The Activity of Alcoholic Extract of Urtica Urens Against Staphylococcus Aureus and Oral Wound Healing

Eman A.Mustafa[1], Faehaa A. Al-Mashhadane[1]*, Ghada A. Taqa[1]

1. Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq.

Abstract

This study was to evaluate the effect of the alcoholic extract of Urtica urens on wound healing and the antibacterial activity of this plant compared to fusidic acid ointment in experimental rabbit models.

Male rabbits "6-7 months age" and "1.25-1.5" Kg body weight were included. The lower jaw of all rabbits were primed for surgery. Under general anesthesia , two full –thickness square longitudinal 1 cm wounds were incised in lower jaw for each animal, then each wound was infected by Staphylococcus aureus bacteria. These wounded animals were grouped according to the following : group 1: Staphylococcus aureus + no treatment, group 2: Staphylococcus aureus + Fusidic acid ointment 2%, group 3: Staphylococcus aureus + Urtica Urens alcoholic extract 20 %. The applications of treatments were recurring every day, and wounded areas were measured in first, 3ed , 7th, 9th days of the experiment. For evaluation of antimicrobial outcome of Urtica urens alcoholic extract against Staphylococcus aureus, swabs were taken from sites of wound incision at the first , third and seventh day ,then the colony forming units(CFU)/ml of microorganisms were counted. All animals were sacrificed on day ten of the study and evaluated by histopathological examination.

Study found complete wound healing at 9th in alcoholic extract of Urtica urens 20 % group in comparison to control and Fusidic acid ointment 2% .

This study showed that Urtica urens plants extracts has positive effect on skin wound healing and has antibacterial activity against Staphylococcus aureus.

Experimental article (J Int Dent Med Res 2020; 13(3): 957-963)

Keywords: Urtica urens, plants extracts, Oral Wound Healing.

Received date: 26 June 2020 **Accept date:** 22 July 2020

Introduction

There is ongoing concern for the use of natural plants products which are one of the well-known and commonly used remedies in treating diseases[1]. In traditional medicine, herbal extracts have been used thousand years ago, nowadays there is an increasing interest in screening the clinical uses of the natural plant products with the aim to recognize another pharmaceutical ways to develop new methods for good health and continue life with less side effects [2]. There is long-term scientific efforts to find the logical basis for scientific practice of traditional plants and the possibility for detection of novel antimicrobial compounds[3]. The importance in the study of plants in medicine as a pharmacological active compound has improved worldwide. Urtica urens "dwarf nettle" is plant related to Urticaceae family and naturally growing with a wide distribution in the world . Urtica urens leaves are stinging, dark green with saw-like edge. The leaves are 2-4 cm long, oval in shape. Wild plants have considerable function in providing minerals, trace elements, vitamins.. The leaf is good source of unusual special important supplements. Nettles enclose protein, flavonoids, fatty acids, vitamins, and minerals. Nettle leaves hold 9 carotenoids: Lutein, lutein and b-carotene . Such antimicrobial and antioxidant agents from nature have significant attention to control the diseases of microbial origin[4]. Staphylococcus aureus is one of the main recurrently isolated bacteria. It can be habitually set up as normal flora in the skin, nose, and throat. Staphylococcus aureus is not all the time pathogenic, despite that it can lead to a diversity of infections, like skin tissue infections, toxic

Corresponding author:
Dr. Faehaa A. Al-Mashhadane ,
Dental College, Mosul University Mosul , Iraq.
E-mail: faehaaazher@uomosul.edu.iq

shock syndrome, bone infections, endocarditis, sepsis, and respiratory tract infections[5]. Wound curing is now a significant challenging health care problem; the seek for better wound – healing agent is one of the oldest challenges for medical and dental practice [6]. Natural medicinal plants have created much concern for the management of injures since they are reasonable and less risk of serious effects. This study was intended to assess the effect of wound healing by alcoholic extract of Urtica urens and to assess the antibacterial activity of this plant compared to fusidic acid ointment in experimental rabbit models.

Materials and methods

This study was approved by scientific committee/department of basic science/college, of Dentistry/University of Mosul.

Plant samples: Urtica urens were gathered from Mosul City, Iraq. The samples of plant were and dried by oven at 40Cº, lastly grinded by electrical mill. The dry powder was reserved until use.

Plant extract preparation: Twenty mg of plant were used for preparation of ethanolic extracts, milled by maceration in 200 ml of ethanol for 24 hrs at room tempreture. Then extracted ethanolic substrates were filtred and dried . The powder was stored in plastic bottle and cool place to prevent contamination and decomposition[7].

