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WOUND HEALING, *IN-VITRO* AND *IN-VIVO* ANTI *STAPHYLOCOCCUS AUREUS* ACTIVITIES OF LIBYAN *MYRTUS COMMUNIS* L.

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ABSTRACT
With take in consideration the varied medicinal value of medicinal plants (due to a different environmental condition) and the infection complications in general and wound healing retardation in specific, **this study aimed** to evaluate the Wound healing, *In-vitro* and *In-vivo* anti *Staphylococcus aureus* activities of Libyan *Myrtus communis* L. **Methods**: Standard methods and designed animal models were used to achieve the study aim. **Results:** The result proved that leaves of the Libyan *Myrtus communis* constitute polar compounds showed *in-vitro* active growth inhibition zones against standard *Staphylococcus aureus* ATCC23925 (22mm± 1.06 with MIC of 6.25mg, n=3) as well as the clinical MRSA isolates (25mm± 6, n=31). In wound healing activity assay and with Post Hoc analysis, high significant differences in performance (P value ≤ 01) were shown in treatment days between control and tested groups, where results showed that *Myrtus communis* enhances the wound healing process by reducing healing period from 12 to 8 days compared to control group. In addition, in the *in vivo* study, *Myrtus* ointment actively treated wounds infected with *Staphylococcus aureus* in 8 days compared with 7 days along with Fucidin ointment. **Conclusion**: This study suggests that Libyan *Myrtus communis* is promising as wound healing accelerator and powerful anti-*Staphylococcus aureus* agent and introduces it as an alternative to Beta-lactam antibiotics and also to Gentamicin and Ciprofloxacin concerning to their side effects after deep clinical evaluation to assess its safety.

KEYWORDS: Wound healing, MRSA, *In-vitro*, *In-vivo* antibacterial activity, *Myrtus communis*.

INTRODUCTION
The skin is the largest organ coats the external surface of the body provide the protection of the body against the pathogens, chemical, UV light and injury (Yousef *et al*., 2019). It is a big challenge in medicine to improve tissue regeneration in the body via therapeutic management of skin intact and integrity, since wound healing process is a complex biological processes consisting of complex interactions among several cell types and their products (Shaw and Martin, 2009; Gonzalez *et al*., 2016). The wound to be successfully healed, it should pass through four stages; hemostasis, inflammation, proliferation and remodelling. Interfering of one or more of these stages may delay skin regeneration and wound healing. However, chronic wounds are thought to be closely related to prolongation of the inflammatory phase (Agren *et al*., 2000). Bacteria and their endotoxins are factors cause prolonged elevation of pro-inflammatory cytokines such as interleukin-1(IL-1) and TNF-α and then prolonged the inflammatory phase, the matter which lead to impairment of wound healing (Edwards and Harding, 2004 ; Menke *et al*., 2007).

It has been reported that aerobic and anaerobic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and beta-hemolytic streptococci are pathogens complicated wound healing process (Edwards and Harding, 2004 ; Davis *et al*., 2008). *Pseudomonas aeruginosa* and *Staphylococcus aureus* have a significant role in wounds bacterial infection where biofilm formation capacity of bacteria is considered as a type of mechanism related to a numbers of chronic wounds

