of QIE-Treated-*S. aureus* and *C. albicans* cells showed fully lysed shrunk pathogen cells after 12 h. QIE showed good efficacy as potent anti-*S. aureus* and anti-*C. albicans* agents using Extract-Treated Cotton-Textiles. QIE ointment formulation showed faster rate of wound and burn healing in mice, with skin tissue development, at the 5th day, as compared to untreated control. A high significant wound closure (from 10 to 0 mm) and burn healing (from 25 to 2.3 mm) occurred after 6 days of treatment. Molecular docking predicted that ten major components in QIE namely (G-gallayol, Isocryptomerin, 10.7-methyl-3-hydroxymethylene-4,5,6,7,8-pentahydrox-h-thalene, Syringic acid, Gallotannic acid, Tannic acid, Pentagalloylglucose 1,*β*-sitosterol, Methyl oleanate, and Amentoflavone hexamethyl ether) are highly integrated in healing by promoting cell proliferation, keratinocyte migration, inhibiting collagenase, converting prothrombin to thrombin, increasing collagens function, enhancing immunity and DNA repair enzymes, as well as reducing inflammation. The combination of more than one bioactive compound in the extract and their synergetic action recommend the usage of QIE as effective topical applications for healing and skin disinfection.

Keywords: *Quercus infectoria*; *S. aureus*; *C. albicans*; Antimicrobial; Healing

Introduction

The skin being the largest body organ, it plays several vital roles, such as protection, thermoregulation, secretory and sensory activities (Njoroge and Bussmann 2007; Tayel *et al*. 2021). Therefore, topical wounds and skin infections require great attention to prevent secondary complications caused by microbial invasion. Both *S. aureus* and *C. albicans* are involved in skin infections and represent globally a major burden on the human health (Golan 2019). However, antibiotics misuse gave rise to antibiotic resistance and resistant strains, which represent a serious problem (Smet 2002).

Nevertheless, the plant Kingdom continuously provide valuable compounds to humans, which can be used in medicinal purposes (Khan *et al*. 2021). Most plants derivatives are commonly considered safe, eco-friendly, and have lower cost as compared to synthetic chemicals (Sun *et al*. 2021). Since prehistoric times, medicinal plants were used as herbal medication to treat several diseases, where their antimicrobial properties make them rich resource for effective medication (Mseddi *et al*. 2020). Medicinal plants usage decreases the side effects often associated with synthetic antimicrobials (Khan *et al*. 2021). According to World Health Organization (WHO) reports, medicinal plants are the greatest source for many drugs (Käppeli *et al*. 2011). The WHO suggests the addition of traditionally used phytomedicine, if they were verified as safe. In this respect, *Quercus infectoria* is very gorgeous in tannins and flavonoids. *Quercus infectoria* tree is located in the Mediterranean region, normally known as oak galls (Greenish 1999; Morales 2021). *Q. infectoria* extract (QIE) was commended in folkloric remedy for leucorrhea, menstruation, dysentery, hemorrhages, gonorrhea, as well as in mouthwash/gargle being potent antimicrobial and antiviral agent (Tayel *et al*. 2013; Morales 2021).

On the other hand, natural derivatives were used for promoting wound healing, as alternatives to chemotherapy,

it has attained great attention to control skin infections and stimulate its regeneration (Gonzalez *et al*. 2016). Even though many medications are present to remediate and renew injured skin, antibiotics and anti-inflammatory treatments are still not sufficient enough to overcome the infection caused by skin's pathogens (Tottoli *et al.* 2020). Topical medicament agents were used as one of the primary treatments and to prevent infection, though, they can cause allergic reactions that can postpone the healing process. Therefore, the discovery of new bio-safe wound healing agents is highly required. In this regard, medicinal plants also provide a wide area of research due to the vast diversity of phytochemicals with antioxidant, anti-inflammatory, antimicrobial and immuno-modulatory activities (Heidari *et al*. 2019). It is believed that medicinal plant extracts (PE) have lower cytotoxicity, with variety of phytochemicals that might act synergistically inhibiting many microorganisms with no resistance development (Yin *et al.* 2018). Fabrications of wound healing formulations based on plants' extracts and biopolymers were recommended as effective treatments for injured tissues besides their action as anti-inflammatory and antimicrobial agents (Tottoli *et al*. 2020; Tayel *et al*. 2021). Accordingly, the main objective of current study was to evaluate the antimicrobial effect of selected medicinal plant extracts (PE), against the two skin pathogens *S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231), using qualitative and quantitative methods. Fabrication of Plant Extract-Treated Cotton-Textiles were designed. The wound and burn healing potential of *Q. infectoria* was evaluated *in vivo,* and molecular docking of major bioactive compounds in QIE towards predicted proteins target in human was investigated.

Materials and Methods

Plants and chemicals

Different plant parts were used for crude extracts including, *Aloe vera, Lapidium sativum, Phyllanthus emblica, Punica granatum* and *Quercus infectoria* were obtained from Agricultural Research Center, Giza, Egypt. All media are ready-use media purchased from Oxoid Company for microbiological media and chemicals, UK. Tween 80, anesthetic ether and 70% ethanol were purchased from Algomhoryia Company for Chemicals, Cairo, Egypt. Vaseline (a purified mixture of saturated hydrocarbons mainly of paraffinic nature), used in medicinal ointments, was obtained from Saif Pharmacy, Cairo, Egypt.

Microbial strains and culture media

S. aureus (ATCC 6538) strain and *C. albicans* (ATCC10231) strain were purchased from MIRCEN, Ain shams university, Egypt. Nutrient agar media was used for bacteria culturing with the following composition (g/L); beef extract 3, peptone 5, sodium chloride 5, and agar 20, with final pH 7. Trypticase Soy broth medium with the following composition (g/L); beef Infusion 30, casamino acids 17.5, starch 1.5, with final pH 7.3 and Yeast Malt Peptone (YMP) medium, with the following composition (g/L); yeast extract 3, malt extract 3, peptone 5, glucose 10 with final pH 6 were used for culturing and maintenance of yeast.

Plants crude extraction

A. vera (leaves), *L. sativum* (seeds), *P. emblica* (Fruits), *P. granatum* peel extract (PPE) and *Q. infectoria* (fruits) were dried and ground using a mixer grinder (Spex Ind. Inc., Metuchen, NJ), the plant parts were dried, ground, and powdered to get ~ 60 mesh size particles. 50 g from each plant powder was mixed with 250 mL of 70% ethanol, left for 72 h, with occasional shaking. Extracts were filtered, through Buchner funnel, the extracts were pooled, and evaporated to remove the solvent at 50°C using flash evaporator. The crude extracts were further dried in a desiccator under vacuum until constant weight (Fig. 1).

