PRANGOS PLATYCHLAENA BOISS ESSENTIAL OILS: A NOVEL STUDY ON ITS TOXICITY, ANTIBACTERIAL ACTIVITY AND CHEMICAL COMPOSITIONS EFFECT ON BURN RATS

| J. K. Rahman | D. M. JAFF | D. Dastan |
|--------------------|----------------------|--------------------|
| Lecturer | Assist Prof. | Assist Prof. |
| College of Science | College of Education | School of Pharmacy |
| Salahaddin Uni. | Salahaddin Uni. | Hamadan Uni. |
| jwanbio9@gmail.com | d95uk@yahoo.co.uk | dara962@gmail.com |
| ABSTRACT | | |

The *Prangos platychlaena Boiss* belonged to the family of Apiaceae is a native medicinal plant of Kurdistan-Iraq. The stem, leaves and flowers of the plant were collected in the mountain of Halgurd of the Kurdistan region of Iraq, and hydrodistilated to obtain the volatile compounds, using both Gas Chromatography (GC) and Gas chromatography/ Mass spectroscopy (GC/MS). The results showed that the *P. platychlaena Boiss* essential oils containing several compounds such as Sylvestrene, α -Pinene oxide, (Z)-Epoxy-ocimene, Borneol, epi-Longipinanol, Germacrene D-4-ol, epi- α -Cadinol, 3-Thujanol acetate and Acorenone with different amount which were not found in any other Prangos species, and all showed antibacterial activity. This experiment was concducted to reveal the toxicity of flowers essential oils of the *P.platychlaena Boiss* on skin burns of the rat. The results revealed that the base cream which contains 1% of the essential oils showd less time in healing wounds in comparing with the other groups. Histological examination of the treated ones showed significantly the formation of new epiderm in those rats which were receiving the base cream containg 1% essential oils. These finding sugested that the essential oils of this plant can be used as a surface treatment agent for the human burns.

Key word: skin, chromatography, wound, pinene

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| إسة عن تاثير سميته ، | : (PRANGOS PLATYCHLAENA BOISS) درا | الزيوت الأساسية لبرنقوس المسطح (|
| | دات الجراثيم والموكناتها الكيميائيةعلى حرق الفئران | ومضا |
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المستخلص

تشتهر المناطق الجبلية في شمال العراق بتنوعها للنباتات البرية و منتجاتها الطبيعية التي لا يزال تستخدم لغرض العلاج التقليدي و غيرها من الأغراض. ان نبات البرنقوس المسطح (Prangos platychlaena) نبات بري يعود الى عائلة Apiaceae وهو يعتبر نبات طبي اصيل لكردستان العراق. تم جمع مختلف الأجزاء النباتية منها السيقان, الأوراق, و الأزهار من جبل هلكورد الواقعة في أقليم كردستان العراق. و لغرض الحصول على المركبات الطيارة, تعرّضت الأجزاء النباتية المذكورة الى عملية التقطير المائي. كما تم تشخيص المكونات الكيميائية بأستخدام جهاز الكروماتوغرافيا الغزية و الكروماتوغرافيا الغازية/ المنباتية المذكورة الى عملية التقطير المائي. كما تم تشخيص المكونات الكيميائية بأستخدام جهاز الكروماتوغرافيا الغازية و الكروماتوغرافيا الغازية/ المطياف الكتلي. أظهرت النتائج بأن الزيوت الأساسية لـ *Platychlaena حتوي على مجموعة من مختلف* العزية و الكروماتوغرافيا الغازية/ المطياف الكتلي. أظهرت النتائج بأن الزيوت الأساسية لـ *Platychlaena حتوي على مجموعة من مختلف* المركبات منها Prango coime oxide.Sylvestrene D-4-ol ، وات لم تتواجد فى أنواع اخرى من 2008 مي ذات فعالية عالية ضد المركبات منها *Prango على العارف التتلي. أظهرت النتائج بأن الزيوت الأساسية لـ Prango و و هي من محتوي على مجموعة من مختلف* المركبات منها borneol (وات معرفة سمية الزيوت الطيارة لأزهار محمول الالم المركبات منها borneol و هي ذات فعالية عالية ضد المركبات منها borneol و معرفة سمية الزيوت الطيارة لأزهار *P platychlaena على الحرو*قات الجلدية للفئران.أظهرت النتائج بأن الكريم البكتيريا. أجريت التجربة لغرض معرفة سمية الزيوت الطيارة لأزهار *P platychlaena على الحرو*قات الجلدية للفئران.أظهرت النتائج بأن الكريم الأساس (base cream) والتي تحقوي على 1% من الزيوت الطيارة استغرق اقل مدة زمنية لازمة لعلاج الجروح مقارنة مع المجاميع. المحص الأساس (base cream) والتي تحقوي على 1% من الزيوت الطيارة استغرق اقل مدة زمنية لازمة لعلاج الجروح مقارنة مع المجاميع. المحص الأساسيجي للمعاملة تبيّت تكوين بشرة جديدة في الفئران المعاملة بالكريم الأساس المحتوي على 1% الزيوت الطيارة مقارنة مع المجاميع المحروقة الأسريجي النسايج بأن الزيوت الطيارة النبات يمكن استخدامها كمعالج سطحي لحروق جلد الأنسان.