infections caused by these pathogens, since this biofilm protecting the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils (Bjarnsholt *et al*.,2008). *Staphylococcus aureus* is a human communal bacterium responsible for many clinically important infections. It is an opportunistic pathogen has been documented by Gjødsbol , *et al* (2006) to be found in 93.5% of patients were suffered from chronic venous leg ulcers. It is well known that *Staphylococcus aureus* has the ability to secrets a number of potential virulence factors and surface proteins which encourage its adherence to the damaged tissue and decrease neutrophil functions and immune responses of the host by which prolonged the inflammatory stages (Fazli *et al*., 2009). As many classes of antibiotics have become therapeutically ineffective due to the increasing rate of resistance, Now a day there is a big attention for using of plants as a source of new alternative antibiotics. Medicinal plants considered as a rich source of medicinal bioactive compounds, which are available at a cheap cost. One of these medicinal plants is *Myrtus communis* L named (Myrtle) and named Merseen by Libyan population. This evergreen plant is belongs to Myrtaceae family, which includes 100 genera and 3000 species. It is distributed in Australia, North western Himalaya and South America and considered as native to Western Asia, Southern Europe and North Africa (Alipour *et al*., 2014). Traditionally *Myrtus* used as a hypoglycemic agent and also it is used as antiseptic and disinfectant. Also, the plant has been used in the food industry, for example, for flavoring meat and sauces (Sabiha *et al*., 2011). Also, the antimicrobial activity of the aerial parts of *Myrtus communis* plant that had been collected from different regions were studied by many researchers for their activities against Gram positive, Gram-negative bacteria and some fungi and results showed varied inhibition zones and this variation has been attributed to the difference in collection regions of plant (Taheri, *et al*., 2013, Besufekad *et al*., 2017). However, *Myrtus communis* L is wild growing in the North East of Libya and is traditionally named Myrseen by Libyan population and used as hypoglycaemic agents and wound antiseptic agent. Despite modern sterilization techniques and prophylactic use of good antibiotics, there is a delay in the wound healing process, especially with the worldwide problem of the increasing rate of bacterial resistance. In this context with the promising biological activity reported from the plant *Myrtus* and because there is few study have ran studying some biological activity of the Libyan *Myrtus communis* with the note that there is no study has done previously to screen the effect of the Libyan *Myrtus communis* for its ability to accelerate wound healing and also no study had done to evaluate the *in vivo* antibacterial activity of this plant species, so this study aimed to evaluate the wound healing, *in-vitro* and *in-vivo* anti *Staphylococcus aureus* activity of Libyan *Myrtus communis*.

MATERIALS AND METHODS

Plant collection and preparation

Myrtus communis was collected in summer from Al-Hamama region, located in Al Jabal Al Akhdar, Northeast of Libya. Plant leaves was cleaned with tap water, air dried at room temperature and then powdered. The dried leaves powder was kept in separate coloured bottles, ready for the extraction process.

Experimental Animals

Experimental Albino Rats used in this study were obtained from the Experimental Animal House, Medicinal Aromatic Plants Research Institute, National Centre for Research, Sudan. Animals were given standard feeding and tap water.

Bacterial strains and isolates

The antibacterial activity was studied against standard American Type Culture Collection *Staphylococcus aureus* ATCC 23925 and clinical Methicillin resistant *Staphylococcus aureus* (MRSA). The standard strain obtained from Medicinal and Aromatic Plant and Traditional Medicine Research Institute, National Centre for Research, Khartoum, Sudan and the clinical isolates obtained from inpatient admitted to Benghazi Medical Center, Benghazi, Libya.

Reference Antibiotics Discs

Augmentin 30µg (Amoxicillin 20µg + Clavulanic acid 10µg), Ceftazidime30µg, Ceftriaxone 30 µg, Vancomycin 30µg, Gentamicin 10µg and Ciprofloxacin antibiotics 30µg are standard antibiotics discs used as references in this study. They were bought from Bioanalyse@YSE TibbiMalzemeler San. Expire dates were 10 - 18 months valid after the date of the assay.

Preparation of plant extract

In Soxhlet apparatus one hundred grams of leaves powder of *Myrtus communis* was thoroughly extracted for enough time (6-10 hours) with enough quantity (250-300ml) of Methanol solvents. The solvent was evaporated with use of rotary evaporator and the extract was air dried and kept in well labelled coloured and tight closed bottles in a fridge at 4Cº.