Culture of bacteria: Staphylococcus aureus was isolated and recognized by gram stain, morphological and biochemical tests[8].

Bacterial broth: Staphylococcus aureus was inoculated in Brain Heart broth for 18 hrs.at 37Cº ,the bacterial growth was harvested by centrifugation (500g/min) for 200 minutes [9] . The pellets result were washed twice in phosphate – buffered saline solution, then the bacterial cells were counted and diluted in P.B.S. solution to a turbidity of o.5 of McFarland standard [10] .

Animals: Male local rabbits of (6-7) months and weighing (1.25-1.5) Kg were involved in this study. Lower jaw of all rabbits were prepared for surgery. For anesthesia, all rabbits received intramuscular injection of 10 mg\kg Xylazine hydrochloride (Interchemie, Holland) and 50 mg\kg ketamine hydrochloride (Holden Netherland, India). Two full –thickness square 1 cm wounds were incised in lower jaw for each animal, then each wound was contaminated by Staphylococcus aureus. Wounds were divided into three groups as follow:Ggroup 1: Staphylococcus aureus + no treatment **.** Ggroup 2: Staphylococcus aureus + Urtica Urens alcoholic extract 20 %**.** Group 3: Staphylococcus aureus + Fusidic acid ointment 2% The applications of treatments were repetitive every day, and wounds surface area were calculated in 1st, 3^{ed}, 7th, 9th days of the experimental procedure. All animals were sacrificed on day ten of the study and evaluated by histopathological examination. Tissue samples from wound areas were fixed in 10% formalin, embedded in paraffin, cut into 5 µm sections perpendicular to the surgical line, and stained with Hematoxylin-Eosin "H&E". These sections were then examined under a light microscope for histological changes by a blinded pathologist. The stage of healing was showed as the wound contraction ratio "WCR": WCR= (A_0 − A_t) *100 A_0 Where A_0 (the initial area) and A_t (the wound area after the application of the treatment) [11].

Microbiological study: For evaluation of antimicrobial effect of Urtica urens extract against Staphylococcus aureus, swabs were taken from sites of wound incision at the first day, third day and seventh day. Each swab was placed in sterile test tube containing 5 ml of sterile Brain Heart broth,got vortex for one minutes then o.5 of Brain Heart broth was taken from each tube and placed in screw – capped vial containing 4-5 ml of sterile Brain Heart infusion broth .To determine the number of microorganisms in the last dilutions replicate specimens 10^7 to 10^8, 100 µ L of each dilution were transferred to two plates of nutrient agar. The plates then incubated at 37Cº for 48 hrs. and the colony forming units per milliliter (CFU/ml) were then calculated[12] .

Results

In the present study it was found that control group (not treated with extract) had no effect on bacterial growth (Fig. 1), whereas examination of alcoholic extract of Urtica urens 20 % against Staphylococcus aureus reveled that the application alcoholic extract of Urtica urens (20 %) against Staphylococcus aureus was inhibit the growth all colonies of bacteria in petri dish (Fig. 2) .

Figure 1. Control group.

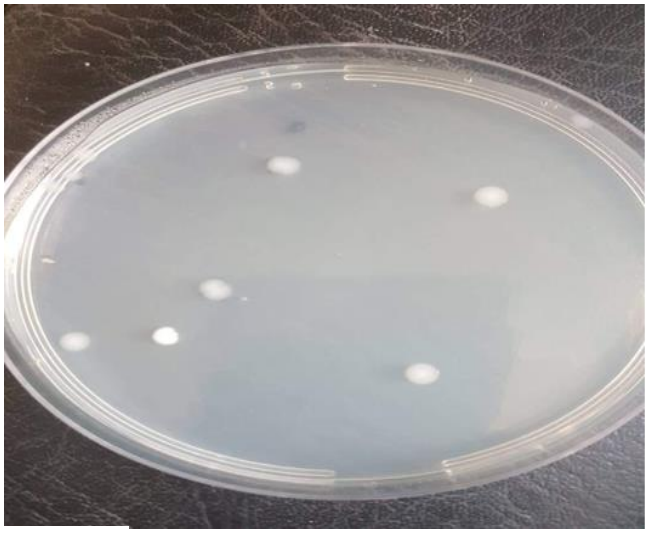

Figure 2. Effects of Urtica urens alcoholic extract 20% against Staphylococcus aureus.

