Sys Rev Pharm 2020;11(12):672-676
A multifaceted review journal in the field of pharmacy

In vivo antimicrobial activity of Matricaria chamomilla extract against Pathogenic Bacteria induced skin infections in Mice

Atheer A. Khashan*, Mohammed A. Hamad*, Mohammad S. Jadaan*.

Corresponding authors:
Atheer Abdulhameed Khashan (M.Sc. pharmacology), Lecturer Department of Pharmacognosy, College of Pharmacy, University of Anbar, Anbar–Ramadi-HIT,964, Iraq.
Mohammed A. Hamad. (PhD. Microbiology), Biotechnology and Environmental center /University of Fallujah, Anbar–Fallujah, 964, Iraq.
Mohammad S. Jadaan (M.Sc. pharmacology), Lecturer Department of Pharmacology, College of Pharmacy, University of Anbar, Anbar–Falloja,964, Iraq.

Abstract

Matricaria chamomilla that has been used to treat various ailments such as burns and wound since ancient times. So, this study was aimed to achieve the effectiveness of various construction of Matricaria chamomilla against the growth of Staphylococcus aureus by using of well agar diffusion technique in comparison with Gentamycin. Results showed a difference in the rate of zones inhibition by using Matricaria chamomilla against staphylococcus where he scored concentration (60 mg / mm) (21mm.) while the Gentamycin a concentration (60 microgram / ml) on the growth of Staphylococcus aureus (19mm.). (15) albino mic was used, divided into 3 groups (n= 5 / group). All animals in all groups experimentally induced skin incision (5 mm) in the flank area of the body, and infected with S. aureus suspension, and treated after infected the wound (24 hrs.) according to the experimental design. The groups were treated with Matricaria chamomilla at a concentration (60 mg/ml), appear complete healing in 14 days of treatment and returning of hair in the wound area, in comparison with a control group and the group when treated with Gentamicin ointment that required (21) days to recovery. So, the Matricaria chamomilla is good as an antibacterial effectiveness against bacterial infections that infected wounds.

Keywords: Antibacterial Activity, Staphylococcus aureus, Matricaria chamomilla flowers Extract, and Wound in mice

Abbreviations: S. aureus= *Staphylococcus aureus.*

Corresponding Authors:
Atheer Abdulhameed Khashan (M.Sc. pharmacology), Lecturer Department of Pharmacognosy, College of Pharmacy, University of Anbar, Anbar–Ramadi-HIT,964, Iraq.
Mohammed A. Hamad. (PhD. Microbiology), Biotechnology and Environmental center /University of Fallujah, Anbar–Fallujah ,964, Iraq.
Mohammad S. Jadaan (M.Sc. pharmacology), Lecturer Department of Pharmacology, College of Pharmacy, University of Anbar, Anbar–Falloja,964, Iraq.

1. INTRODUCTION

Matricaria chmomilla

Medical plants are considered one of the most important means to treat many bacterial infections because of it has different antibacterial compounds and there are no side effects against human (Aljanaby, 2018).

Figure 1. Chamomile plant at full blooming stage (a) and dried flowers which used in this work (b).

Matricaria (M. chmomilla) is also called Matricaria recutita. It is usually called Asteraceae or Chamomile. It belongs to the Asteraceae family and is widely used as an antibacterial agent in Europe, Africa and Asia. (Mehmood et al., 2015). 1000 years ago, this medicinal plant was used to treat different infections in Egypt, Greece and Rome, and is considered one of the nine sacred herbs (Crevin and Philpott., 1990). Matricaria chmomilla has been used to treat different illness such as gastrointestinal infection, cold and diarrhoea (Romha *et al.*,2018). Matricaria chmomilla flowers are one of the most important parts of this plant has been used in many medical treatments like antipyretic and carminative (Baquar SR,1989).

2. MATERIAL &METHODS

***Culture media**

Prepare according to the instructions of the production company, and sterilize in an autoclave at 121°C under a pressure of 12-15 PSI, and then incubate at 37°C for 24 hours for the cultivation and diagnosis of the bacteria used for this analysis (Spacil et al., 2008).

Methods

Preparation of plant

Matricaria chmomilla was determined as a test herb. Fresh flowers of Matricaria chmomilla. were accumulated desert from Anbar-Hit,all flowers were air-dried.