In vitro anti-*Staphylococcus aureus* activity of *Myrtus communis*

Disc diffusion assay

Antibacterial activity was tested by disc diffusion method, as described by Mukhtar and Ghori (2012) with some modifications. One ml of a bacterial suspension Prepared according to standard method and adjusted to a bacterial density of 10^8 C.F.U/ml is seeded in the Petri dishes containing Mueller-Hinton agar. A sterile paper disc (6mm diameter) was aseptically placed on the inoculated plates on which 10µL of previously prepared plant essential oil were added. Then plates were kept for 15 minutes at room temperature. After 18 hr of incubation at 37 °C, the inhibition zones were measured in mm. Disc impregnated with 40% v/v of DMSO was

used as negative control. Both assays of standard and clinical bacteria were done in triplicate. The sensitivity of bacterial strain and isolate to the plant essential oil was classified as not sensitive for a diameter smaller than 8 mm, moderately sensitive for a diameter range from 8 to 14 mm, sensitive for a 14–20mm diameter, and very sensitive for a diameter larger than 20mm.

Determination of Minimum inhibitory concentration (MIC)

Andrews, (2006) agar dilution method was adopted in this study with little to determine the minimum inhibitory concentration of the plant essential oil which can inhibit the growth of the seeded clinical bacteria on the Mueller-Hinton agar media. Serial dilutions were prepared for the plant essential oil in decreasing concentrations in the following order: 200,100, 50, 25, 12.5, 6.25, and 3.13mg/ml. In sterile covered glass bottles, 5ml Melted double strength Mueller-Hinton agar cooled to 45°C were mixed with 5ml of each dilution of the tested plant essential oil to get a final serial dilution of 100, 50, 25, 12.5, 6.25, 3.13 and 1.65mg/ml of the essential oil. The mixture was poured to sterile small petri dishes, left to solidify and then by using of a standard loop (0.01ml), a loop full of each of tested bacterial fresh suspension adjusted with McFarland 0.5 solution was spotted in duplicate spots onto the surface of each agar plate. The inoculum allowed to be absorbed into the agar before incubation and then the plates incubated at 37°C for 18 hours. After the incubation period the least concentration mg/ml of the plant extract that inhibits the growth of organism was considered as the end point (MIC).

Pharmaceutical preparation of 3% ointment of *Myrtus communis* methanol bark extract

To prepare a 100gm ointment of 3% of *Myrtus communis* extract, a mix of emulsifying wax (30gm), white soft paraffin (50gm) and Liquid paraffin (20ml) was put (in Pyrex beaker with magnetic stiror) on a heater (Hot plate magnetic stiror) at 60 Cº. After all ingredients melted were poured into morter contain 3gm of the extract powder. The mix was mixed well with a glass rod till get homogenized mixture. The ointment preparation was kept in a clean dry well tight container at 4 Cº (British Pharmacopia, 1988).

Wound healing and *In vivo* Antibacterial Activity of *Myrtus communis*

Preparation of wounded animal

Two assays lines were designed in this study. Each assay line was composed of two groups and each group composed of 5 rats. For each rat, full-thickness wound was made in the skin of the test animals according to the model of Abdrabo *et al*. (2005). Hair of the lower back and right flank of animals was fully shaved. Rats were lightly anaesthetized by inhalation using halothane. The animals were held in a standard crouching position, and the mobile skin of flank was gently stretched and held by fingers. A metal circular object measuring 1 cm in diameter was placed on the stretched skin and an outline of the object was traced on the skin using a fine-tipped pen. The wound was made by excising the skin within the border of the object to the level of loose subcutaneous tissue, using sterile forceps and a scalpel blade. The artificial wounds were circular, with a diameter of 1 cm. The first day of the experiment was regarded as the Zero day.

First *in vivo* assay line: Wound healing activity of *Myrtus communis* (Rats with non-infected wounds)

Ten experimental albino rats were divided into two groups, each consists of 5 rats. **Group 1 (wound only)** named untreated control group where wounds were left without treatment but were daily cleaned with alcohol 70%. **Group 2 (wound + *Myrtus communis* ointment 3%) in which w**ounds were treated topically with *Myrtus communis* ointment 3% every 12 hours starting from the first day. Wound cleaning with alcohol 70% was adapted as a previous step before applying the plant ointment.