Groups	1st day	3rd day	7th day
Control(Staphylococcus aureus)	7.4 x10^5 ±0.0 a A	59.25 x10^5±0.02 a B	18.1 x10^5 ±0.01a A
Fusidic acid ointment 2%	6.4 x10^5 ±0.01 a A	1.6 x10^5±0.02 bB	1.2 x10^5±0.01 bB
Urtica urens alcoholic extract 20%	15.1 x10^5 ±0.0 b A	0.68 x10^5±0.01 bB	0.5 x10^5±0.02 b B

Table 1. Means of colony forming unit per milliliter (C.F.U) of **(**Staphylococcus aureus**)** in control positive group ,Fusidic acid ointment 2% group and Urtica urens alcoholic extract 20% group.
- Data were describe (Mean + SD.)
- Different capital letters mean there are significant different in the same raw.
- Different small letters mean there are significant different in the same Colum.

In our study we were monitored oral wound healing for nine days after incision full – thickness square 1 cm wounds in lower jaw for each animal. In present study we found completely healing at 9th in alcoholic extract of Urtica urens 20 % group in comparison to control and Fusidic acid ointment 2% . Evaluation of the surface areas of wound healing show that the mean of wound contraction in urtica urens was significantly differ after 3 , 7 ,9 days (11.0±0.01), (6.0±0.02) , (0.0±0.0) mm2 respectively after contamination of the wound compared with control group (Staphylococcus aureus group) (21.0±0.02), (16 ±0.01) (6.0±0.02) mm2 and Fusidic acid ointment 2% group (21.0±0.02), (12.0±0.01), (5.0 ±0.0) mm2 respectively at the same days whereas no significant difference found between control and Fusidic acid ointment 2% at long of wound healing day (Table 2) .

Concentration %	1st day	3rd day	7th day	9th day
Control(Staphylococcus aureus)	27.0 ±0.0 a A	21.0±0.02 aB	16 ±0.01 a C	6.0±0.02 a D
Fusidic acid ointment 2%	27.0 ±0.01 a A	21.0±0.02 aB	12.0±0.01 a C	5.0 ±0.0 a D
Urtica urens alcoholic extract 20%	24.0 ±0.0 b A	11.0±0.01 b B	6.0±0.02 b C	0.0±0.0 b D

Table 2. Effect of topical Uritica urens alcoholic extract 20% on wound healing (mm^2). - Data were describe (Mean + SD.)
- Different capital letters mean there are significant different in the same raw.
- Different small letters mean there are significant different in the same Colum.

In control group we found that the wound area significantly difference between all groups after 3 , 7 ,9 days (21.0±0.02)(16 ±0.01)(6.0±0.02)mm^2 in comparison to wound area in 1st day (27.0 ±0.00) mm^2. In Fusidic acid ointment 2% also we found that the wound area significantly difference between all groups after 3 , 7 ,9 days (21.0±0.02)(12.0 ±0.01)(5.0±0.00) mm^2 in comparison to wound area in 1st day (27.0 ±0.01) mm^2. Urtica urens alcoholic extract 20% group we found that the wound area significantly difference between all groups after 3, 7, 9 days (11.0±0.01) (6.0 ±0.02) (0.0±0.00)mm^2 in comparison to wound area in 1st day(24.0 ±0.0) mm^2. Application of Urtica urens alcoholic extract 20% we found significant acceleration the wound healing at 9th day in Urtica urens alcoholic extract 20%

group)(0.0±0.00)mm^2 in comparison to Fusidic acid ointment 2%(5.0±0.00)) and control group (6.0±0.02)mm^{2}at the same day. (Table 1) The WCR of Urtica urens alcoholic extract 20% group were (76), (89), (94), (100)% higher than Fusidic acid ointment 2% group (76), (79), (88), (95)% and than, Control (Staphylococcus aureus) group (73), (80), (89),(94) respectively at 1st ,3rd , 7th ,9th days Table (2).

Group	Wound contraction ratio (WCR)%			
	1st day	4th day	7th day	9th day
Control(Staphylococcus aureus	73%	80%	89%	94%
Fusidic acid ointment 2%	76%	79%	88 %	95%
Urtica urens alcoholic extract 20%	76%	89%	94%	100%

Table 3. Effect of topical Uritica urens ointment on WCR %.

Histopathological results showed that in control group their was disappearance of normal skin architecture with loss of epidermis at incision line , also granulation tissue formation fill the gap between two side of incision with infiltration of mononuclear cells. (Fig. 3 and 4).