In vitro qualitative evaluation of antimicrobial activity

The antimicrobial potentiality of plant extracts (PE), toward *S. aureus* and *C. albicans* strains were evaluated, using qualitative methods. Pathogens were grown in nutrient broth and YMP broth medium for 24 h, inoculum was standardized with sterile-saline to turbidity equivalent to 0.5 McFarland scale (1–2 × 10^8 and 1–5 × 10^6 CFU/mL), respectively. Disc diffusion test was done according to CLSI (2010); 100 μL inoculum suspension from either *S. aureus* or *C. albicans* strain were spread uniformly over 20 mL agar medium in sterile petri-dishes. Sterile discs were loaded with 25 μL of PE aliquots, placed on the medium. For well diffusion assay, 100 μL of the pathogen inoculum suspension from either *S. aureus* or *C. albicans* were spread uniformly over the medium, 50 μL from each PE was added to 6 mm-wells. All inoculated plates were incubated at 37°C or 30°C, for 24–48 h. The microbial activity was measured in mm by the inhibition zone (ZOI) width.

Quantitative evaluation of antimicrobial activity

S. aureus and *C. albicans* were grown in nutrient broth and YMP broth media for 24 h, respectively, inoculum was standardized with sterile saline to turbidity equivalent to 0.5 McFarland scale. The MIC was determined using 10-folds serial dilution prepared from each plant extracts, diluted using sterilized culture medium, transferred to plates, inoculated with pathogen. The plates were examined for the presence of growth and the lowest concentration of PE leading to complete inhibition was designated as the minimal bactericidal or fungicidal concentrations (MBC) or (MFC).

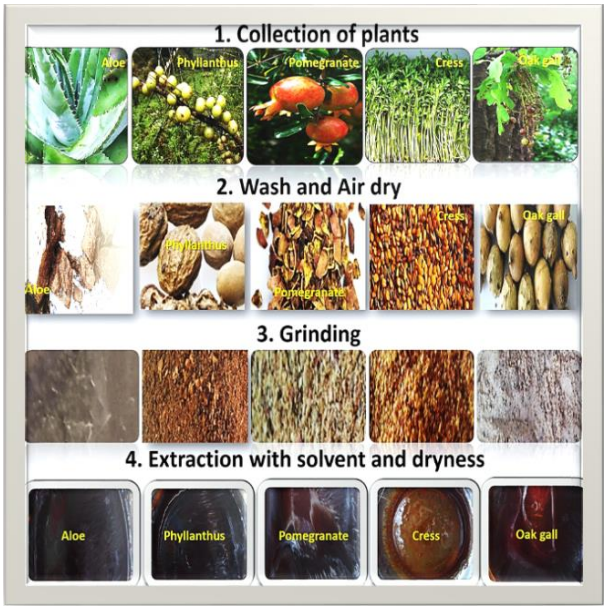


Fig. 1: Collection of plant materials and preparation of extracts from various parts

Fabrication of extract-treated cotton textiles

Standard and scoured cotton textiles were used for impregnating with QIE or PPE. The method of "pad-dry-cure" was performed for textile finishing. 1 × 1 cm^2 cotton fabrics were cut and immersed in extracts solution, at their MBC levels, stirred for 2 h at 50°C, then padded and squeezed using 2 nips and dips to 100% wet pick up. Treated cotton fabric pieces were dried for 3 min at 37°C, as described by Tayel *et al*. (2013). The antimicrobial evaluation of extract-treated fabrics was conducted using ZOI assay on inoculated plates with pathogen.

SEM imaging

SEM imaging was done according to Marrie and Costerton (1984) method for revealing the antimicrobial action of PE on tested microbes. 18 h-old pathogen strains were treated with plant extract (QIE) at their corresponding MBC and MFC, respectively. Treated bacteria and yeast were incubated for 6 h and 12 h at 37ºC and 30°C, respectively. Samples were fixed using fixative solution (2.5% glutaraldehyde, 2% paraformaldehyde dissolved in 0.1M sodium-cacodylate buffer, pH 7.3) for 30 min. Fixed samples were dehydrated using ethanol concentrations (10–100%), mounting onto stubs and sputter-coated with palladium/gold. Micrographs were captured using SEM (S-500-Hitachi, Japan) at 25 kV and 10 kx, at Theodor-Bilharz Research Institute, Cairo, Egypt.

Wound and burn healing potentiality of QIE

Adult female Swiss albino mice (180-200 g) at National Research Center, Cairo, Egypt were kept in standard stainless-steel cages maintained in the animal house under laboratory conditions (relative humidity 60–70%, Temp. 23 ± 2°C, 12 h/12 h light/dark cycle). Mice were fed with balanced diet and water adlibitum. All the animal experiment was performed according to the departmental ethical committee guidelines (Principles of Laboratory Animal Care NIH publication no. 85-23, revised 1985). The ointment was formulated using 10% (w/w) of QIE with soft paraffin base. Anesthesia was made by intraperitoneal injection of aesthetic ether (50 mg/kg body weight). Dorsal parts of animals were shaved, burn or wounds were created on the shaved area of rats using a burn set with an aluminum rod (1.5 cm) heated at 110°C and exposed to 1 atm. pressure for 10 s. Treatment started after 1h after burn wound induction. For wound model, skin excision wounds were created using a punch biopsy needle. The entire wound was left open and ointment was daily applied twice daily, to cover all over the wound and burn. The study comprised four different groups; each group consists of 6 animals. All groups were left for 7 days as follow: Group I and III: wound and burn control with no treatment, Group II-wound treated and Group IV-burn treated with prepared ointment, twice daily. The reductions and progressive changes in wound area were monitored and the wound area was measured and evaluated on a mm scale graph paper.

Molecular docking and statistical analysis

Molecular docking for predicted protein target in human was done on *Homo sapiens* database using Swiss Docking online program (Gfeller *et al*. 2013). Antimicrobial assessment was conducted in triplicates, standard deviations and means were calculated using Microsoft Excel software (2010). Data were expressed in their mean values ± SD (standard deviation).