Second *in vivo* assay line: Antibacterial activity of *Myrtus communis* (*Staphylococcus aureus* infected rats)

The same steps were done as in the first trial line, but in this line an artificial wound infection was done by seeded the wound with *Staphylococcus aureus* ATCC25923 suspension freshly prepared and adjusted with 0.5 McFarland standards (10^7-10^8 C.F.U. /ml). **Group 1 (infected wounds + Fucidin ointment 2%);** where **w**ounds of these rats group were infected with tested bacteria and then treated topically with Fucidin ointment 2% every 12 hours as a standard healing agent starting from the first day. **Group 2 (infected wounds +** *Myrtus communis* ointment 3%) in which wounded animals were artificially infected with *Staphylococcus aureus* ATCC25923 same as in Group 1, and after 12 hours treated topically with *Myrtus communis* ointment 30% every 12 hours starting from the first day. Means of diameters of wounds of each group were measured once daily (every 24 hours) and percentages of healing area were evaluated.

Statistical Analysis

Data were expressed as mean ± SD. Statistical examination was performed utilizing SPSS version 20, One-way analysis of variance (ANOVA) followed by the LSD Post Hoc test. The P values more than 0.05, less than ≤0.05 and ≤ less than 0.01 were considered as not significant, significant and highly significant values, respectively.

RESULTS AND DISCUSSION

Wound healing activity

Although the plant medical value is differ from one plant to another and from one species to another referring to different secondary metabolites, they produce in

response to the different collection area with different ecosystem and environmental condition, nevertheless several medicinal plants has been found reported as helper in wound healing process (Nagori and Solanki, 2011). *Myrtus communis* is one of these plants which has been reported from some different areas as having wound healing activity, but in specific, no study found studied the wound healing activity of the Methanol extract of the leaves of the *Myrtus communis* wild grow in the North East of Libya and figure out if it has any enhancing activity for the healing process. Table (1) showed that complete wound healing takes 12 days in the control group(wound only), while it takes 8 days with the treated rats group (wound treated with *Myrtus communis* 3% ointment) to access complete healing. This pointed to that the tested *Myrtus communis* ointment was accelerates the wound healing process and reduces the healing time by 4 days. Also in this study it has been noticed that no significant differences ($P \geq 0.05$) had shown in the percentages of wound healing area between the control wounded rats group received no treatment and the wounded rats group treated with *Myrtus communis* 3% ointment in the zero, first and second days of the assay while starting from the third up to the sixth day, high significant differences ($P \leq 0.01$) in the percentages of wound healing area were shown between the two tested groups (Table 1). This result matches with Rezaie *et al.* (2012), who documented that the leaves of *Myrtus communis* have significant wound healing activity where they enhanced the wound contraction and fibroblast cell proliferation. This study result is in harmony with Aidi *et al.* (2010), Maxia *et al.* (2011), and Farzaei *et al.* (2014), who reported that the *Myrtus communis* aerial parts demonstrated a significant anti-inflammatory activity via reduction of TNF-α and IL-6 levels and inhibition of myeloperoxidase (MPO) activity and leukocyte migration by then help in wound healing process. Also it has been confirmed that Myrtel remarkably could enhance the protein expression levels of the angiogenic markers such as HIF-1α and VEGF in HUVECs(Raeiszadeh *et al.*, 2018). The crucial process for development of new blood vessels which named angiogenesis is well known to be initiated by stimulation of endothelial cells, especially by VEGF isoforms that are mainly regulated by HIF-1α (Li *et al.*, 2015; Zimna and Kurpisz, 2015).

Tabe 1: Means ± standard deviation of area of wound healing of clean wounds treated with *Myrtus communis* 3% ointment.