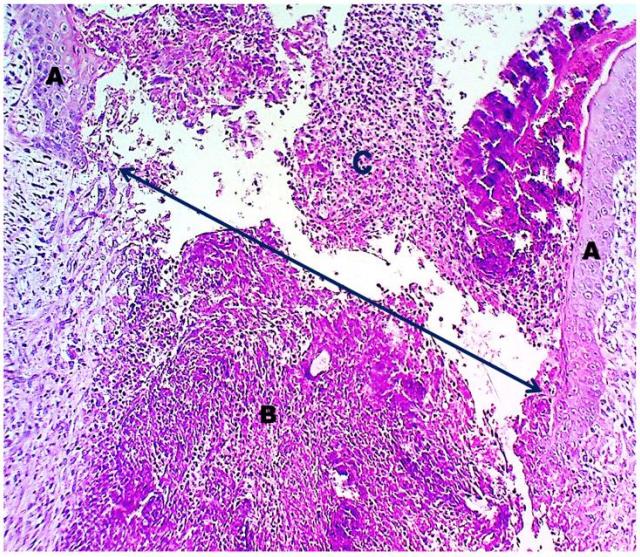

Figure 3. Microphotograph of rabbit skin wound healing of control group. Disappearance of normal skin architecture . (A) loss of epidermis at incision line (arrow) ,(B) granulation tissue formation fill the gap between two side of incision. (C)infiltration of mononuclear cells. H&E stain, 100x.

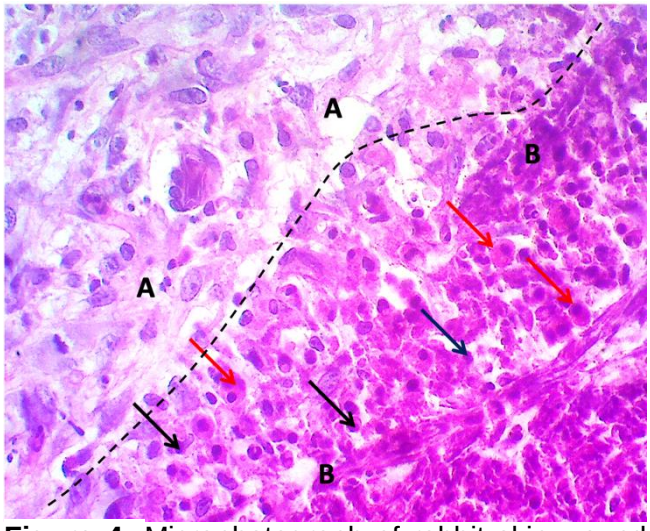

Figure 4. Microphotograph of rabbit skin wound healing of control group at incision line show demarcation line (dotted line) between (A)normal skin tissue and (B) granulation tissue with sever infiltration of mononuclear cells (black arrows)(red arrows) and plasma cells. H&E stain, 400x.

While microphotographs of rabbit skin wound healing of Urtica urens at incision line show complete healing represented by normal skin tissue with epidermis formation, focal epithelialization and sebaceous glands (Fig. 5 and 6).

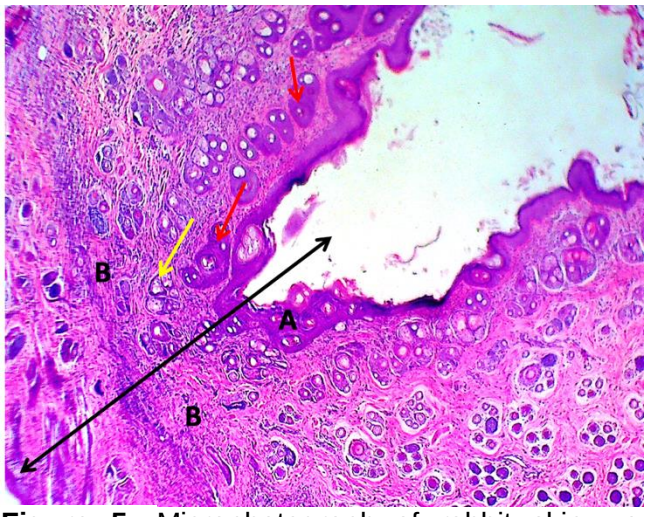

Figure 5. Microphotograph of rabbit skin wound healing of Urtica urens at incision line(black arrow) show complete healing represented by normal skin tissue with(A) epidermis formation and (B)dermis, focal epithelialization (red arrows) and sebaceous glands(yellow arrow). H&E stain, 40x.