Results

In vitro antimicrobial activity

In this study, five medicinal plants were evaluated for their potential antimicrobial activities toward the two skin pathogens *S. aureus* and *C. albicans* strains (Table 1). The antibacterial activity varied among examined extracts; the most significantly powerful extract was that of *P. granatum* extract (PPE) as evidenced by its widest ZOI of 21 ± 1.7 mm and the lowest MBC of 0.1 mg/mL. Also, *Q. infectoria* extract (QIE) showed significant antibacterial activity with ZOI of 18.3 ± 1.5 mm and 1 mg/mL MBC, against the *S. aureus* strain. The most significant antifungal extract was QIE against *C. albicans* as verified by its widest ZOI of 27 ± 0.5 mm and MFC of 10 mg/mL, followed by PPE. All other extracts showed no significant activity against both pathogens (Table 1 and Fig. 2). PPE and QIE exhibited strong antibacterial and antimycotic activities, thus, they

Table 1: (**A)** Antimicrobial activity of selected plant extracts against *S. aureus* (ATCC 6538), measuring Zone of Inhibition (ZOI), Minimal Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC). **B**. Anti-*S. aureus* and anti-candidal effect of QIE and PPE loaded on cotton fibers, at different MBC/MFC

Extracted plants A			*S. aureus* (ATCC 6538)		*C. albicans* (ATCC 10231)	
Commercial name	Scientific Name	Used part	ZOI (mm)	MBC (mg/mL)	ZOI (mm)	MFC (mg/mL)
Oak gall	*Quercus infectoria*	Fruits	18 ± 1.5	0.1	27 ± 0.5	0.1
Aloe	*Aloe vera*	Bark	00	00	00	00
Cress	*Lepidium sativum*	Seeds	00	00	00	00
Phyllanthus	*Phyllanthus emblica*	Fruits	00	00	00	00
Pomegranate	*Punica granatum*	Peels	21 ± 1.7	0.01	20.3 ± 1.5	ND

(**B**) Concentration	Plant Extract	Zone of inhibition (mm)	
		S. aureus (ATCC 6538)	*C. albicans* (ATCC 10231)
MBC/MFC	QIE	ND	ND
	PPE	ND	ND
1.5 X MBC/MFC	QIE	ND	ND
	PPE	ND	ND
2 X MBC/MFC	QIE	21 ± 1	21 ± 1
	PPE	16.5 ± 0.5	ND

Data are average of 3 replicates ± SD (standard deviation)

were chosen for further investigations to elucidate their potential antimicrobial actions.

Plant Extracts-treated cotton textiles

Results in Table (1B) revealed that the applications of QIE and PPE in cotton textile was successful as anti-*S. aureus*. The mean ZOI using QIE-treated textiles was 21 ± 1 mm at 2MBC with *S. aureus*. Whereas, PPE-loaded textiles showed ZOI of 16.5 ± 0.5 mm against *S. aureus*. QIE application was effective for inhibiting *C. albicans*. The mean ZOI using QIE–treated textiles was 21 ± 1 mm at 2MBC against *C. albicans*, whereas, no inhibition zones were observed with PPE-loaded textiles.

SEM imaging

Treated cells with MIC concentration of QIE (Fig. 3) showed that the treatment caused remarkable morphological alterations as compared with control. After only 6 h (Fig. 3), treated-*S. aureus* and treated-*C. albicans* cells were shrunk, tiny and dehydrated, while, after 12 h of exposure to the extract, cells were completely disrupted and lysed, the cellular components as well as debris were only observable. After 12 h, cells lost their water contents, it could be expected that all biological processes inside the cells are affected, no cell wall synthesis, and cells tended to deform and lyse.

Wound, burn healing activities of QIE and docking analysis

Results (Table 2) revealed the reduction of wound area of different groups over the period of 7 days. At the 5th day, a significant closure of wound from 10 to 2.3 mm was observed. The control group has shown gradual closure of wound; but complete wound closure was not observed until the 7th day (Table 2. In case of QIE-treated burn complete healing occurs in the 7th day (from 25 mm to 0 mm) as

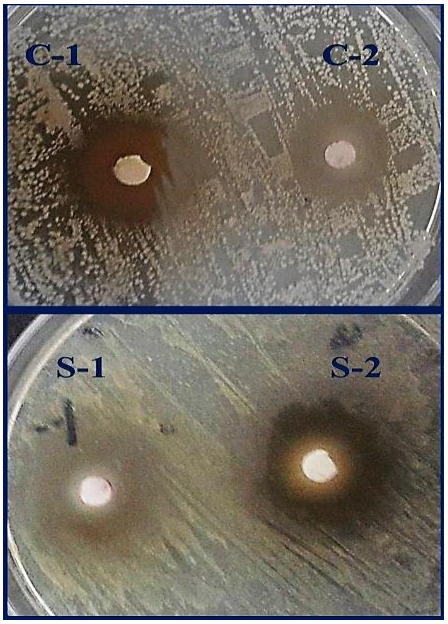


Fig. 2: Disc diffusion assay using QIE (1) and PPE (2) against *C. albicans (C)* (ATCC 10231) and *S. aureus (S)* (ATCC 6538)

compared with control in which no full cure was observed (6.7 mm). QIE ointment (10%) showed significantly better wound and burn healing effect, with reduction in the burn wound size from 25 mm to 2.3 mm at the 6th day, as compared to control (Table 2; Fig. 3).

The application of *Q. infectoria* extract in wound/burn healing shows significant curing activity for wound and burn in mice. To explain the medicinal effect of QIE on wound healing, molecular docking of the major components in QIE was estimated in *Homo sapiens* database to detect the predicted protein targets in human and its role in healing process using Swiss Docking online program (Table 3 and Fig. 5). Ten major bioactive molecules namely, G-gallayol, Isocryptomerin, 10.7-methyl-3-hydroxymethylene-4,5,6,7,8-pentahydrox-h-thalene, Syringic acid, Gallotannic

Table 2: Effect of QIE treatment on the development of induced wound and burn in mice for 7 days

Treatment (day)	Wound length (mm)		Burn mean diameter (mm)	
	Control	Treated	Control	Treated
1st	10	10	25	25
2nd	9.8 ± 0.3	7.8 ± 0.25	25 ± 1	23 ± 1
3rd	8.5 ± 0.5	5.7 ± 0.2	22.6 ± 0.6	14.7 ± 0.6
4th	7.5 ± 0.5	3.7 ± 0.6	19.3 ± 1.1	10.6 ± 0.6
5th	6.2 ± 0.3	2.3 ± 0.2	13.6 ± 0.6	5.3 ± 0.6
6th	4.7 ± 0.3	0	10.6 ± 0.6	2.3 ± 0.6
7th	3.2 ± 0.3	0	6.7 ± 0.6	0

Data are average of replicates ± SD (standard deviation)

acid, Tannic acid, Pentagalloylglucose 1, *β*-sitosterol, Methyl oleanate and Amentoflavone hexamethyl ether were detected in GC/MS analysis of QIE.