Preparing of plant extract

Dry flowers of the herb were mechanically ground. Flowers powder was extracted with ethanol. Aliquots of getting were rinsed for 24 hr. at room temperatures. The extracts were purified by using Whatman filter paper Number 1 and the

filtrates were evaporated in an incubator at 30 °C. (Forbes *et al*.,2007). The resultant focuses were stored in the refrigerator until used.

Extraction methods

Mash 50 g of plants in an electric mixer for about one to two minutes, then immerse them in 450 ml of ethanol (95%). It has been placed at room temperature for three months, the composition is separated in a test tube by centrifugation at about three thousand revolutions per minute, and the filtrate is dried in an oven at 37°C for 24 hours. Finally, the product is stored in a refrigerator at (-20) °C (Krell R, 1996).

Culture preparation

Activate the bacteria by recultivating it on nutrient agar and place it in a 37°C incubator for 24 hours, then use it in a sterile tube containing heart infusion broth, and then keep it at 37°C Place in the box for 24-72 hours. C. Under the guidance of the spectrophotometer, the test quantity of all bacteria is converted into predictable. Under the wave period of 580 nanometers, the probability of mellow transmittance is 27%, and the light transmittance of supplement broth used for microbial assembly the rate is 100% (Jassim SJ., 2003).

Preparation of standard dilutions of Matricaria chmomilla

Dilute with ethylene glycol, an inert solvent that is inert to microorganisms (Charles et al., 1996), and extract a series of concentrations from the extract with a concentration of 10-100 mg, and then reduce it to 2 with ethylene glycol. Ml to obtain a final concentration from 1-10%.

Matricaria chmomilla extract activity test well diffusion method screening

The screening of antibacterial activity has developed into a magnificent diffusion technology (Saeed & Tariq, 2005). There are no fillers on Mueller-Hinton's agar plates. 1 ml of uniform bacterial inoculum. Using a sterile glass spreader, the inoculum will overflow the tray overturned. The inoculated plate was dried in a thermostat at 37°C for 20 minutes. Add selected 9 mm crack edges to destroy ordinary holes on the pipe surface and modify all focal points of 0.1 ml to provide ethylene glycol as a power source. After 24 hours, the inoculated intervertebral discs were incubated at 37°C, and the inhibited diameter area was modified to the nearest millimeter.

Antibacterial agents

Gentamicin, ampoule (80 mg /2 ml), (Meheco Company), from China.

Experimental animals

fifteen local albino mice 4-6 weeks of age weighing 20-22g were used in the experiment, each cage was containing 5 mice. Animals were all subjected to 22-25°C for 14 /10 hrs. light/dark photoperiod They were divided into 3 groups (n= 5 / group). They were supplied by Pharmacology &Toxicology Dept., College of Pharmacy University of Anbar, Iraq.

Preparation the antibiotics

We used of Gentamicin (80 mg /2ml): and taken (0.25) ml (10 mg-10000 µg) of antibiotic solution and completed to (100 ml) of D.W in order to getting 100 µg /ml (stock solution), and from it premeditated the different concentration: 20, 40 ,60, and 80 µg /ml.

Bacterial count

Staphyllococcuse aureus is counted in indirectly by:

1. Spectrophotometer: was utilized to quantify the turbidity of *Staphyllococcuse aureus* suspension to reach an appropriate optical density (O.D), whereas the percentage of focus transmission has been 25% at a Wavelength of 450 nanometers.
2. Used McFarland Solution (tube No .0.5) Standard McFarland solution No.0. was set up as indicated by Baron *et al* ,.(1994)

Bacterial inoculation of media

Nutrient agar has been prepared after sterilized by autoclave for 15 minutes at 121 C and 15 Ib/In2, then followed:

Left the medium to cooling to reach 48 C by using of a water bath, from the bacterial suspension 0.2 ml of *Staphyllococcus aureus was* taken and that compared to the McFarland solution No.0.5, using a sterile micropipette, and added to every 20 ml of the medium.

20 ml of the medium which contained bacteria were added to each sterile Petri- dish and left for 10 minutes to allow solidifying of the media.

The activity of 100µl/well of different concentration of Matricaria chmomilla also, antibiotics to the exploratory bacteria (S. aureus) performed by Wells of six mm in width in the solid medium. The plates containing bacteria were incubation at 37°C for 24 h. After incubation, the width of inhibitory zones was estimated in millimeter ruler. (Wagih *et al.,* 2014).