الكليمات المفتاحية: بشرة, كروماتوغرافي, جرح, باينين

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INTRODUCTION

Mountain region of the north of Iraq, is well known for the diversity of the wild plants and their natural products used as fodder, for a long time by its habitants, traditional medicine and other purposes(3). The family of Apiaceae is regarded as one of the important famililes, with its different species. The genus of Prangos belongs to the family of Apiaceae, under the order of apiales, having 30 species, distributed from the Mediterranean to central Asia, 14 of them were found in Turkey (2), while seven of them distributed in the Kurdistan region of Iraq which are P.platychlaena Boiss., P.ferulacea (L.), P. uloptera DC., P.asperula Boiss., P.pabularia P.peucedanifolia Lindl.. Fenz. and P.carymbosa Boiss . P. platychlaena Boiss is a perennial plant, length can reach up to (1-1.5m), This plant is found naturally growing in the mountain of Kurdistan region, it contains different quantities of the essential oils and other phytochemical compounds, which plays an important role in the antibacterial activity and in the wound healing process (11). Toxicity assay is regarded as an important aspect among the process of wound healing. This process promoted by plant products, which possessed the effect with no or less toxicity compared with the synthetic drugs. A part of this study is concerning the essential oil compounds from the leaves, stems and flowers of P. platychlaena Boiss which deals with the chemical compositions, antibacterial activity and the toxicity of essential oils of the *P*. platychlaena Boiss. Up to date of reported literatures concerning this function of the P. platychlaena Bioss, covering the investigations about this plant species, were not found.

MATERIALS AND METHODS Collection of plant materials

The *Prangos platychlaena Boiss* leaves, stem and flowers *were* collected during May -July 2017 from the Halgurd mountain in Kurdistan region of Iraq, at the altitude of 2170m. above sea level, latitude 36:42.30758N and longitude 44:52.29358E which then were identified by the professional taxonomist from the department of biology, College of Education ,Salahaddin university-Erbil.

Essential oil isolation: The oils of the *P.platychlaena* plant parts were extracted by

the hydrodistillation process for 4 hours, using a Clevenger-type apparatus then was dried by using Na₂SO₄. The obtained essential oil was kept in a darkly closed vial at $4^{\circ}C$ (4).

Density of essential oil

The density of essential oils was determined as the weight of essential oil extract (g) per volume of essential oils.

The yield of essential oils

The oils yield of leaves, stem and flowers were determined as percent volume (ml) of essential oil per fresh weight (g) of the plant part (% v/m).

GC and GC/MS analysis

The oils from leaves, stem and flowers samples were analysis by using Gas-Chromatography (GC), with а flameionization detector (FID). The processes were carried out by using fused silica capillary DB-5 column (60m \times 0.5mm, film thicken 0.5 um). The temperatures of the detector and injector were300.0° С and 250.0°C respectively. The N₂ as the carrier gas at a flow rate of 1ml/min, oven temperature was from 60° C to 250° C at the rate of 5° C/ min was programmed, and finally held isothermally for Analysis 10mins. of oil bv Gas Chromatography/Mas Spectroscopy (GC/MS) was performed by using a thermoquest-Finnigan gas chromatograph with the column mentioned above, with the same conditions coupled to a TRACE mass spectrometer. The scanning of mass ranged from 43 to 456 m/z. The voltage of ionization was 70 eV. The tempretures for the ion source and the interface were kept at 200° C and 250° C, respectively(9).