Days	Control (wound only)		Wound + *Myrtus communis* 3% ointment		LSD Post Hoc analysis	
	Mean ± SD	% of healing area	Mean ± SD	% of healing area	P value	Sig.
0day	2.9±1.3	100	4.0±1.3	100	4.0±1.3	100
1day	2.5±1.2	-72.9621	2.2±0.8	-36	2.2±0.8	-36
2day	2.2±1.1	-73.5	1.6±0.3	-47.8	1.6±0.3	-47.8
3day	1.9±0.9[a]	-70.5273	1.1±0.1[b]	-48.4	1.1±0.1[b]	-48.4
4day	1.6±0.7[a]	-61.2579	0.8±0.2[b]	-44.3545	0.8±0.2[b]	-44.3545
5day	1.2±0.6[a]	-60.9	0.5±0.2[b]	-24.2	0.5±0.2[b]	-24.2
6day	1.0±0.4[a]	-57.1333	0.2±0.1[b]	-19.5	0.2±0.1[b]	-19.5
7day	0.7±0.2[a]	-49	0.1±0.1[b]	0.2	0.1±0.1[b]	0.2
8day	0.5±0.1	-42.1571	0.0±0.1	0.1	-	-
9day	0.3±0.1	-39.5	0.0±0.0	-	-	-
10day	0.2±0.1	-33.0333	-	-	-	-
11day	0.1±0.1	0.2	-	-	-	-
12day	-	-	-	-	-	-

In vitro* anti-*Staphylococcus aureus* activity of *Myrtus communis

In Libya this plant named Myrseen by Libyan population and is mainly used in low doses as hypoglycemic plant but in minor used for treatment of wounds by some villagers and **the result of the *in vitro*** antibacterial screening of this study showed that the growth of tested standard *Staphylococcus aureus* ATCC 25923 was actively inhibited by the methanol extract of leaves of *Myrtus communis* and revealed diameter of inhibition zones (DIZ) of 22±1.06 and minimum inhibitory concentration (MIC) of 6.25mg/ml (Figure1), and this result is in agreement with Mansouri (1999) who reported in his study that the alcoholic extract (Ethanol 95%) of leaves of the Iranian *Myrtus communis* has good growth inhibition activity against *Staphylococcus aureus* ATCC 25923 and revealed inhibition zone of 21mm which is close to what revealed from this study. **Even though** Both, this study and Masouri's study used different solvents; Methanol and 95% Ethanol, respectively but both studies agreed in that the polar constituents of leaves of *Myrtus communis* has a powerful growth inhibition activity against standard *Staphylococcus aureus* strain. **On the other hand** this study results proved that the tested clinical Methicillin resistant *Staphylococcus aureus* (MRSA) was actively inhibited too but with larger DIZ of 25.0± 6 compared to the standard strain (Figure 1). Abouzeed *et al.* (2013) also did an *in vitro* study to evaluate the antibacterial activity of methanol extract of leaves of the Libyan *Myrtus communis* against MRSA and they proved that the tested MRSA was moderately inhibited with DIZ of

9mm, the activity which is less than what shown from this study. Although Abouzeed and his team have studied the anti- *Staphylococcus aureus* activity of the same solvent extract of the same part of *Myrtus communis* which had been collected from the same region from which the plant had been collected in this study, but this investigation showed higher antibacterial performance against MRSA, and this may refer to the different time (season) of collection, since the plant has been harvested in Summer in this study while in Abouzeed's study it had been collected in Spring. Different environment condition between different seasons makes variation in the type and concentrations of the plants secondary metabolites, the matter which leads to variation in the performance activity.