Discussion

Natural antimicrobial compounds, especially from plant origins, are generally-recognized-as-safe (GRAS), with rapid biodegradability and least mammalian cytotoxicity; marking them as ideal eco-friendly safe agents, due to its bioactive phytochemicals and their possible synergistic effect (Isman 2000). The proliferation in resistance to many antimicrobial agents by microorganisms has been increased with time, therefore the necessity of searching for novel agents became essential. As a result, evaluating plant extracts known to have medicinal value is highly recommended for the developing of new antimicrobial agents. PPE and QIE exhibited strong antibacterial and antimycotic activities, thus, they were chosen for further investigations to elucidate their potential antimicrobial actions. Similarly, Baharuddin *et al*. (2015) screened the anti-activity of QIE against *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis* and reported ZOI ranging 9.33-23.00 mm and MFC of 4.00, 1.00, 0.25, 8.00, 2.00 mg/mL, respectively. The main benefits for using natural extracts, such as PPE or QIE as antimicrobials are their efficacious, bio-safe and low-cost as compared to synthetic chemicals (Ribeiro *et al.* 2015). PPE is very rich in phenolic compounds, which are powerful bio-agents (Cowan 1999). The application of GRAS extracts as antimicrobial agent does not permit resistance by pathogenic bacteria; because the presence of variety of bioactive compounds will be very hard for most microorganisms to resist them all. QIE is popular medicinal plant used traditionally in postpartum care, and for treatment of various disorders. QIE is highly rich in tannins therefore, demonstrate anti-inflammatory, anti-microbial, and anti-oxidant activities (Baharuddin *et al.* 2015). QIE is used in folkloric-medicine as remedial agent for hemorrhages, dysentery, gonorrhea and as mouthwash (Morales 2021). Finished cotton textiles with anti-*S. aureus* plant extracts could be recommended for the application in manufacturing surgery coats, intensive care, bed covers, wound dressings, and medical antibacterial bandages. In addition, QIE can be used as an effective anti-candidal agent in antiseptic suspensions and solutions and as a final agent for disposable anti-candidal cotton textiles.

Tannins originated from plants were verified as effective antimicrobials (Min *et al.* 2008); probably through their interaction with microbial cell proteins.

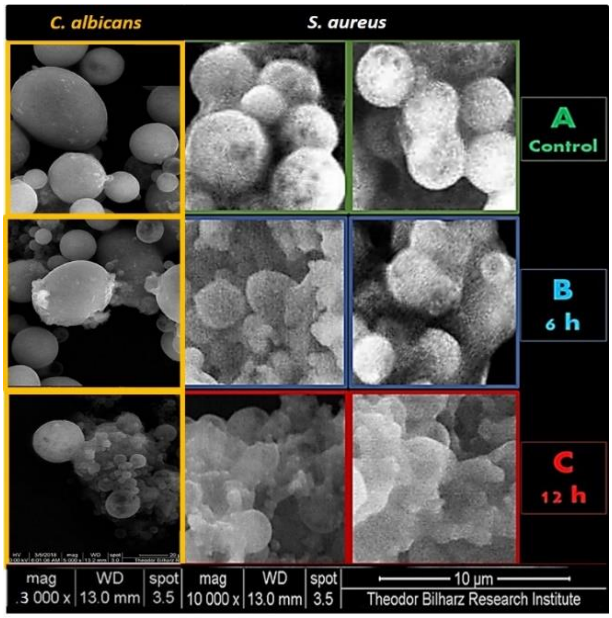


Fig. 3: Anti-staphylococcal and anti-candidal action of *Q. infectoria* extract (QIE) against *S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231), control with no plant extract (A), after exposure to corresponding MBC for 6 h (B), and 12 h (C) as evidenced by SEM micrographs

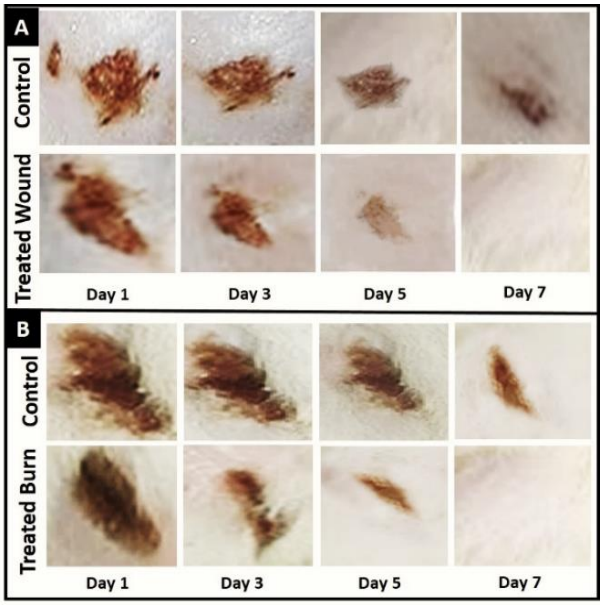


Fig. 4: Healing assessment of wound and burn in mice through 7 days' treatment with formulated ointment containing QIE, wound (**A**), Burn (**B**), and control with no treatment

Table 3: Selected proteins target and predicted mode of action for major bioactive compounds in QIE using Swiss docking target online program