Identification of compounds

The identification of oil constituents in leaves, stem and flowers of the P.platychlaena plants were performed by calculating of their retention indices under the same chromatographic conditions for the n-alkanes (C_6-C_{24}) and oil on a DB-5 column. Identification of the oils were performed by comparing their mass spectra with those of the library standards or the authentic compounds, while for confirming compounds, the percentage of their relative areas were obtained by Flame Ionization Detector (FID), which used without using of correction factors(9).

Bacterial strain

The in vitro antimicrobial activities of the essential oils compounds were carried out, by pathogenic using bacteria such as Staphylococcus aureus ATCC 6538P, clinical Staphylococcus aureus. Pseudomonas aeruginosa ATCC 9027, clinical Pseudomonas aeruginosa. The pathogen isolated from human, which bacteria was obtained from the Media Diagnostic Health Center in Erbil city.

Culture media and activation of bacterial strain

The medium of Mueller-Hinton broth was used for cultivation of bacteria. The inocula were prepared by transferring several single colonies of microbes into a sterile broth media and mixed together, then incubated at 37° C for overnight. Inoculum of the culture solution was adjusted to the McFarland scale 0.5 and confirmed by spectrophotometrical reading at 580nm (12, 16).

Evaluation of antibacterial activities

Bacterial activities of the essential oils were by the Microdilution assessed method according to previous methods (8, 9) ,this used for determining of the minimum Inhibitory Concentration (MIC) of the *P*. platychlaena Boiss. The assay was carried out in 96-well sterile micro plates, then, 100 µl of nutrient broth was added to each well of the microplate. The first well was filled with 100µl of the essential oils. Next, by transferring 100µl content from the first well into the next well of the same row of the microplate, after mixing the content of the well, this procedure was repeated for the following well of the same row to obtain serial dilution of 1:2, 1:4, 1:8, 1:16, etc., in this case, the concentration of the sample will decrease, while the pure dimethyl sulfoxide and broth media were used as negative control, whearas Ciprofloxacin (antibacteria) were considered as references. For each well, a microbial suspension of working solution was inoculated. The absorbance for each well was measured at 630nm before incubation time; by ELISA Microplate Reader. using the microplate was incubated at 37 ^oC for 24h for the growth of bacteria. The absorbance was re-measured after incubation time to compare with the initial measurement. The MIC was

calculated at the break point concentrations by comparing the absorbance before and after incubation time.

Preparation of cream

Different percentages of the flower essential oils of *P. platychlaena Boiss* were mixed with base cream (Cream contains: Micro Crstalline Wax, Hard Paraffin Wax, Light liquid Paraffin; Made in India; N.C.No.:MH/COS/KD-C-323), which obtained from local Pharmaceutical, was homogenized for 15min at 25° C, the mixture stored at 4° C during the experiments (21).

Animals

The experiment was conducted at the Biology department, College of education, University of Salahaddin, using adult female albino rat (Rattu norvegicus), weighting (180-200) g, age (8-10) weeks. They were housed under standard conditions (12h.light, 12h.dark) photoperiod, at temperature 23- 25° C, humidity 60 ± 5 %, having standard diets and free access to tap water. All animals were housed in plastic cages (38× 18×30) cm, bedded with the chip of wooden which sterilized with UV light about 1.5 hr. During the experiment, 2 animals were kept in each cage to avoid removing of cream on the surface of burn. Each animal received treatment once per day during the light phase (9.00am)(1).

Burn wound model

The rat was anaesthetized by intramuscular Ketamine (85mg/Kg of body injection of weight), and Xylazine (6 mg /Kg of body weight); (Interchemic, Holland) (11). The sterilized clipper was used to shave the back of rats, the plate circular weighting 50.0 g., 2.0 cm diameter, was heated with the flame of Bunsen burner until the temperature reached to 120°C. measured with an infrared thermometer. The plate was induced for 10 seconds on the back of rats under controlled condition. This method allowed the seconddegree burn injury of skin (7).

Experimental design

Group 1: without burn using for anatomical analysis

Group 2: Burned skin rat treated with base cream only (lacking any effective agent).

Group 3: burned skin rat receive MEBO ointment (MEBO contain 0.25% w/w β -

sitosterol; Gulf Pharmaceutical industries, Ras Al Khaimah, U.A.E).

Group 4, 5, 6, 7 and 8: Burned skin rats were treated with a cream containing (0.5%, 1%, 2%, 4% and 8%) of flowers essential oils, respectively.