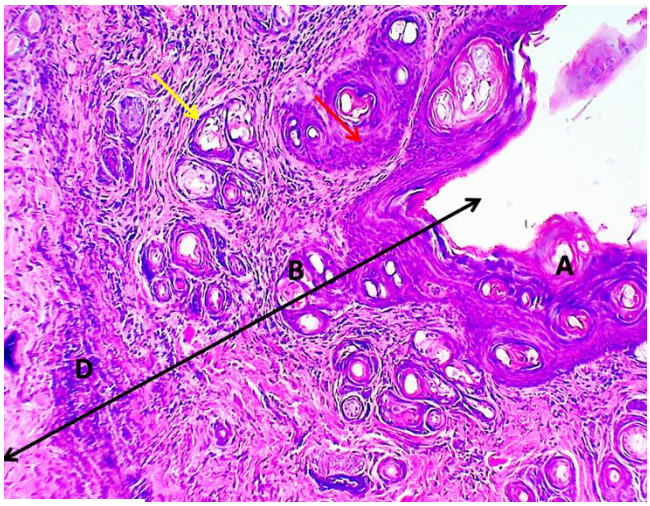

Figure 6. Microphotograph of rabbit skin wound healing of Urtica urens at incision line(black arrow) show activity of keratinocytes with in (A) epidermis formation and (B)dermis, focal epithelialization(red arrows) and sebaceous glands(yellow arrow) with mild inflammatory cell infiltration(D). H&E stain, 100x.

In relation to fusidic acid group, transverse section of rabbit skin wound healing of fusidic acid ointment at incision line show granulation tissue formation, congested blood vessels with sever infiltration of polymorph nuclear cells (Fig. 7 and 8).

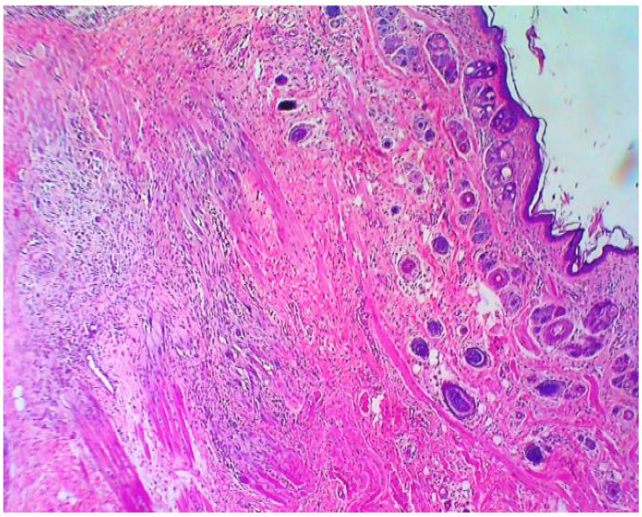

Figure 7. Transverse section of rabbit skin wound healing of Fusidic acid ointment at incision line (dotted circle) show granulation tissue formation(A), congested blood vessels(arrow) with sever infiltration of polymorph nuclear cells .H&E stain, 100x.

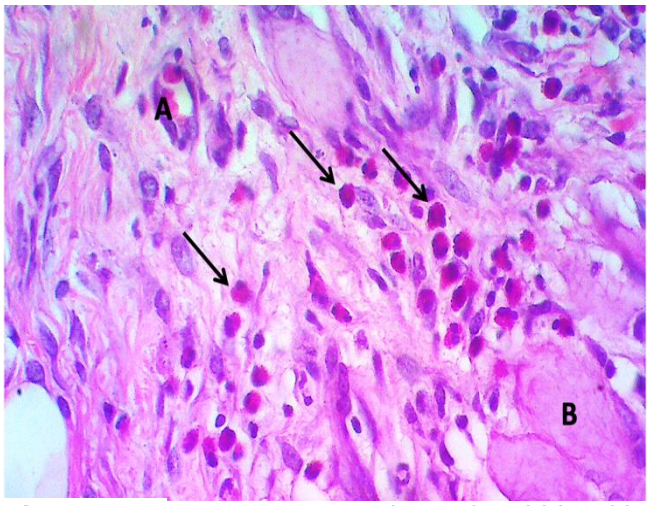

Figure 8. Transverse section of rabbit skin wound healing of Fusidic acid ointment at incision line show newly formed blood vessels((A), sever infiltration of polymorph nuclear cells especially eosinophils (arrows) with degeneration of muscles (B) .H&E stain, 400x.

Discussion

Wound healing is a challenge to researchers. Many researchs had proven that polymolecular traditional medical treatments provided more useful effects than the allopathic medicine in numerous cases[13]. Healing passes through phases of epithelialization, granulation, collagenation and scar maturation which are simultaneous but not dependent to each other[14] . Appropriate healing of the wounds is necessary for a persistent recovery of damaged tissue parts [15] . Local therapy can support healing process and minimise systemic side effects[16].