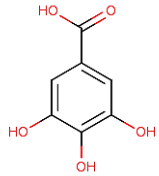
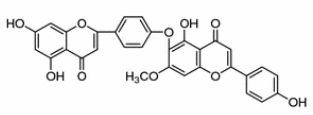
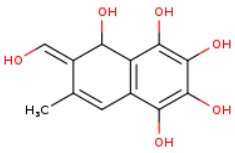
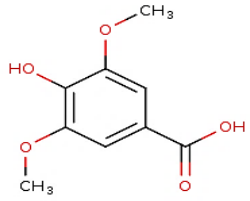
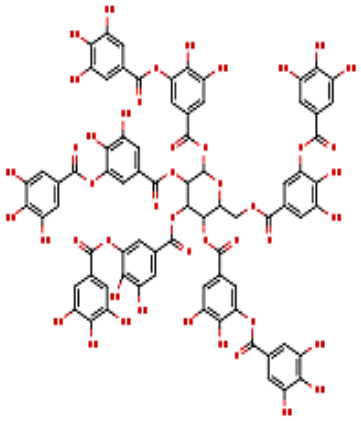
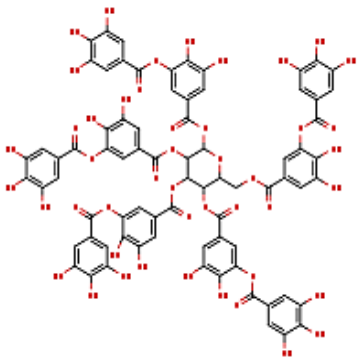
Phenolic Compounds	Expected Protein Target	Gene	Uniport ID	Reference
	Insulin-like growth factor 1 receptor	IGF1R	P08069	Abbot *et al.* (1992)
	Alpha-(1,3)-fucosyltransferase 7	FUT7	Q11130	Malý *et al.* (1996)
	Carbonic anhydrase-9	CA9	Q16790	Humphray *et al.* (2004)
	Plasminogen activator inhibitor 1	SERPINE1	P05121	Providence *et al.* (2008)
G-galloyol				
	Mast/Stem cell growth factor receptor	KIT	P10721	Taniguchi *et al.* (1999)
	Aldo-keto reductase family 1 member B1	AKR1B1	P15121	Shen *et al.* (2011)
Isocryptomerin				
	Stromelysin-1	MMP	P08254	Newman *et al.* (1994)
	Matrix metalloproteinase-9	MMP9	P14780	Newman *et al.* (1994)
	Interstitial collagenase	MMP1	P03956	Desrochers *et al.* (1991)
10.7-methyl-3-hydroxymethylene-4,5, 6,7, 8-pentahydrox-h-thalene				
Table 3. Continued Phenolic Compounds	Expected Protein Target	Gene	Uniport ID	Reference
	Carbonic anhydrase 9	CA9	Q16790	Humphray *et al.* (2004)
	Plasminogen activator inhibitor 1	SERPINE1	P05121	Providence *et al.* (2008)
				
Syringic acid				
	Thrombin and Coagulation factor X	F10	P00742	Walker *et al.* (1980)
	Tyrosine-protein phosphatase non-receptor type 2	PTPN2	P18031	Simoncic *et al.* (2002)
	Tyrosyl-DNA phosphodiesterase -1	TDP1	Q9NUW8	Raymond *et al.* (2004)
	Plasminogen activator inhibitor 1	SERPINE1	P05121	Providence *et al.* (2008)
Gallotannic acid				
	Thrombin & Coagulation factor X	F10	P00742	Walker *et al.* (1980)
	Tyrosine-protein phosphatase non-receptor type 2	PTPN2	P18031	Simoncic *et al.* (2002)
	Tyrosyl-DNA phosphodiesterase -1	TDP1	Q9NUW8	Raymond *et al.* (2004)
	Plasminogen activator inhibitor 1	SERPINE1	P05121	Providence *et al.* (2008)
Tannic acid				

Table 3: Continued

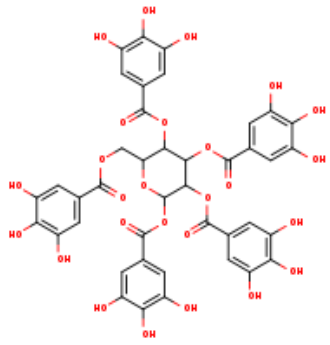
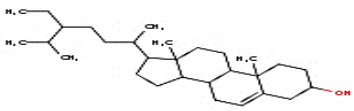
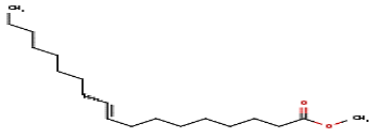
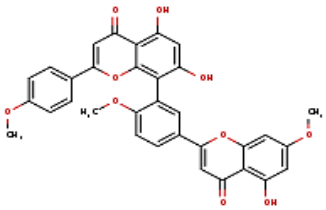
 Pentagalloylglucose 1	Thrombin & Coagulation factor X	F10	P00742	Walker *et al*. (1980)
	Tyrosine-protein phosphatase non-receptor type 2	PTPN2	P18031	Simoncic *et al*. (2002)
	Tyrosyl-DNA phosphodiesterase-1	TDP1	Q9NUW8	Raymond *et al*. (2004)
	Plasminogen activator inhibitor 1	SERPINE1	P05121	Providence *et al*. (2008)
 β-sitosterol	Androgen receptor	AR	P10275	Gottlieb *et al*. (2004)
 Methyl oleanate	Prostaglandin G/H synthase 2 (Cyclooxygenase 2)	PTGS2	P35354	Xie *et al*. (1992)
 Amentoflavone hexamethyl ether	Tyrosine-protein phosphatase non-receptor type 2	PTPN2	P18031	Simoncic *et al*. (2002)

Fig. 5: Predicted mode of action for major bioactive compounds in QIE using Swiss docking target online program

As a result, tannins inactivate some vital mechanisms, such as microbial adhesions, enzymes activity, proteins transport and oxidative phosphorylation (Scalbert 1991; Shimada 2006). QIE can increase the osmotic pressure in the surrounding media, due to its high contents of bioactive phytochemicals, thus, derive the microbial cells to release their interior contents. After 12 h, cells lost their water contents, it could be expected that all biological processes inside the cells are affected, no cell wall synthesis, and cells tended to deform and lyse. QIE can interact with the microbial membrane and cell wall, increasing their permeability and causing the release of their interior components. Plant extracts penetrate the cells and interact with vital components such as, DNA, RNA, enzymes, etc., causing their inactivation or inhibiting their synthesis (Isman 2000; Tayel *et al.* 2018a, b). Similarly, Tayel *et al.* (2018a) reported that 1% QIE was effective against some pathogens such as, *S. aureus*, *C. albicans* and *E. coli*. Generally, the majorities of natural antimicrobials, especially from plant origins, are GRAS with quick biodegradability and least mammalian cytotoxicity; which recommend them as ideal ecofriendly safe antimicrobials (Isman 2000).