Anatomical study

After 14 days, the sample of the treated rats skin area were collected and fixed in 10% formal saline for (24 - 48) h, then dehydrated in a concentration gradient including absolute ethanol, the clearing process was performed by using xylol solution, followed by paraffin wax for infiltrate and embedded process. The paraffin sections cut into 4µm- thick section, then stained by Gill haematoxylin and eosin, all slide were photographed and examined with the digital camera that connected to the light microscope(19).

Statistical analysis

The antimicrobial activity and toxicity of *P*. *platychlaena Boiss* were statisticaly analyzed using Kruskal- Wallis test and Dunn's multiple comparision test by Graph Pad-Prism version 7. All results arranged as mean \pm SE for each Properties and *P* value less than 0.05 considered as statistically significant differences among them.

RESULTS AND DISCUSSION

The chemical composition of essential oils of P. platychlaena Boiss

The essential oils of *P.platychlaena Boiss*. was isolated by the hydrodistillation process, the yields (v/w)% of the leaves, stem and flowers based on the fresh weight represented 0.27 %, 0.04% and 1.02%, respectively. While, the density of each of leaves, stem and flowers were 0.97, 0.94 and 0.95, respectively (Table 1). Identified (36, 38 and 43) compounds in leaves, stems and flowers, respectively, which accounted for 90.44%, 92.72% and 96.49 of the essential oil content in the three parts of P. Boiss., respectively. platychlaena The qualitative and quantitative values of each essential oil compounds with the retention time indices of the identified compounds were tabulated in Table 2. The major compounds of the essential oil (E)- β -Ocimene (25.93%), Bornyl acetate (24.58%) and α-Pinene (5.84%), sylvestrene (4.62%), γ -Terpinene (3.75%),δ-Cadinene (2.74%),Myrcene (2.61%) and p-Cymene (2.55%) were in leaves

extraction, whereas the stems main compounds were Bornyl acetate (25.49%), (E)-β-Ocimene (22.94%), α-Pinene (9.5%), p-Cymene (6.48), γ -Terpinene (4.13%), Sylvestrene (4.01%), Myrcene (2.97%) and δ -3-Carene (2.76%), and major compounds of the flower essential oils which were (E)- β -Ocimene (28.5%), Bornvl acetate (24.18%).y-Terpinene (14.15%), (6.48%), *α*-Pinene p-Cymene (4.16%), Sylvestrene (3.02%) and Terpinolene (2.41%). The leaves essential oil of *P*. platychlaena Boiss. containes 14 hydrocarbon monoterpenes which represents (49.5%), 7 oxygenated monoterpene (27.09%),9 hydrocarbon sesquiterpene represents (6.01%), and 6 oxygenated sesquiterpene represents (7.87%), while their stem essential oils 14 hydrocarbon contains monoterpene represents (57.35%), 8 oxygenated 9 monoterpenes (28.48%). hydrocarbon sesquiterpene (1.89%) and 7 oxygenated sesquiterpenes (5%). As well as the flowers of platychlaena Р. **Boiss** contains 13 hydrocarbon monoterpenes which represent oxygenated monoterpenes (64.54%),10 (27.11%),9 hydrocarbon sesquiterpene (3.17%). 7 oxygenated represents sesquiterpene (1.31%),3 hydrocarbons (0.29%) and other which represents (0.07%)(Table 3).

The new chemical components

The results showed that the essential oils of the different part of P. platychlaena Boiss contains chemical compounds various such as Sylvestrene, α-Pinene oxide. (Z)-Epoxyepi-Longipinanol, ocimene. Borneol, Germacrene D-4-ol, epi-α-Cadinol, 3-Thujanol acetate and Acorenone were not found in any Prangos species.

Determination of antibacterial activities

The highest antibacterial activities of essential oil toward the bacteria strains of the *S. aureus* ATCC 6538P was present in flowers, with the MIC values 0.86mg/ml, while the MIC value 1.16 mg/ml was recorded in leaves against *P. aeruginosa* ATCC 9027, as well as, the MIC value against *P. aeruginosa* was 5mg/ml that present in leaves.while the stem essential oils showed hight values MIC against pathogen bacteria. Whereas, the MIC values of Ciprofloxacin for both strains of *S. aureus* were 1.0 and 1.66, respectively, also for both

strains of *P. aeruginosa* were 1.0 and 2.33mg/ml, respectively. The statistical analysis showed that the essential oils of different part *P. platychlaena* Boiss. have significant differences (p<0.05) among them against pathogen bacteria. Also, when the data compared to Ciprofloxacin it shows close or better than antimicrobial references, as show in Table 4.