In medicine many plants are valuable for wound healing, among these plants, Urtica species "Urticaceae" which exhibited strong anti-inflammatory potential [17]. The phenolic substances in plants are variable during steps like growing, harvesting, storage and technological procedures of plants. The phenolic components found in plants have established large consideration due to their antioxidant property and they can interrelate with biological systems and participate an key role in antiinflammatory and antimicrobial activities[18,19]. Oxygen free radicals are toxic substances, generate oxidative stress during inflammation of wound though process of healing. Many types of scavengers at the wound sites have been showed to be effective in this environment and

injury curing. This healthy effective role of scavengers have been documented to the antioxidant function of them. It help the formation of mature collagen fibers and fibroblasts with improved angiogenesis. According to that, accelerators of healing from natural sources are of great attention for modern major and minor surgery[7] . So, the healing ability of plants are mostly linked with considerable antioxidant activities of them[14,19-21] . The ethanolic extract of Urtica urens perform its therapeutic function by augmentation of tissue regeneration with complete healing represented by normal skin tissue with epidermis and dermis formation, focal epithelialization and sebaceous glands as described in results of this study(Fig. 7and 8). The beneficial effect of our extract may be due to, at least in part, antioxidant capacity, in addition to the enhancement of collagen fibers deposition. Antioxidants and flavonoids have been reported to recover wound healing through increasing collagen cross-linking and then breaking strength [20]. Also Urtica urens exhibited stronger anti-inflammatory potential [17, 22] compared to control (Fig. 1and 2) and fusidic (Fig. 9 and 10) groups that shows infiltration of mononuclear cells with sever infiltration of polymorph nuclear cells especially eosinophils associated with muscles degeneration. Human pathogens need to overcome a network of defense mechanisms of the host in order to establish their actions on it [23].

Staphylococcus aureus an anaerobic gram-positive bacterium that considered as one of the sources of infections in the skin & oral cavity representing a major public health danger [24,25].The survival of Staphylococcus aureus is facilitated by its ability to resist oxidative products. Organisms in the log phase of growth clearly demonstrate a resistance to oxidative products [26]. Bacterial resistance of Staphylococcus aureus make the treatment of such infections more difficult. A preclinical animal model of Staphylococcus aureus which is rapid and cost effective, could afford another method to assess the efficacy of antimicrobial agents[27-29]. Urtica urens have antibacterial activity against most of the tested Gram-positive and Gram-negative bacteria in variable degrees[4]. Current studies evaluated the antimicrobial effect of both aqueous and ethanolic extracts of irritant leaves against different gram positive and gram negative bacteria and their susceptibility to them, for example Staphylococcus aureus, Salmonella spp and Bacillus subtilus. demonstrated high susceptibility to nettle extracts, and they specify that both types of extracts exert antibacterial activities in different degrees. Ethanolic extracts have favorable effect compared to aqueous one due to the presence of more active ingredient in ethanol than in water [30].

In this study the activity of Urtica urens ethanolic extract was evaluated against Staphylococcus aureus. In case of Staphylococcus aureus which is recognized as being resistant to a high number of antibiotics with its ability to produce several types of enterotoxins that can induce many dangerous types of medical problems including septicemia[4,31,32]. The antibacterial action of Urtica urens could apparently related to certain types of components like terpenes and flavonoids . Phytochemical analysis showed that such extracts had violet and yellow-orange spots representing the presence of these substances which support the fact that plant investigated in this study "Urtica urens" displayed antibacterial activity[4,33]. Staphylococcus aureus action is thought to be related to inhibition of protein synthesis of bacteria in a mode like that of the conventional antibiotic "chloramphenicol". In addition to that, luteolin-7-diglucuronide is a main flavonoid with powerful antibacterial activity against Staphylococcus aureus. A study showed that luteolin decrease synthesis of Staphylococcus aureus toxin [34]. Since that wound healing process is an extremely complex mechanism where multiple factors may. Intervene including bacterial infection[35], urtica urens extract seem to at hand a true interest and potential for wound healing with great microbiological importance against bacteria. This may be due to existence of many active compounds in this plant.

Conclusions

According to this study, Urtica urens plants extracts can possibly be used as natural alternative to antibiotic with positive impact on oral wound healing contributed to its activity against Staphylococcus aureus.

Acknowledgements

The authors are very grateful to the University of Mosul / College of Dentistry for their provided facilities, which helped to improve the quality of this work.

Declaration of Interest

All authors state that they have no known competing financial attention or personal relations that could influence the work of this manuscript.