Wound, Burn Healing Activities of QIE and Molecular Docking

Topical antibiotics are used for managing of burn/wound; however, finding new medication with higher efficacy and lower side effects is still considered as a priority (Dwivedi *et al.* 2017; Tayel *et al.* 2021). Umachigi *et al*. (2008) reported that wound healing and repair was enhanced by applying QIE, e.g. skin coverage of the wound area by structured epidermis and dermal mature tissue. The bioactive components in QIE such as, tannins and phenolics exert antioxidant and anti-microbial activities, which accelerate the healing process (Umachigi *et al.* 2008; Tayel *et al.* 2018a, b). QIE has demonstrated antioxidant and anti-inflammatory effects, along with its antimicrobial properties, are probably responsible for wound contraction and enhancement of tissue epithelization, developing rapid crust through protein precipitation, therefore, increase fasten wound healing (Anlas *et al.* 2019). Docking analysis of the major components in QIE was estimated in *Homo sapiens* database to detect the predicted protein targets in human and its role in healing using Swiss Docking online program (Gfeller *et al.* 2013). Ten major bioactive molecules namely, G-gallayol, Isocryptomerin, 10.7-methyl-3-hydroxymethylene-4,5,6,7,8-pentahydrox-h-thalene, Syringic acid, Gallotannic acid, Tannic acid, Pentagalloylglucose 1, *β*-sitosterol, Methyl oleanate and Amentoflavone hexamethyl ether were detected in GC/MS analysis of QIE (Zhu *et al*. 2009; Hameed *et al.* 2015; Muthu and Gardetti 2016; Elham *et al*. 2021). The 1st bioactive molecule is G-gallayol with (-6.84 cm/s skin permeation) calculated as log k_p according to Potts and Guy (1992). Several mode of actions have been predicted for G-gallayol, it targets the 2-*a*-(1,3)-fucosyltransferase 7 that enable the leukocytes to accumulate at the inflammation site, thus reduce inflammation (Malý *et al.* 1996). It stops cell apoptosis by enhancing carbonic anhydrase-9 enzyme produced by *CA9* gene and Insulin-like-growth factor-1 receptor (IGFIR) produced by *IGF1R* gene, it enhances tissue renewing process (Providence *et al.* 2008). Also, the reversible hydration of CO_2 by carbonic anhydrase-9 enzyme involved in cell proliferation and its transformation, while IGFIR enhances protein synthesis through mechanistic target of rapamycin activation required for myofibrillar muscle protein synthesis, and triggers the antiapoptotic effects. Moreover, G-gallayol enhance plasminogen activator inhibitor-1 (PAI-1) produced by *SERPINE1* gene, it regulates cell adhesion/spreading, and is required for the stimulation of keratinocyte migration during cutaneous injury repair (Malý *et al.* 1996; Humphray *et al.* 2004; Providence *et al.* 2008). The 2nd bioactive molecule is Isocryptomerin with (-5.68 cm/s) skin permeation. It enhances Mast and Stem cells growth factor receptor KIT produced by *KIT* gene, which is important in cell-surface receptor for the cytokine KITLG/SCF, which is vital in the regulation of cell survival, proliferation, hematopoiesis, Stem cell maintenance, Mast cell development and function. Also, it enhances the Aldo-keto reductase family 1 member B1 enzyme produced by *AKR1B1* gene, that plays a role in detoxifying dietary and lipid-derived unsaturated carbonyls (Taniguchi *et al.* 1999; Shen *et al.* 2011). The 3rd bioactive molecule is 10.7-methyl-3-hydroxymethylene-4, 5, 6, 7, 8-pentahydrox-h-thalene which has (-7.6 cm/s) skin permeation. It inhibits 3 types of enzymes (Stromelysin-1, Matrix metalloproteinase-9 and Interstitial collagenase produced by *MMP*, *MMP9* and *MMP1* genes, respectively (Whitham *et al.* 1986; Brinckerhoff *et al.* 1987; Saus *et al.* 1988; Huhtala *et al.* 1991). These enzymes are responsible for degrading fibronectin and different types of collagens, such as I, II, III, IV, V, VII and X collagens. It is well known that both collagen and fibronectin play an essential role in wound healing (Saus *et al.* 1988; Harsha and Brundha 2020). Collagen is a unique, triple-helix protein, forming the major part of extracellular dermal matrix (Harsha and Brundha 2020). Collagen is crucial for activating cell migration and tissues regeneration *via* stimulating fibroblasts and macrophages, thus, enhance and speed up the healing process (Harsha and Brundha 2020). Furthermore, the fast wound healing period, after treatment with QIE and the absence of inflammation and infection signs in treated wounds/burns indicated the synergistic potent effect of QIE to overcome wound infections as well as inflammation, thus, promote faster skin epithelization and regeneration. The 4th bioactive compound is Syringic acid that has (-6.77 cm/s) skin permeation, it targets Carbonic anhydrase-9 enzyme produced by CA9 gene that participates in pH regulation, and involved in cell proliferation and transformation. The 5th, 6th, and 7th

bioactive compounds namely, Gallotannic acid, Tannic acid and Pentagalloylglucose 1 target the same proteins (Table 3 and Fig. 4), they target coagulation Factor-X protein produced by *F10* gene, which is a vitamin K-dependent glycoprotein that converts prothrombin to thrombin in the presence of calcium and phospholipid during the process of blood clotting. They have selective cleavage for Arg-|-Thr and Arg-|-Ile that bonds prothrombin to form thrombin (Walker *et al*. 1980). Also, they target Tyrosine-protein phosphatase non-receptor type-2 (PTPN2) which negatively regulates many signaling and biological processes such as, cell proliferation/differentiation, hematopoiesis, inflammatory response and glucose homeostasis. They are important in the immune system development, control T-cells differentiation as well as activation (Simoncic *et al*. 2002). In addition, target Tyrosyl-DNA phosphodiesterase-1 produced by *TDP1* gene which is a DNA repair enzyme (Raymond *et al*. 2004). The 8th bioactive compound is β-sitosterol has (-2.20 cm/s) skin permeation, it targets androgen receptor produced by *AR* gene, this steroid hormone receptor are ligand-activated transcription factors that regulate eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues (Gottlieb *et al*. 2004). The 9th bioactive compound is Methyl oleanate (-2.84 cm/s skin permeation), it targets Prostaglandin G/H synthase-2 produced by *PTGS2* gene that works as dual peroxidase and cyclooxygenase for biosynthesis of prostanoids, a class of C20 oxylipins that have particular role in inflammatory response. It converts docosapentaenoate to 13R-HDPA, a precursor that activates phagocytosis during infection (Xie *et al*. 1992; Barnett *et al*. 1994; Landino *et al*. 1997; Dalli *et al*. 2015). Finally, the 10th bioactive compound is Amentoflavone hexamethyl ether with (-5.57 cm/s skin permeation), it targets Tyrosine-protein phosphatase non-receptor type-2 produced by *PTPN2* gene, which negatively regulates some biological processes such as, hematopoiesis, inflammation, cell proliferation and its differentiation. Also, it has important role in the immune system development, T-cell receptor signaling, T-cells differentiation/activation (Simoncic *et al*. 2002). Medicinal plants are GRAS and natural acting in a synergized way. Hence, the source of ethno pharmacology does not always be in a single active compound, but rather due to the combination of more than one bioactive compound in the plant extract (Rahman *et al*. 2017).