Toxicity of flower essential oils on burning

This study evaluated the time of healing of rat's wound using different concentration of a new herbal ointment compared with the references ointments. The results were indicated significant differences in the healing time (P < 0.05) among the flower essential oils, base cream (negative control) and references ointment (positive control). This results showed the better healing and shorter time for the base cream containing 1% flower essential oil with 14 days and then base cream containing 8% essential oil with 20 days, respectively, whereas the healing time of negative control and positive control were 27 days and 29 days, respectively. Increasing the concentration of flower essential oils caused to decrease the healing effects. At lower concentrations, no effect on the healing of the is shown. while in higher wound concentrations a positive effect on the close of the wound and the time of healing decreases, Figure 1.

Anatomical evaluation

The histological study indicated that the rats treated with the flowers essential oils of *P*. *platychlaena Boiss* after 14 to 20 days, showed considerable recovery of the skin and epidermis of treated rats, which covers the area of the burn wound complete re-structured and similar to the normal skin of the untreated rats, as shown in Figure 2.

This is the first study on the chemical compositions, antibacterial activities and an in vivo toxicity performed for the flowers essential oils of the *P. platychlaena Boiss* in Iraqi-Kurdistan region. These results indicated that the flowers of *P. platychlaena Boiss* containing more essential oils than the leaves and stem, this may possibly related to for the development stage of the plant and genetic factor(8). These results showed that the *P. platychlaena Boiss* essential oils containing a

large amount of (E)-β-Ocimene, Bornyl acetate, γ -Terpinene, p-Cymene, α -Pinene, Sylvestrene, δ-Cadinene, Myrcene and Terpinolene, have important roles in the biological activity(20). According to our results some chemical compounds such as Svlvestrene. α-Pinene oxide, (Z)-Epoxyocimene. Borneol. epi-Longipinanol, Germacrene D-4-ol, epi-α-Cadinol, 3-Thujanol acetate and Acorenone were not found in any Prangos species. All of these data revealed, that the flowers of P. platychlaena Boiss were containing a larger amount of monoterpenes than both of the stems and leaves parts, whereas, the leaves containing the largest amounts of the sesquiterpene than both stem and flowers parts. In Comparison of our study with the previous studies performed on the essential oils from other Prangos species, the variations showed in the qualities and quantities of essential oils composition. The (E)- β -Ocimene, α -Pinene, Myrcene, p-Cymene and δ -Cadinene compounds were found in the aerial part of P. latiloba Korov according to (5) while Bornyl acetate, δ -3-Carene, γ -Terpinene and Sylvestrene were not founded. The oil chemical composition of α-Pinene (0.2%), Myrcene(0.1%) were found in the aerial part of P.heyniae, while Bornyl acetate, p-Cymene, γ-Terpinene, δ-3-Carene, δ-Cadinene, (E)-β-Ocimene and Sylvestrene were not founded in this plant(21). Based on the available literature, this is the first study performed on the antibacterial activities for the essential oils of P. platychlaena Boiss. The leaves and flowers essential oils have antibacterial activites due to the presences of major compounds which were identified in our study, in the leaves and flowers such as oil (E)- β -Ocimene, Bornyl acetate, α -Pinene, γ-Terpinene sylvestrene, ,δ-Cadinene, Myrcene and p-Cymene, they were causing to inhibite the growth of bacteria. Up to now, no study on the healing time of wound recovery is available until the preparation of this manuscript. The disruption in normal skin may causes infection and alter homeostasis, the infection of a wound was and still regarded as one of the common diseases in our country(18). The healing of the wound is a complex process in dermal regeneration and the epidermal tissues, because some biological mechanisms such as inflammation. antimicrobial and wound contraction were involved in this process (14). According to our results, the cream which contains 1% and 8% of the essential oil showed less time in healing for wounds in compare with the other groups, as the flowers essential oils contain the major active phytochemical compounds such as (E)β-Ocimene, Bornyl acetate, γ-Terpinene, p-Cymene, α -Pinene. Svlvestrene and Terpinolene, Table1. Previous studies reported the results of antimicrobial and antiinflammation properties related to the acceleration of the wound healing process in rats(7), also results of this work were showed the essential oils of leaves and flowers of this plant species have antibacterial activities. As well as, most of these chemical constituents of the essential oils have properties of antimicrobial activities such as (E)-β-Ocimene ,Bornyl acetate (15) and α -Pinene(22) or antiinflammation factors such as p-Cymene (17) and myrcene(13). Our results revealed the essential oil of flowers containing a large amount of monoterpenes may be led to

