Effect of Carpobrotus edulis Extract on Pathogenic Bacterial Species and	
Candida	(151-156)

Effect of *Carpobrotus edulis* Extract on Pathogenic Bacterial Species and Candida

Karayem Jebril Karayem¹, Waleed Altanashi², Sana OmranTreem³, Hawa Ahmed Alneiry³, Mona abdlhamed Mrewes³, Nasreddin Rajab Rhouma¹ ¹Teaching staff at Microbiology Division, biology department, Misrata University ²Research assistant at research center faculty of science ³Bachular students at Microbiology Division, biology department, Misrata University <u>kryemvet@gmail.com</u>

تأثير مستخلص نبات الصبار Carpobrotus edulis على أنواع البكتيريا الممرضة والكانديدا كريم جبريل كريم¹، وليد الطناشي²، سناء عمران طريم³، حواء احمد النعيري³، منى عبدالحميد مربويص³، نصرالدين رجب رحومه¹ ¹عضو هيئة تدريس شعبة الأحياء الدقيقة، قسم الأحياء، كلية العلوم، جامعة مصراته ²مساعد باحث، مركز الأبحاث كلية العلوم، جامعة مصراته ³طلبة بكالوريوس، شعبة الأحياء الدقيقة، قسم الأحياء، كلية العلوم، جامعة مصراته

المستخلص:

أوراق نبات الصبار C. edulis استخدمت كعلاج بديل وتقليدي لعلاج إصابات الجلد والأنسجة الرخوة. هذه الدراسة أجريت لبحث مدى تأثير خلاصة أوراق C. edulis على أنواع من البكتيريا والخمائر جمعت من حالات الكلينيكية. تأثير الخلاصة تمت دراسته بطريقة الحفر والانتشار خلال الأجار بحيث صنعت حفر بقطر 6 مم في أجار Muller Hinton glucose methylene blue بلندم المتدرية، بينما استخدم Muller Hinton glucose methylene blue بالنصبة للعزلات البكتيرية، بينما استخدم علال الأجار بحيث صنعت حفر بقطر 6 مم في أجار Muller Hinton glucose methylene blue من الخاصة للعزلات البكتيرية، بينما استخدم Muller Hinton glucose methylene blue بالنصبة للعزلات البكتيرية، بينما استخدم علاصة للحفر بالأطباق. بعد زراعة الأطباق بالبكتيريا وحقن الحفر بالمستخلص حضنت لمدة 24 ساعة عند درجة حرارة 37°م وبعدها حدد قطر هالة التثبيط. أظهر وحقن الحفر بالأطباق. بعد زراعة الأطباق بالبكتيريا وحقن الحفر بالأطباق. بعد زراعة الأطباق بالبكتيريا وحقن الحفر بالمستخلص مضاداً لنمو البكتيريا موجبة وسالبة الجرام وكذلك للخمائر المستخدمة بهذه الدراسة. كانت وحقن الحفر بالأطباق بعد زراعة الأطباق بالبكتيريا وحقن الحفر بالأطباق. بعد زراعة الأطباق بالبكتيريا وحقن الحفر بالمستخلص دقطر هالة التثبيط. أظهر وحقن الحفر بالأطباق. بعد زراعة الأطباق بالبكتيريا وحقن الحفر بالمستخليق المن المالية الجرام وكذلك للخمائر المستخدمة بهذه الدراسة. كانت مستخلص نشاطاً مضاداً لنمو البكتيريا موجبة وسالبة الجرام وكذلك للخمائر المستخدمة بهذه الدراسة أن المستخدمة بهذه الدراسة أن المستخلص نشاطاً مضاداً لنمو البكتيريا موجبة وسالبة الجرام وكذلك للخمائر المستخدمة بهذه الدراسة أن مستخلص نشاطاً مضاداً لنمو البكتيريا والخمائر ويمكن استخدامه لعلاج الأفات الموضعية النامم من المستخلم نبات والخمائر ويمكن استخدامه لعلاج الأفات الموضعية النارمية أن الإصابة بالتي من المتدمة بهذه الدراسة أن المامن الموضعية النامم أن مستخلص نبات وموجبة الموانة بباقي العائر ويمكن استخدامه لعلاج الأفات الموضعية النامم مالمان ويمكن استخدامه لعلاج الأفات الموضعية النامم مالمان ويمان المامان ويمكن استخدامه لعلاج الأفات الموضعية النامم مالمان الإصابة بالبكتيريا سالبة وموجبة الجرام وأيصاني الكمائر ويمكن استخدامه لعلاج الأفات الموضعية النامم ويالمانما ولمائم ويم