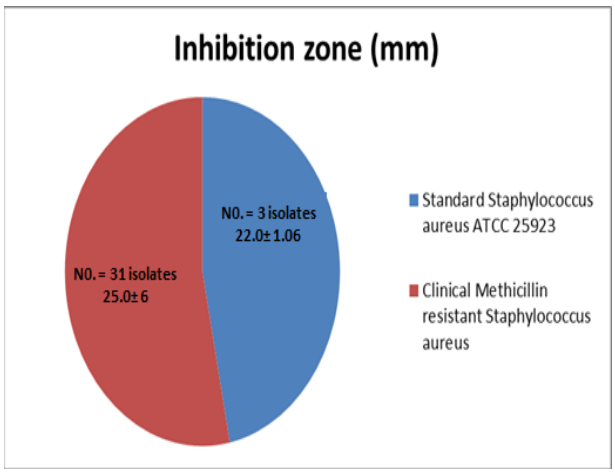

Figure (1): Antibacterial activity of *Myrtus communis* against both standard *Staphylococcus aureus* and clinical MRSA.

When the growth inhibition performance of leaves methanol extract of *Myrtus communis* has been compared with that offered from six antibiotics references, results showed that there was a high significant difference (P value ≤ 0.01) between the performance of the three tested Beta-lactam antibiotics; Augmentin, Ceftazidime and Ceftriaxone and *Myrtus communis* performance against tested MRSA. *Myrtus communis* actively suppresses MRSA growth with DIZ of 25mm ± 6 compared with zero (0) DIZ shown from Augmentin, Ceftazidime and Ceftriaxone (Figure 2). This result pointed to the thought that this plant may have a substance/s with structure which can bind to the cell wall binding protein and withstand the breakdown activity of the bacteria beta-lactamase enzyme. **Also** Post Hoc analysis of the results in this study showed high significant difference (P value ≤ 0.01) between the antibacterial activity of Ciprofloxacin (Fluoroquinolone antibiotic) and *Myrtus communis*, despite both were actively inhibited the growth of MRSA but the Ciprofloxacin was the highest with DIZ of 31.8mm ± 3. However regarding to the serious adverse effects sometimes occur with use of the fluoroquinolone antibiotics such as tendonitis, insomnia, restlessness, and rarely, seizure, convulsions, and psychosis, this study can suggest *Myrtus communis* as an alternative to Ciprofloxacin in treatment of infections caused by multidrug resistant *Staphylococcus aureus* organism. In addition, both the drug of choice for treatment of MRSA infections the peptidoglycan; Vancomycin and the aminoglycoside Gentamicin used in this screening were actively inhibited the growth of MRSA and revealed close DIZ of 19.1± 2 and 19.6± 2, with no significance differences (P value ≥ 0.05).

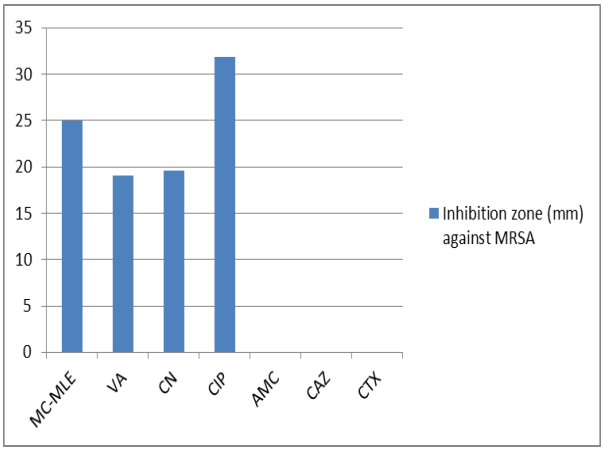

Figure (2): Antibacterial activity of *Myrtus communis* compared with antibiotics references.

MC-MLE = *Myrtus communis* Methanol leaves extract
CAZ = Ceftazidime 30µg
VA = Vancomycin 30µg **CN**= Gentamicin 10 µg
CIP= Ciprofloxacin 30µg
CTX = Ceftriaxone 30µg **AMC** = Amoxicillin 20µg + Clavulanic acid 10µg
MRSA = Methicillin resistant *Staphylococcus aureus*

However, the results proved that *Myrtus communis* was the most active (DIZ of 25mm) compared with Vancomycin and Gentamicin (Figure 2) with a high significant difference (P value ≤ 0.01), and this result highlighted the promising anti MRSA activity offered by *Myrtus communis* especially with take in consideration the annoying side effect and route of administration of Gentamicin and the increasing rate of Staphylococcus resistance to Vancomycin.