***In vivo* study**

A total number of 15 mice were used in this study. They were divided into 3 groups (n= 5 / group) and kept at a temperature between 23-28C. The animals were housed in a cage individually in an air-conditional room.

3. RESULT AND DISCUSSION

Wound (Open wound)

All Animals in all groups will make skin incision (3 mm) by scrabble above the first layer of muscle in the flank area of the body.

Group A: skin incision (3 mm) in the flank area immediately above the first layer of muscle and infected with 1 ml of *Staphylococcus aureus* suspension which contain 1.5×10^8 cell/ml, and after following the infection wounds (24) were treated with extract, once daily for (14) days.

Group B: skin incision (3 mm) infected with 1 ml *Staphylococcus aureus* suspension which contain 1.5×10^8 cell/ml and after 24 hrs. of infection treated with Gentamicin 5 gm, once daily for (14) days.

Group C: skin excision (3 mm) and infected with *Staphylococcus aureus* suspension which contains 1.5×10^8 cell/ml, without treatment for (14) days.

Effect of Matricaria chmomilla on Different Concentration of Bacterial Growth

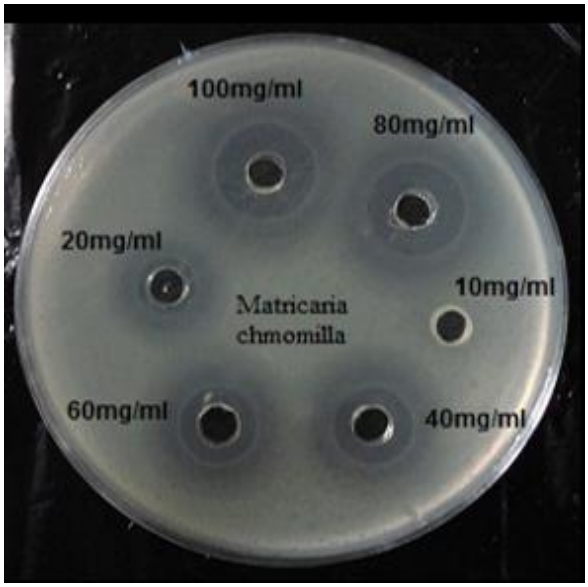
The growth inhibitory effect of different concentrations of *Matricaria chmomilla* on *Staphyllococcus aureus* was illustrated in tables (1), and figure (2).

4. DISCUSSION

The growth inhibitory effect of different concentrations of *Matricaria chmomilla* on *Staphyllococcus aureus* was illustrated in tables (1), figure (2).

Results showed that the concentration (10 mg/ml) of Matricaria chmomilla has not any effect antibacterial against *S.aureus* while 20mg/ml showed the superiority of activity with inhibition zone (15 mm.)and this may due to increase in apigenin, the most active compound of chamomile penetrates into deeper skin layers when applied topically which supports the use of chamomile as a topical anti-inflammatory agent in treating inflammations in deep tissues (Merfort and Heilman, 1994). The mean zones of inhibition of Matricaria chmomilla against *S aureus* at (60mg/ml) are (21mm). The concentration 60 mg/ml for Matricaria chmomilla showed significance effect at ($p<0.05$) compared with their 20 &40 mg/ml concentrations.

The effect of Gentamicin at concentration 20 µg /ml on bacterial growth and Matricaria chmomilla against *Staphyllococcus aereus* are listed in table (2). Matricaria chmomilla is the most effective agent against *S. aureus*, compare with Gentamicin at concentration (60) µg /ml with inhibition zone 19mm. while Matricaria chmomilla was 21mm. In the group treated with Matricaria chmomilla After 14 days showed complete healing and presence of hair, without scar tissue (Figure 7). While in group treated with Gentamicin, was healing with the formation of scar tissue as shown in (Figure 8). After 14 days in the control group, the wound has been found signs of inflammation (swelling, redness and hotness) and exiguity of food consumption. These results are in agreements with previous reports (Subrahmanyam; and Ugane ,2004), the finding of the present study indicate that Matricaria chmomilla increased the formation of granulation tissue, density and activation of fibroblasts, keratinisation in the surface of a wound,



thickness of epidermis and thickness of collagen fibers. Moreover, keeping with earlier reports, (Ndayisaba *et al.*,1993) Matricaria chmomilla decreases infection, inflammation, edema and dehiscence. As well, Matricaria chmomilla increases the rate of wound healing, which confirms previous reports (Subrahmanyam *et al.*,2001). This result is by Benguo *et al.*, 2008 who reported that flowers of chamomile were used for apigenin extraction, which contain a percentage of less than 0.3% (Marshall, 2002).