Abstract:

The leaf of *C. edulis* used as a traditional alternative medicine for local skin and soft tissue infection treatment. This study was performed to investigate the effect of *C. edulis* extract on clinical bacterial isolates and yeast. Effect of the extract was done by agar well diffusion method where 6mm diameter holes were made on Muller Hinton agar in bacterial isolates while Muller Hinton glucose methylene blue agar (MH-GMB) was

151 مجلة النماء للعلوم والتكنولوجيا (STDJ) العدد الثالث المجاد (3) اكتوبر 2022 كلية الزراعة – جامعة الزيتونة – ترهونه – ليبيا (ISSN: 2789-9535) used for yeast isolates, and 50μ l of the extract was added into the holes. Inoculated plates were incubated for 24 hours at 37°C and the inhibitory zone was estimated. Antimicrobial activity of *C. edulis* extract was shown against all tested Gram-positive and negative bacterial isolates and yeast. *S. aureus* exhibited the highest susceptibility to the crude *C. edulis* extract compared to other tested bacterial isolates. In this study observation, *C. edulis* has an inhibitory effect on bacterial and yeast isolates and can be used as a topical treatment of lesions caused by Gram-positive and Gram-positive bacteria as well as yeast.

Keywords: Carpobrotus edulis, Extract, Pathogenic Bacteria and Candida.

Introduction:

Plants are widely used as a primary treatment in about 70-90% of the population in developing countries (Robinon & Zhang, 2011). In the rural area, traditional medicine is the only available treatment system, thus it is still popular and approved in these communities and most commonly used in treatment of skin lesions (Naidoo & Coopoosamy, 2011). In India, herbal drugs wildly used to alleviate diabetes mellitus, liver disorders, and viral hepatitis. People usually seek traditional plant therapy when no or little benefit of modern health system was found (Tandon & Yadav, 2017).

Carpobrotus edulis (C. edulis) world wild spreading plant, grows on sand soils and coastal dunes. Beside its uses as an ornamental plant and support native plants in the ecosystem, it still used in traditional medicine in some countries (Martins et al., 2010; Novoa & Gonzalez, 2014). Traditionally, C. edulis leaf extract used to reduce mucus membrane inflammation in respiratory and urinary tract also reduces water retention and alleviate urinary tract pains (Pirie et al., 2014). Sex active ingredient compounds in C. edulis have been identified; rutin, neohesperidin, hyperoside, cactichin, ferulic acid and semi-purified tannin fraction. Furthermore, in vitro, its crude extract found to have a selective antibacterial effectivity against tested pathogenic bacterial isolates; substantial inhibitory activity was reported against Moraxella catharralis (M. catharralis), Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis) and negative antibacterial activity against Gram-negative tested group (Van Der Watt & Pretorius, 2001). Barker and his colleagues when a plant studied for use as a traditional treatment, some criteria need to be considered: proof of plant usage as a treatment in the target population; clinical case for which it is used as a treatment as well as availability of the plant in the ecosystem (Baker et al., 1995).

In Misurata city, *C. edulis* used as an herbal medicinal plant in the treatment of dermatomycosis, wound, diabetic foot, and other skin lesion. It is available as a natural plant in coastal ecosystem, usually grow in sandy soil and hills. The plant crude extract applied directly on the affected area to accelerate wound healing, treat skin mycotic lesions, and diabetic foot infection medicament.

This study aimed to evaluate the inhibitory effect of *C. edulis* crude and alcoholic extract on different pathogenic bacterial isolates and yeast. Also to estimate the association between tested bacterial isolates antimicrobial susceptibility and *C. edulis* inhibitory effect.

152 مجلة النماء للعلوم والتكنولوجيا (STDJ) العدد الثالث المجلد (3) اكتوبر 2022 كلية الزراعة – جامعة الزيتونة – ترهونه – ليبيا (ISSN: 2789-9535)

Materials and methods:

Area of study and lab work:

Misurata city, located in the north central area of Libya on the Mediterranean Sea coast. The plant leaves were collected from plants grow in the natural environment and sent to the laboratory for crude and alcohol extractions. Laboratory work was performed in the research center of the faculty of science and Misrata University and Misurata central private laboratory.