References

1. Jaradat NA , DModarresi-Chahardehi1i B , Abualhasan MN. Antioxidant evaluation for Urtica urens, Rumex cyprius and Borago officinalis edible wild plants in Palestine. Pak J Pharm Sci. 2016, 29(1 Suppl):325-30.
2. Taqa G A, Mustafa Eman A., Al-Haliem SM.. Evaluation of Anti-Bacterial and Efficacy of plant extract (Urtica urens) on Skin Wound Healing in Rabbit. International Journal of Enhanced Research in Science Technology &Engineering, 2014,3(1):(64-70).
3. Israt J. Biva, Chi P. Ndi, Susan J. Semple and Hans J. Griesser .Antibacterial Performance of Terpenoids from the Australian Plant Eremophilalucida .Antibiotics, 2019,8(2).
4. Mzid M, Sameh Ben Khedira, Maryem Ben Salemb, Wafa Regaiegb and Tarek Rebaia. Antioxidant and antimicrobial activities of ethanol and aqueous extracts from Urtica urens. Pharmaceutical Biology, 2017,55(1): 775–781.
5. 28-Yu-Jen Chen, Po-An Chen, Chih-Jung Chen and Yhu-Chering Huang. Molecular characteristics and clinical features of pediatric methicillin-susceptible Staphylococcus aureus infection in a medical center in northern Taiwan. BMC Infectious Diseases. 2019, 19:402-411.
6. Al-Mashhadane FA. The Clinical Effect of Phenytoin on Oral Wound. Al–Rafidain DentJ., 2012,12(1): 165-170.
7. Al-Bayati FA and Al-Mola HF. Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. J.Zhejiang Vniv Sci B. , 2008, 9(2) : 154-159.
8. Karmakar A, Dua P and Ghosh C. Biochemical and molecular analysis of Staphylococcus aureus clinical isolates from hospitalized patients . Canadian Journalof Infectious Diseases and Medical Microbiology. 2016: 9041636.
9. Ahmed ZM, Mustafa EA and Jawad I. Adherence of Candida albicans to flexible denture base material. Al Rafidain Dent J. 2012, 12(2) : 229-235.
10. Andrews JM.. BSAC standardized disc susceptibility testing method (version 5). Journal of antimicrobial chemotherapy 2006, 58(3): 511- 529.
11. Noorbala,M.T; Rahmatabadi,M.H.D; Binesh,F; Morshedi,A. Effecacy of Iranian hydrogen on wounds dressing in rats as an animal model . IRCMJ, 2009,11(4): 387-390.
12. Benson HJ.Bacterial population counts in: Microbiological Applications . 8th ed . McCrawHill , NewYork ,USA, 2002 : 87.
13. Hanafia OA, Abidin T, Ilyas S, Niangollan M and Syamsudin E. Wound Healing Activity of Binahong (*Anredera cordifolia* (Ten.) Steenis) Leaves Extract towards NIH-3T3 Fibroblast Cells. J Int Dent Med Res2019; 12(3): 854-858
14. Ipek Süntar, Esra Küpeli Akkol, Lutfun Nahar, Satyajit D. Sarker . Wound healing and antioxidant properties: do they coexist in plants? Free Radicals and Antioxidants, 2012, 2 (2): 1-7.
15. Susanto A, Muhaimina RK, Amaliya A & Sutjiatmo AB. The Effectiveness of Ethanolic Extract of Moringa Leaves (Moringa Oleifera Lam.) Gel on the Wound Healing Process of the Rat's Palate. J Int Dent Med Res2019; 12(2):504-509.
16. Budi HS, Astuti ER. The MMP-2, MMP-9 Expression and Collagen Density of the Ambonese Banana Stem Sap Administration on Wound Healing. J Int Dent Med Res2019; 12(2):492-497.
17. Carvalho AR , Costa G , Figueirinha A , Liberal J , Prior JAV , Lopes MC, Cruz MT , Batista MT.Urtica spp.: Phenolic composition, safety, antioxidant and anti- inflammatory activities. Food Res Int., 2017, 99(Pt 1):485-494.
18. Mzid M , Badraoui R , Khedir SB , Sahnoun Z , Rebai T. Protective effect of ethanolic extract of Urtica urens L. against the toxicity of imidacloprid on bone remodeling in rats and antioxidant activities. Biomed Pharmacother , 2017, 91:1022-1041.
19. Mzid M , Ben Khedir S , Bardaa S , Sahnoun Z , Rebai T. Chemical composition, phytochemical constituents, antioxidant and anti-inflammatory activities of Urtica urens L. leaves. Arch Physiol Biochem, 2017, 123(2):93-104.