decrease the time of wound healing(8). Also, our results demonstrated that by decreasing the concentration to 0.5%, the time of healing increases probably for the fact that the cream at that level contains less amount of the constituents of active compounds, while by increasing oil concentration to 2% and 4 %, the time of wound healing are delayed because the chemical constituent of the flowers essential oils become toxic to the burned skin of rats at this concentration. As well as, by increasing the concentration of the flowers essential oil to 8%, the time of healing will decrease to 20 days. This might be due to the synergists properties of the essential oils constituents. No toxicity was observable on the burned skin of rats at the maximum dosage(24). The results obtained from the histological study, concludes that flowers essential oils ointment of P. platychlaena Boiss. have the ability to reduce the healing time and accelerate the formation of skin tissues in treated animals which could be useful to patients suffering from burn skin.

Table 1. Yield (v/w) % and density of essential oil in a different part of *P. platychlaena Boiss*

| Part of plant | Yield % | density |
|---------------|---------|---------|
| Leaves | 0.27 | 0.97 |
| Stem | 0.04 | 0.94 |
| Flower | 1.02 | 0.95 |

Table 2. Chemical composition of essential oil of different part of P. platychlaena Boi

| Compounds | | Flower | | | eaf | | em |
|-----------------------------|------|--------|-------|-------|-------|-------|-------|
| | KI | RT | %Area | RT | %Area | RT | %Area |
| α-Thujene | 924 | 3.97 | 0.18 | 3.97 | 0.08 | 3.97 | 0.14 |
| α-Pinene | 932 | 4.11 | 4.16 | 4.11 | 5.84 | 4.13 | 9.5 |
| Camphene | 946 | 4.35 | 0.58 | 4.36 | 0.82 | 4.36 | 1.08 |
| Verbenene | 961 | - | - | 4.45 | 0.11 | 4.44 | 0.3 |
| Sabinene | 969 | 4.77 | 0.7 | 4.77 | 0.6 | 4.77 | 0.6 |
| β-Pinene | 974 | 4.85 | 0.37 | 4.85 | 0.74 | 4.85 | 1.41 |
| Myrcene | 988 | 5.06 | 2.05 | 5.07 | 2.61 | 5.07 | 2.97 |
| a-Phellandrene | 1002 | 5.37 | 1.8 | 5.37 | 0.32 | 5.37 | 0.23 |
| δ-3-Carene | 1008 | 5.48 | 0.14 | 5.48 | 0.73 | 5.49 | 2.76 |
| ρ-Cymene | 1020 | 5.81 | 6.48 | 5.79 | 2.55 | 5.81 | 6.48 |
| Sylvestrene | 1025 | 5.9 | 3.02 | 5.88 | 4.62 | 5.89 | 4.01 |
| (Ě)-β-Ocimene | 1044 | 6.17 | 28.5 | 6.11 | 25.93 | 6.14 | 22.94 |