Tested bacterial and yeast isolates:

Pathogenic bacterial and yeast isolates were provided by Central Misurata laboratory. In addition to *Candida albicans* (*C. albicans*) different groups of bacterial species were included, Gram-negative; *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and Gram-positive; *Staphylococcus aureus* (*S. aureus*).

Extracts of C. edulis:

Crude and alcoholic extract of *C. edulis* were used to investigate there inhibitory effect on previously mentioned isolates. Crude extract obtained by *C. edulis* leaf squeezing and collected concentered extract stored at 4°C. Alcoholic extract was performed as following 40 grams of *C. edulis* leaf powder was added into 200 ml of 96% ethanol and in a conical flask and placed on a rotary shaker at 150 rpm for 24h. The mixture of leaf powder and ethanol was filtered to separate the solid component from the fluids contains the solvent and active ingredients. The solvent was removed by a rotary evaporator and, the condensed *C. edulis* extract was collected and stored at 4°C (Mbanga et al., 2010).

Both extracts were stored in tightly closed dark containers at 4°C for late uses. Obtained extracts (crude and alcoholic) were considered as 100% and dimethyl sulfoxide (DMSO) used to prepare 50%, and 75% concentrations.

Evaluation of C. edulis antimicrobial activity:

Antimicrobial effect of the different concentrations (50%, 75% and 100%) of crude and alcoholic extracts were assayed by transferring 50µl into 6mm holes made in Mueller-Hinton agar by sterile cork borer. Of each tested bacterial isolates, three plates were used, each plate inoculated by bacterial isolates and contains the three concentrations and one for the DMSO as a control. All plates were incubated at 37°C for 24h. Inhibition zone was estimated as an average of the three inhibition zones reading of the triplicate. Susceptibility of *C. albicans* to *C. edulis* extracts was estimated as above mentioned except Muller Hinton glucose methylene blue agar (MH-GMB) (glucose 2% as nutrient support and methylene blue 0.5 μ g/ml) was used instead of Muller Hinton agar for better clearance of inhibition zone) (Usach et al., 2020).

Data analysis:

To estimate the effects of the three concentration on the same tested bacterial isolate T-test was used, while ANOVA test was used to estimate the efficacy of one concentration on all tested bacterial isolates. The results considered significant when p value ≤ 0.05 . Data analysis was performed by using Statistical Package for the Social Science (SPSS, USA) version 20 for Microsoft Windows.



Effect of <i>Carpobrotus edulis</i> Extract on Pathogenic Bacterial Species and	
Candida	(151-156)

Results and Discussion:

Effect of crude extract on tested bacterial isolates.

Crude extract inhibitory effect was very clear on tested Gram-positive and Gram-negative bacterial isolates (table 1) and (figure 1). This observation indicates C. *edulis* extract contains antibacterial components.



Figure 1: The inhibitory zone of alcoholic extract concentrations on *S. aureus* and *E. coli* isolates

This study finding showed the crude extract had the highest growth inhibitory effect on *S*. *aureus* which recorded 26mm (Figure 1). Crude extract was significantly more effective in *S*. *aureus* growth inhibition compared to all alcoholic concentrations (P=0.004).



Figure 2: Growth inhibition zone of alcoholic extract concentrations on C. albicans



Effect of *Carpobrotus edulis* Extract on Pathogenic Bacterial Species and Candida(151-156)

Extract type	Alcoholic extract			Crude	DMSO
Bacterial and yeast isolates	50%	75%	100%	extract	DIVISO
S. aureus	11	12	13	26	0
E.coli	12	12	13	10	0
K. pneumonia	9	9	10	14	0
P. aeruginosa	9	12	15	13	0
C. albicans	11	11	13	18	0

Table 1: Inhibitory effect of *C. edulis* crude extract and alcoholic extract concentrations on tested bacterial and *C. albicans* isolates.

Alcoholic extract also showed growth inhibitory on tested bacterial isolates fluctuated between 9 to 15 mm. Another study conducted in Tunisia which found *C. edulis* had inhibitory effect on Gram-positive bacterial isolates but had no effect on Gram-negative bacterial species (Meddeb et al., 2017). Different results on Gram-negative group of bacteria may due to different geographical area of *C. edulis* which could leads to different chemical components or tested bacterial isolates in Tunisia acquired some resistance factors to the extract.