Conclusion

PPE and QIE showed antimicrobial activity against the skin pathogens *S. aureus* and *C. albicans*. SEM imaging confirmed the action of QIE against both skin pathogens, where, the microbial cells were fully disrupted and lysed, after 12h of exposure to QIE because of its high content of bioactive phytochemicals, as compared to the untreated control. Both plant extracts are GRAS and can used as antimicrobial agents. The successfulness of QIE and PPE applications for the fabrication of anti-*S. aureus* and anti-*C. albicans* textiles, highlight their effectiveness and applicability for skin pathogens control. Results revealed that 10% QIE has good efficacy in wound closure and tissue repair; thus, can be recommended for wounds or burns treatment associated with microbial infections. Molecular docking predicted the main targets of ten major components commonly found in QIE, these bioactive compounds are highly integrated in wound healing, they are involved in the enhancement of immune system, promoting proliferation, migration of keratinocyte, increasing the function of collagens, converting prothrombin to thrombin, activating DNA repair enzyme, as well as reducing inflammation in addition to its potent antimicrobial activity to control skin pathogens.

Acknowledgments

The authors are greatly thankful for the mercy help and guidance from ALLAH.

Author Contributions

Conceptualization, writing, editing, and supervision: Noha Sorour and Ahmad Tayel, Laboratory work, bioinformatics, and data analysis: Shymaa Elbuckley and Rateb Abbas, all authors read and approved the final manuscript.

Conflict of Interest

All authors declare that there are no financial/commercial conflicts of interest.

Ethics Approval

The manuscript contains experiments using animals. The permission of the national authorities (the accreditation no. of the laboratory and of the investigator) are stated in the manuscript.

References

Abbott AM, R Bueno, MT Pedrini, JM Murray, RJ Smith (1992). Insulin-like growth factor I receptor gene structure. *J Biol Chem* 267:10759–10763

Anlas C, T Bakirel, F Ustun-Alkan, B Celik, MY Baran, O Ustuner, A Kuruuzum-Uz (2019). *In vitro* evaluation of the therapeutic potential of Anatolian kermes oak (*Quercus coccifera* L.) as an alternative wound healing agent. *Ind Crops Prod* 137:24–32

Baharuddin NS, H Abdullah, WNAWA Wahab (2015). Anti-Candida activity of *Quercus infectoria* gall extracts against Candida species. *J Pharm Bioallied Sci* 7:15–20

Barnett J, J Chow, D Ives, M Chiou, R Mackenzie, E Osen, B Nguyen, S Tsing, C Bach, J Freire, H Chan (1994). Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. *Biochim Biophys Acta Protein Struct Mol Enzymol* 16:130–139

Brinckerhoff CE, PL Ruby, SD Austin, ME Fini, HD White (1987). Molecular cloning of human synovial cell collagenase and selection of a single gene from genomic DNA. *J Clin Invest* 79:542–546

CLSI – Clinical and Laboratory Standard Institute (2010). *Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement*. CLSI Document M100-S20. CLSI, Clinical and Laboratory Standard Institute, Wayne, PA

Cowan MM (1999). Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564–582

Dalli J, N Chiang, CN Serhan (2015). Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. *Nat Med* 21:1071–1075

Desrochers PE, JJ Jeffrey, SJ Weiss (1991). Interstitial collagenase (matrix metalloproteinase-1) expresses serpinase activity. *J Clin Invest* 87:2258–2265

Dwivedi D, M Dwivedi, S Malviya, V Singh (2017). Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. *J Tradit Compl Med* 7:79–85

Elham A, M Arken, G Kalimanjan, A Arkin, M Iminjan (2021). A review of the phytochemical, pharmacological, pharmacokinetic, and toxicological evaluation of *Quercus infectoria* galls. *J Ethnopharmacol* 273:113592

Gfeller D, O Michielin, V Zoete (2013). Shaping the interaction landscape of bioactive molecules. *Bioinformatics* 29:3073–3079

Golan Y (2019). Current treatment options for acute skin and skin-structure infections. *Clin Infect Dis* 68:206–212

Gonzalez ACO, TF Costa, ZDA Andrade, ARAP Medrado (2016). "Wound healing-a literature review. *Anais Bras Dermatol* 91:614–620

Gottlieb B, LK Beitel, JH Wu, M Trifiro (2004). The androgen receptor gene mutations database (ARDB): 2004 update. *Hum Mutat* 23:527–533

Greenish HG (1999). *Materia Medica: Being an Account of the More Important Crude Drugs of Vegetable and Animal Origin, Designed for Students of Pharmacy and Medicine.* Scientific Publishers, India

Hameed IH, H Jasim, MA Kareem, AO Hussein (2015). Alkaloid constitution of *Nerium oleander* using gas chromatography-mass spectroscopy (GC-MS). *J Med Plants Res* 9:326–334

Harsha L, MP Brundha (2020). Role of collagen in wound healing. *Drug Invent Today* 13:55–57

Heidari M, R Bahramsoltani, AH Abdolghaffari, R Rahimi, M Esfandyari, M Baeeri, G Hassanzadeh, M Abdollahi, MH Farzaei (2019). Efficacy of topical application of standardized extract of *Tragopogon graminifolius* in the healing process of experimental burn wounds. *J Tradit Compl Med* 1:54–59

Huhtala P, A Tuuttila, LT Chow, J Lohi, J Keski-Oja, K Tryggvason (1991). Complete structure of the human gene for 92-kDa type IV collagenase. Divergent regulation of expression for the 92-and 72-kilodalton enzyme genes in HT-1080 cells. *J Biol Chem* 266:16485–16490

Humphray SJ, K Oliver, AR Hunt, RW Plumb, JE Loveland, KL Howe, I Dunham (2004). DNA sequence and analysis of human chromosome 9. *Nature* 429:369–374

Isman MB (2000). Plant essential oils for pest and disease management. *Crop Prot* 19:603–608

Käppeli S, SG Gebhardt-Henrich, E Fröhlich, A Pfulg, MH Stoffel (2011). Prevalence of keel bone deformities in Swiss laying hens. *Brit Poult Sci* 52:531–536

Khan N, N Jamila, F Amin, R Masood, A Atlas, W Khan, NU Ain, SN Khan (2021). Quantification of macro, micro and trace elements, and antimicrobial activity of medicinal herbs and their products. *Arab J Chem* 1:103055

Landino LM, BC Crews, JK Gierse, SD Hauser, LJ Marnett (1997). Mutational analysis of the role of the distal histidine and glutamine residues of prostaglandin-endoperoxide synthase-2 in peroxidase catalysis, hydroperoxide reduction, and cyclooxygenase activation. *J Biol Chem* 272:21565–21574

Malý P, AD Thall, B Petryniak, CE Rogers, PL Smith, RM Marks, JB Lowe (1996). The α (1, 3) fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E and P-selectin ligand biosynthesis. *Cell* 86:643–653