| γ-Terpinene | 1054 | 6.62 | 14.15 | 6.54 | 3.75 | 6.55 | 4.13 |
| Terpinolene | 1088 | 6.93 | 2.41 | 6.92 | 0.8 | 6.92 | 0.8 |
| α-Pinene oxide | 1099 | 7.23 | 0.2 | 7.2 | 0.08 | 7.2 | 0.21 |
| (Z)-Epoxy-ocimene | 1128 | 8.17 | 0.6 | 8.15 | 0.42 | 8.15 | 0.41 |
| neo-allo-Ocimene | 1140 | 8.23 | 0.2 | 8.23 | 0.1 | 8.22 | 0.32 |
| trans-Verbenol | 1140 | 8.64 | 0.94 | 8.63 | 0.35 | 8.62 | 0.6 |
| Borneol | 1165 | 9.17 | 0.4 | 9.15 | 0.18 | 9.16 | 0.09 |
| Terpinen-4-ol | 1174 | 9.44 | 0.05 | - | - | - | - |
| p-Cymen-8-ol | 1179 | 9.71 | 0.26 | - | - | - | - |
| trans-Chrysanthenyl acetate | 1235 | 11.79 | 0.18 | 11.76 | 1.35 | 11.77 | 1.27 |
| Bornyl acetate | 1284 | 12.3 | 24.18 | 12.24 | 24.58 | 12.29 | 25.49 |
| 3-Thujanol acetate | 1295 | 12.84 | 0.1 | | | 12.82 | 0.09 |
| α-Copaene | 1374 | 14.47 | 0.1 | 14.68 | 0.13 | 14.45 | 0.1 |
| β-Bourbonene | 1387 | 14.7 | 0.07 | 14.84 | 0.42 | 14.85 | 0.09 |
| β-Elemene | 1389 | 14.87 | 0.07 | 14.92 | 0.08 | 14.92 | 0.11 |
| (E)-Caryophyllene | 1417 | 15.57 | 0.11 | 15.55 | 0.48 | 15.55 | 0.12 |
| Germacrene D | 1484 | 17.1 | 2.14 | 17.06 | 1.07 | 17.06 | 0.05 |
| β-Selinene | 1489 | 17.43 | 0.27 | 17.19 | 0.11 | 17.19 | 0.11 |
| δ-Amorphene | 1511 | 17.56 | 0.07 | 17.51 | 0.72 | 17.52 | 0.29 |
| β-Curcumene | 1514 | 17.7 | 0.11 | 17.67 | 0.26 | 17.67 | 0.09 |
| δ-Cadinene | 1522 | _ | _ | 18.07 | 2.74 | 18.06 | 0.93 |
| Germacrene B | 1559 | 18.08 | 0.23 | - | - | - | - |
| epi-Longipinanol | 1562 | 18.89 | 0.13 | 18.88 | 0.34 | 18.81 | 0.07 |
| Germacrene D-4-ol | 1574 | 19.35 | 0.05 | 19.25 | 0.16 | 19.24 | 0.07 |
| Spathulenol | 1577 | 19.42 | 0.46 | 19.41 | 1.35 | 19.42 | 0.68 |
| epi-α-Cadinol | 1638 | - | - | 20.83 | 2.51 | 20.86 | 1.07 |
| x-Muurolol | 1644 | 20.87 | 0.18 | - | - | 20.95 | 0.14 |
| a-Cadinol | 1644 | 21.16 | 0.36 | 21.15 | 2.09 | 21.15 | 1.51 |
| Valeranone | 1674 | - | - | 21.53 | 1.42 | 21.54 | 1.46 |
| Acorenone | 1692 | 21.65 | 0.07 | | | | - |
| Oplopanone | 1739 | 23.01 | 0.06 | - | - | 17.67 | - |
| Isopropyl hexadecanoat | 2024 | 28.58 | 0.07 | - | - | - | - |
| n-Tricosane | 2300 | 33.42 | 0.09 | - | - | - | - |
| n-Pentacosane | 2500 | 35.07 | 0.05 | - | _ | - | - |
| n-Heptacosane | 2300 | 36.65 | 0.14 | - | _ | _ | - |
| Total | 2100 | 20102 | 96.49 | | 90.44 | | 92.72 |