In vivo* anti-*Staphylococcus aureus* activity of *Myrtus communis

The *in vivo* antibacterial assessment done in this study showed that there was only one day gap difference in the healing period between tested group and control group, where infected wounds treated with Fucidin ointment (control group) takes 7 healing days while infected ones treated with *Myrtus communis* ointment (tested group) takes 8 healing days for complete healing (Table 2). The statistics analysis in this study showed that no significant differences (P ≥0.05) shown in the percentages of wound healing area in the zero, first and second days of the assay between the tested and control groups. However from the third to the seventh day high significant differences (P ≤0.01) shown in the

percentages of wound healing area between the control group and the tested group treated with *Myrtus communis* ointment (Table 2), while by the eight day of the assay no significant differences (P ≥0.05) had shown between the two groups. This result pointed to that *Myrtus communis* plant actively fights *Staphyloccoocus aureus* bacterium and enhances the wound healing process. These study outputs clarify that *Myrtus communis* ointment has good anti-*Staphylococcus* performance as same as Fucidin ointment, the drug of choice commonly used in treatment of infections caused by the Gram positive *Staphylococcus aureus*. This finding is in agreement with Taheri *et al*. (2013) who documented that leaves of *Myrtus communis* are effective against *Staphylococcus aureus* bacterium and is in harmony with Pereira *et al*. (2013) who proved that alcoholic extract of leaves of *Myrtus communis* has a significant inhibitory effect against Gram-positive bacteria. However with this *in vivo* antibacterial screening as the first study done in Libya to evaluate the effectiveness of leaves of the Libyan *Myrtus communis* in treatment of wounds infected with *Staphyloccoocus aureus*, the results comes in harmony with that shown from the *in vitro* antibacterial screening in this study, the situation which highlighted and confirmed the promising anti-*Staphylococcus aureus* activity of Libyan *Myrtus communis*.

Table (2): *In vivo* anti-*Staphylococcus aureus* activity of *Myrtus communis* 3% ointment compared with Fucidine 2% ointment.

Days	wound infected with *S.aureus* + Fucidine 2% ointment		wound infected with *S.aureus* + *Myrtus communis* 3% ointment		LSD Post Hoc analysis	
	Mean ±SD	% of healing area	Mean ±SD	% of healing area	P value	Sig.
0day	3.4±1.2	100	5.6±3.7	100	.24	NS
1day	2.2±0.6	-61.30	3.3±1.8	-30.11	.33	NS
2day	1.3±0.3	-56.89	2.0±0.1	-39.12	.06	NS
3day	0.7 ±0.2	-52.54	1.4 ±0.6	-48	.002	**
4day	0.5 ±0.2	-70.72	1.0 ±0.5	-48.6	.002	**
5day	0.3 ±0.1	-59.5	.7 ±0.3	-49	.000	**
6day	0.1 ±0.1	-33.03	.5 ±0.2	-27.87	.000	**
7day	0.0 ±0.0	0.1	.2 ±0.2	-19.5	.000	**
8day	-	-	.1±0.0	0.1	-	-
9day	-	-	.0±0.0	0.0	-	-
10day	-	-	-	-	-	-

CONCLUSION

This study showed that the Methanol extract of leaves of Libyan *Myrtus communis* effectively accelerated the wound healing process and showed both *in-vitro* and *in-vivo* promising antibacterial performance against *Staphylococcus aureus* compared to control group. In addition, this study suggests that leaves of *Myrtus communis* plant wild growing in the North East of Libya could be considered as a potential source of raw and purified materials of a big challenge in pharmaceutical industry for producing new promising anti–Methicillin resistant *Staphylococcus aureus* agents and wound healing enhancers agents.

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