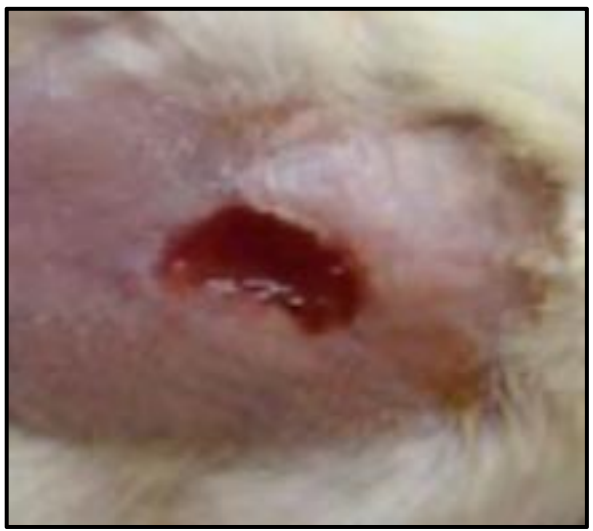
Table 1. Diameter zone of inhibition (mm.) of Matricaria chmomilla against *S. aureus*

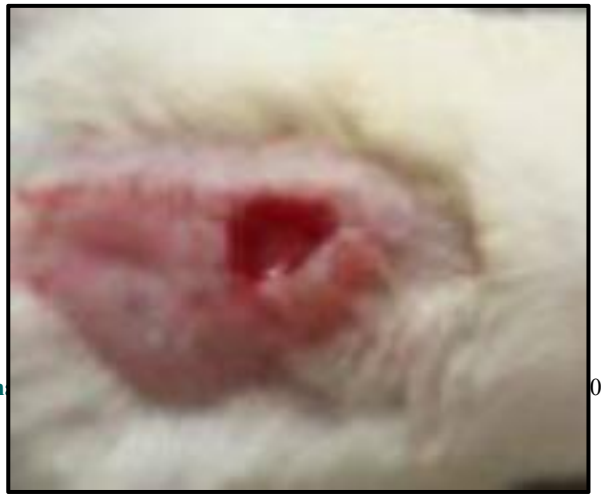
Extract\. Concentration	10 mg/ml	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100mg /ml
Matricaria chmomilla	0	15	18	21	25	28
Gentamicin	4	13	17	19	21	26

Figuer 2. effect of different concentrations of Matricaria chmomilla on *Staphyllococcus aureus*

Figure 3. Skin of mice, after 24 hours of infection with *S aureus* Showed inflammation and presence of pus

Figure 4. Skin of mice, after 14 days **in** control group the wound has been found signs of inflammation (swelling, redness and hotness)





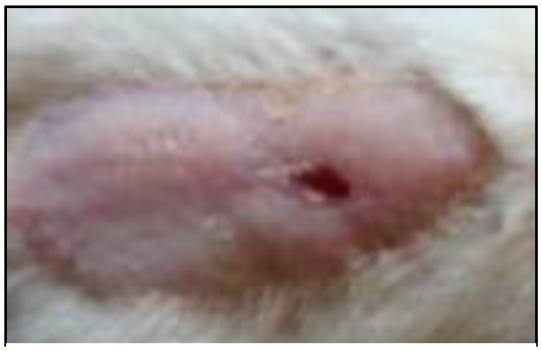


Figure 5. Skin of mice, after 24 hours of treatment with extract showed the

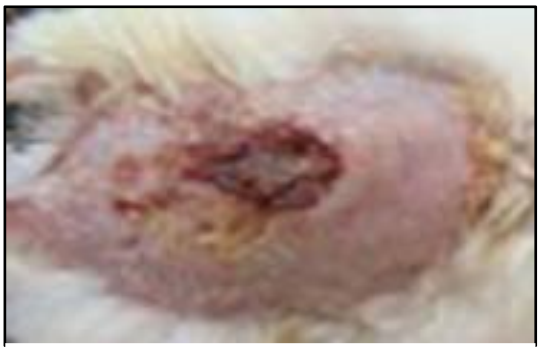


Figure 6. Skin of mice, after 24 hours of infection with S aureus in control group, showed the wound is inflamed