RT: retention time, KI: Kovats Index, symbol -: not detected

Table 3. Chemical classes of the essential oil compounds of P. platychlaena Boiss. in a

| | diff | ferent part | ts | | | |
|---------------------------|--------|-------------|------|----------|--------|-------|
| class chemical | Leaf | | Stem | | Flower | |
| | number | %area | numb | er %area | number | %area |
| Monoterpene hydrocarbon | 14 | 49.5 | 14 | 57.35 | 13 | 64.54 |
| Oxygenated Monoterpene | 7 | 27.06 | 8 | 28.48 | 10 | 27.11 |
| Sesquiterpene hydrocarbon | 9 | 6.01 | 9 | 1.89 | 9 | 3.1 |
| Oxygenated Sesquiterpene | 6 | 7.87 | 7 | 5 | 7 | 1.31 |
| Hydrocarbone | - | - | - | - | 3 | 0.29 |
| Other | - | - | - | - | 1 | 0.07 |
| Total | 36 | 90.44 | 38 | 92.72 | 43 | 96.49 |

Symbol - : not detected

| Table 4. Determination of minimum inhibitory concentration (| (MIC) of different parts of <i>P</i> . |
|--|--|
| platychlaena Boiss essential oils against pathoge | enic bacteria. |

| | | MIC (mg/ml) | | | | | | |
|--------------------|-------------|----------------------------------|-----------|----------------------------|---------------|--|--|--|
| Sample Plant parts | | S. aureus ATCC 6538P S.aureus | | P. aeruginosa ATCC 9027 | P. aeruginosa | | | |
| Essential oil | Leaves | 2.54±0.32 | 3.14±0.02 | 1.16±0.08* | 5.0±0.0* | | | |
| | Stem(front) | 3.08±0.04* | 3.18±0.03 | 5.3±0.33* | 8.5±0.76* | | | |
| | Flowers | 0.86±0.13* | 3.0±0.57 | 1.43±0.06 | 7.66±0.66 | | | |
| Ciprofloxacin | | 1±0.28 | 1.66±0.33 | 1±0.28 | 2.33±0.33 | | | |

Values are mean ±SD, Symbol * : it mean significantly with data caring the same symbol among different essentials against the same pathogen.

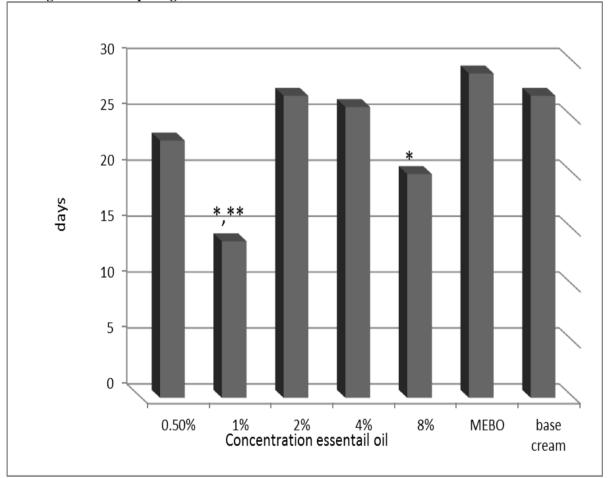


Figure 1. Time of wound healing of treated rats in different groups * significant difference between the treated group with control groups (base cream * *significant difference between treated groups with control groups (MEBO)

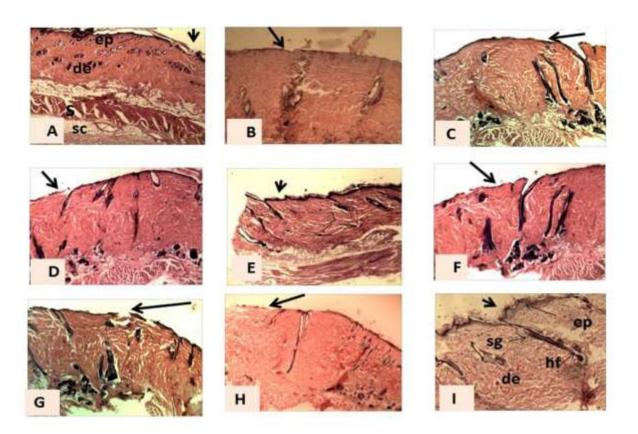


Figure 2. Histological section from burned skin of rats obtained from 15th day excision wound (*x40* magnification except (I) was x100). (A) Normal control group (untreated group),

(B)negative control (base cream), (C) positive control group(MEBO), (D),(E),(F),(G) and (H)) groups of base cream containing 0.5%, 1%, 2%,4% and 8% essential oil flower *P*.

platychlaena Boiss., respectively, (I) base cream containing 1% essential oil after treatment, ep: epiderm, de: derm, s: skin muscle, sc: subcutaneous connective tissue, sg: sebaceous gland, hf: hair follicle, indication of Bold arrow marks (\downarrow): incomplete epiderm, indication of head

Conclusion

These results showed that different parts of *P*. platychlaena Boiss., essential oils containing a large amount of (E)-β-Ocimene, Bornyl acetate, γ -Terpinene, p-Cymene, α -Pinene, δ-Cadinene, Svlvestrene. Mvrcene and Terpinolene, which have important roles in the antibacterial activities against known pathogenic bacteria, as well as, some chemical compounds such as Sylvestrene, a-Pinene oxide, (Z)-Epoxy-ocimene, Borneol, epi-Longipinanol, Germacrene D-4-ol, epi-a-Cadinol, 3-Thujanol acetate and Acorenone were found in P. platychlaena Boiss but not detected in any other Prangos species. Moreover, during this study it was found that the flower essentail oils of P. platychlaena Boiss have important and significant roles in the healing of rats wound.

arrow marks (⁺): complete epiderm.

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Authors declare no conflict of interest **REFERENCES**

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