Figure 3: Inhibition zone diameter of crude and different alcoholic extract concentration.

The highest inhibition of *K. pneumonia* growth, was shown at crude extract (14 mm). Similar finding was reported in a study conducted by Nayyef et al in 2020 where *K. pneumonia* was more sensitive to crude extract than other Gram-negative isolates (Nayyef et al., 2020). Crude extract of *C. edulis* showed the highest antifungal activity. This study showed *C. edulis* had antimicrobial activity against tested bacterial and yeast isolates. Inhibitory effect against Gram-positive bacteria was higher than Gram-negative-bacteria. Clinical studies are required to investigate the efficacy and safety of local application of the extract.



Effect of *Carpobrotus edulis* Extract on Pathogenic Bacterial Species and Candida(151-156)

References:

Baker, J. T., Borris, R. P., Carté, B., Cordell, G. A., Soejarto, D. D., Cragg, G. M., ... & Tyler, V. E. (1995). Natural product drug discovery and development: new perspectives on international collaboration. *Journal of natural products*, *58*(9), 1325-1357.

Martins, A., Vasas, A., Schelz, Z. S., Viveiros, M., Molnar, J., Hohmann, J., & Amaral, L. (2010). Constituents of Carpobrotus edulis inhibit P-glycoprotein of MDR1-transfected mouse lymphoma cells. *Anticancer research*, *30*(3), 829-835.

Mbanga, J., Mangoma, N., & Saidi, B. (2010). An evaluation of the antimicrobial activities of Aloe barbadensis, A. chabaudii and A. arborescens leaf extracts used in Folklore Veterinary Medicine in Zimbabwe. *Journal of Animal and Veterinary Advances*, 9(23), 2918-2923.

Meddeb, E., Charni, M., Ghazouani, T., Cozzolino, A., Fratianni, F., Raboudi, F., ... & Fattouch, S. (2017). Biochemical and molecular study of carpobrotus edulis bioactive properties and their effects on dugesia sicula (turbellaria, tricladida) regeneration. *Applied biochemistry and biotechnology*, *182*(3), 1131-1143.

Naidoo, K. K., & Coopoosamy, R. M. (2011). A comparative analysis of two medicinal plants used to treat common skin conditions in South Africa. *African Journal of Pharmacy and Pharmacology*, 5(3), 393-397.

Nayyef, S. H., Salih, N. I., & Mustafa, M. A. (2020). Antimicrobial Activity Of Aloe Vera And Opuntia Ficus Extract Against Certain Pathogenic Bacteria. *European Journal of Molecular & Clinical Medicine*, 7(9), 422-428.

Novoa, A., & González, L. (2014). Impacts of Carpobrotus edulis (L.) NE Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. *PLoS One*, *9*(9), e107557.

Pirie, A. D., Davies, N. W (Ahuja, K. D., Adams, M. J., Shing, C. M., Narkowicz, C., . . . Geraghty, D. P. (2014). Hypolipidaemic effect of crude extract from Carpobrotus rossii (pigface) in healthy rats. *Food Chem Toxicol*, 66, 134-139. doi:10.1016/j.fct.2014.01.034 **Robinon**, M & (Zhang, X. (2011). The World Medicine Situation (Traditional Medicines: Global Situation, Issues and Challenges). Geneva. *World Health Organization, Geneva, Switzerland*.

Tandon, N., & Yadav, S. S. (2017). Contributions of Indian Council of Medical Research (ICMR) in the area of medicinal plants/traditional medicine. *Journal of ethnopharmacology*, *197*, 39-45.

Usach, I., Margarucci, E., Manca, M. L., Caddeo, C., Aroffu, M., Petretto, G. L., ... & Peris, J. E. (2020). Comparison between citral and pompia essential oil loaded in phospholipid vesicles for the treatment of skin and mucosal infections. *Nanomaterials*, *10*(2), 286.

Van Der Watt, E., & Pretorius, J. C. (2001). Purification and identification of active antibacterial components in Carpobrotus edulis L. *Journal of ethnopharmacology*, 76(1), 87-91.