Figure 7. Skin of mice, after 14 days of treatment with extract, showed

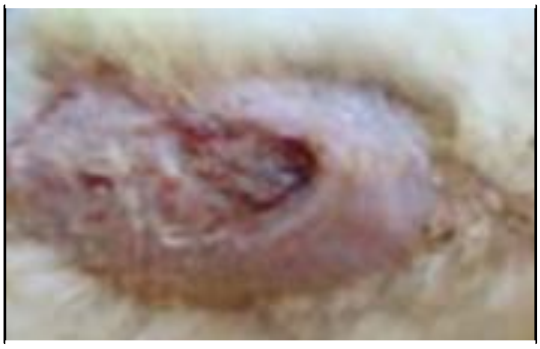


Figure 8. Skin of mice, after 14 days of treatment with Gentamicin showed

5. REFERENCE

1. Aljanaby AAJ. Antibacterial activity of an aqueous extracts of Alkanna tinctoria roots against drug resistant aerobic pathogenic bacteria isolated from patients with burns infections. ROMJ 2018; 1: 1-6.
2. Mehmood MH, Munir S, Khalid UA, Asrar M, Gilani AH. Antidiarrhoeal, antisecretory and antispasmodic activities of Matricaria chamomilla are mediated predominantly through K (+)-channels activation. BMC Complement Altern Med 2015; 15: 75.
3. Crevin JK, Philpott J. Herbal medicine past and present (1st ed). Duke Univ Press, USA 1990.
4. Romha G, Admasu B, Hiwot Gebrekidan T, Aleme H, Gebru G. Antibacterial activities of five medicinal plants in ethiopia against some human and animal pathogens. Evid Based Complement Alternat Med 2018; 2018: 2950758.
5. Baquar SR. Matricaria chamomilla L. In: Medicinal and poisonous plants of Pakistan. Printas Karachi, Karachi, Pakistan 1989; 278.
6. Spacil, Z., L.Novakova and P.Solich (2008).Analysis 3:425-434. Of phenolic compounds by high performance liquid and ultra –performance food of Handbook preservation food for chromatography, Talanta 76:189-199.
7. Forbes BA, Shan DF, & Weissfeld AS, Bailey and Scotts (2007). diagnostic microbiology, 12 th edition Elsevier.
8. Krell R, Value-Added products from Bee keeping, Agricultural Services Belletin, (1996); No. 124.
9. Jassim SJ., (2003) Effect of Haloxylonarticulatum extract on some experimental bacterial and fungal skin infection in dogs. MSc. Thesis, Pharmacology and Toxicology Department / College of Veterinary Medicine, University of Baghdad.
10. Charles OW, Ole G, & Robert FD (1969). Textbook of organic medicinal and pharmaceutical chemistry, 5th Edition, J.B. Lippincott company, Philadelphia and Toronto.
11. Saeed S, & Tariq P, (2005). Anti-bacterial activities of Menthapiperita, Pisumsativum and Momordicacharantia, Pak.J.Bot., 37:997-213.
12. Merfort, I. and Heilman, J. (1994). In vivo skin penetration studies of chamomile flavones. Pharmazie., 49 (7): 509-511.
13. Baron, E.; Peterson, L.R. and Finegold, S.M. (1994). Bailey and Scoffs Diagnostic Microbiology. 9th ed. the C. V Mosby.,Co.USA. p. 386-403.
14. Wagih Abd ElFattah El-Shouny, Eman H.F. Abd El-Zaher, Maha Abd El-Fattah &Olaa Abd El-Salam,.Antimicrobial activity of chamomile acetone extract against some experimentally-induced skin infections in mice EJER; 2014, Vol. 2: 58-70.
15. Ndayisaba, G; Bazira,14 Habonimana, E; Muteganya, D (1993) Clinical and bacteriological results in wounds treated with honey. Journal of Orthopaedic Surgery 7(2): 202-204.
16. Subrahmanyam M, Shahapure AG, Nagane NS. Effects of topical application of honey on burn wound. Ann Burns and Fire Disasters 2001; 14: 134-7.
17. Subrahmanyam M, Ugane SP. Honey dressing beneficial in treatment of Fournier's gangrene. Indian Journal of Surgery 2004; 66:75–7.
18. Marshall C. 2002. The use of honey in wound care: a

review article. British Journal of Podiatry 2002;52(2):47– 9.