Marrie T, JW Costerton (1984). Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intra-arterial catheters. *J Clin Microbiol* 19:687–693

Min BR, WE Pinchak, R Merkel, S Walker, G Tomita, RC Anderson (2008). Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. *Sci Res Essay* 1:66–73

Morales D (2021). Oak trees (*Quercus* spp.) as a source of extracts with biological activities: A narrative review. *Trends Food Sci Technol* 109:116–125

Mseddi K, F Alimi, E Noumi, VN Veettil, S Deshpande, M Adnan, A Hamdi, S Elkahoui, A Alghamdi, A Kadri, M Patel (2020). *Thymus musilii Velen.* as a promising source of potent bioactive compounds with its pharmacological properties: *In vitro* and *in silico* analysis. *Arab J Chem* 13:6782–6801

Muthu SS, MA Gardetti (2016). *Green fashion*, Vol. 2, pp:239–241. Singapore: Springer

Newman KM, Y Ogata, AM Malon, E Irizarry, RH Gandhi, H Nagase, MD Tilson (1994). Identification of matrix metalloproteinases 3 (stromelysin-1) and 9 (gelatinase B) in abdominal aortic aneurysm. *Arteriosclerosis Thrombosis* 14:1315–1320

Njoroge GN, RW Bussmann (2007). Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. *J Ethnopharmacol* 4:303–307

Potts RO, RH Guy (1992). Predicting skin permeability. *Pharm Res* 9:663–669

Providence KM, SP Higgins, A Mullen, A Battista, R Samarakoon, CE Higgins, CE Wilkins-Port, PJ Higgins (2008). SERPINE1 (PAI-1) is deposited into keratinocyte migration "trails" and required for optimal monolayer wound repair. *Arch Dermatol Res* 300:303–310

Rahman N, H Rahman, M Haris, R Mahmood (2017). Wound healing potentials of *Thevetia peruviana*. Antioxidants and inflammatory markers criteria. *J Tradit Compl Med* 1:519–525

Raymond AC, MC Rideout, B Staker, K Hjerrild, ABJ Burgin (2004). Analysis of human tyrosyl-DNA phosphodiesterase I catalytic residues. *J Mol Biol* 338:895–906

Ribeiro AS, M Estanqueiro, MB Oliveira, JMS Lobo (2015). Main benefits and applicability of plant extracts in skin care products. *Cosmetics* 2:48–65

Saus J, S Quinones, Y Otani, H Nagase, EDH Jr, M Kurkinen (1988). The complete primary structure of human matrix metalloproteinase-3. Identity with stromelysin. *J Biol Chem* 263:6742–6745

Scalbert A (1991). Antimicrobial properties of tannins. *Phytochemistry* 30:3875–3883

Smet PAD (2002). Herbal remedies. *New Engl J Med* 347:2046–2056

Shen Y, L Zhong, S Johnson, D Cao (2011). Human aldo-keto reductases 1B1 and 1B10: A comparative study on their enzyme activity toward electrophilic carbonyl compounds. *Chem Biol Interact* 30:192–198

Shimada T (2006). Salivary proteins as a defense against dietary tannins. *J Chem Ecol* 32:1149–1163

Simoncic PD, A Lee-Loy, DL Barber, ML Tremblay, CJ McGlade (2002). The T cell protein tyrosine phosphatase is a negative regulator of Janus family kinases 1 and 3. *Curr Biol* 12:446–453

Sun S, S Huang, Y Shi, Y Shao, J Qiu, RCAA Sedjoah, Z Yan, L Ding, D Zou, Z Xin (2021). Extraction, isolation, characterization and antimicrobial activities of non-extractable polyphenols from pomegranate peel. *Food Chem* 351:129232

Taniguchi Y, R London, K Schinkmann, S Jiang, H Avraham (1999). The receptor protein tyrosine phosphatase, PTP-RO, is upregulated during megakaryocyte differentiation and Is associated with the c-Kit receptor. *J Amer Soc Hematol* 94:539–549

Tayel AA, RA Ghanem, MS Al-Saggaf, D Elebeedy, AIA El-Maksoud (2021). Application of fish collagen-nanochitosan-henna extract composites for the control of skin pathogens and accelerating wound healing. *Intl J Polym Sci* 30:1–8

Tayel AA, MA El-Sedfy, AI Ibrahim, SH Moussa (2018a). Application of *Quercus infectoria* extract as a natural antimicrobial agent for chicken egg decontamination. *Rev Argent Microbiol* 50:391–397

Tayel AA, RA Ghanem, SH Moussa, M Fahmi, HM Tarjam, N Ismail (2018b). Skin protectant textiles loaded with fish collagen, chitosan and oak galls extract composite. *Intl J Biol Macromol* 117:25–29

Tayel AA, F Wael, OA Abdel-Monem, SM El-Sabbagh, AS Alsohim, EM El-Refai (2013). Production of anticandidal cotton textiles treated with oak gall extract. *Rev Argent Microbiol* 1:271–276

Tottoli EM, R Dorati, I Genta, E Chiesa, S Pisani, B Conti (2020). Skin wound healing process and new emerging technologies for skin wound care and regeneration. *Pharmaceutics* 12:735

Umachigi SP, KN Jayaveera, CA Kumar, GS Kumar, DK Kumar (2008). Studies on wound healing properties of *Quercus infectoria. Trop J Pharm Res* 7:913–919

Walker FJ, WG Owen, CT Esmon (1980). Characterization of the prothrombin activator from the venom of *Oxyuranus scutellatus scutellatus* (taipan venom). *Biochemistry* 19:1020–1023

Whitham SE, G Murphy, P Angel, HJ Rahmsdorf, BJ Smith, A Lyons, AJP Docherty (1986). Comparison of human stromelysin and collagenase by cloning and sequence analysis. *Biochem J* 240:913–916

Xie W, DL Robertson, DL Simmons (1992). Mitogen-inducible prostaglandin G/H synthase: A new target for nonsteroidal anti-inflammatory drugs. *Drug Dev Res* 25: 249–265

Yin C, L Xie, Y Guo (2018). Phytochemical analysis and antibacterial activity of *Gentiana macrophylla* extract against bacteria isolated from burn wound infections. *Microb Pathog* 1:25–28

Zhu F, YZ Cai, J Xing, J Ke, Z Zhan, H Corke (2009). Rapid identification of gallotannins from Chinese galls by matrix assisted laser desorption/ionization time of flight quadrupole ion trap mass spectrometry. *Rapid Commun Mass Spectr Intl J Dev Rapid Dissemin Minute Res Mass Spectr* 23:1678–1682