20. Tsala ED, Nga N Thiery B N, Martin, Bienvenue M T, Theophile D. Evaluation of the antioxidant activity and the healing action of the ethanol extract of Calotropis procera bark against surgical wounds J Intercult Ethnopharmacol, 2015, 4 (1): 64-69.
21. Mzid M , Zohra Ghlissib, Maryem Ben Salemb, Sameh Ben Khedira, Khansa Chaabounic,Fatma Ayedic, Zouheir Sahnounb, Ahmed Hakimb, Tarek Rebaia. Chemoprotective role of ethanol extract of Urtica urens L. against the toxicity of imidacloprid on endocrine disruption and ovarian morphometric in female rats, GC/MS analysis Biomedicine & Pharmacotherapy. 2018, 97: 518–527.
22. Marrassini C , Acevedo C, Miño J, Ferraro G, Gorzalczany S .Evaluation of antinociceptive, antinflammatory activities and phytochemical analysis of aerial parts of Urtica urens L. Phytother Res. 2010, 24(12):1807-12.
23. Sharma, S., Bhatnagar, R. & Gaur, D. Complement Evasion Strategies of Human Pathogenic bacteria. Indian. J Microbiol . 24 April, 2020.
24. Puspita S, Lutfialifta P O . The Inhibitory Effect of Kaffir Lime Extract towards Staphylococcus Aureus Bacteria. J Int Dent Med Res 2020; 13(2): 539-542.
25. Astuti SD, Mawaddah A, Nasution AM, Mahmud AF, Fitriyah N, Kusumawati I., et al.Effectiveness of Photodynamic Inactivation with Exogenous Photosensitizer Curcuma longa Extract Activated by Laser Diode 403 nm on Staphylococcus Aureus. J Int Dent Med Res 2020; 13(1): 155-161.
26. (Das, D., Bishayi, B. Contribution of Catalase and Superoxide Dismutase to the Intracellular Survival of Clinical Isolates of Staphylococcus aureus in Murine Macrophages. Indian J Microbiol 2010, 50(40): 375–384.
27. Yi Guo, Romela Irene Ramos, John S. Cho, Niles P. Donegan, Ambrose L. Cheung , Lloyd S. Millerc. In Vivo Bioluminescence Imaging To Evaluate Systemic and Topical Antibiotics against Community-Acquired Methicillin-Resistant Staphylococcus aureus-Infected Skin Wounds in Mice. Antimicrobial Agents and Chemotherapy, 2013, 57 (2): 855–863
28. Modarresi-Chahardehi1 A, Darah I, Fariza-Sulaiman S & Mousavi L. Screening antimicrobial activity of various extracts of Urtica dioica. Rev. Biol. Trop., 2012, 60(4): 1567-1576.
29. Steenkamp V , Mathivha E, Gouws MC, van Rensburg C. Studieson antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. J Ethnopharmacol, 2004,95(2-3):353-7.
30. Salih NA, Arif E Dh and Ali DJ. Antibacterial effect of nettle (Urtica dioca) .Al-Qadisiya Journal of Vet.Med.Sci , 2014, 13 (1):1-6.
31. Kaushik A, Aron A, Mihreteab S, Mohamedkassm N, Michael Kidane E. Phytochemical screening and antimicrobial activity of medicinal plants used by eritrean traditional healers. World J Pharm Res. 2015, 4(4):501–509.
32. Yoon DJ , Fregoso DR , Nguyen D , Chen V , Strbo N , Fuentes JJ , Tomic-Canic M , Crawford R , Pastar I. A tractable, simplified ex vivo human skin model of wound infection. Wound Repair Regen, 2019, 27(4):421-25.
33. Marrassini C , Acevedo C, Miño J, Ferraro G, Gorzalczany S.Evaluation of antinociceptive, antinflammatory activities and phytochemical analysis of aerial parts of Urtica urens L. Phytother Res. 2010, 24(12):1807-12.
34. Majed M. Masadeh, Ahmad S. Alkofahi, Karem H. Alzoubi, Haitham N. Tumah & Kamal Bani-Hani. Anti-Helicobactorpylori activity of some Jordanian medicinal plants. Pharmaceutical Biology, 2014, 52(5):566-569.
35. Al-Mashhadane FA., Mustafa EA. and Taqa GA. Histological and antimicrobial effects of tramadol infiltration on incisional oral mucosal wound healing in rabbits, Iraqi Journal of Veterinary Sciences, 2019, 33 (2): 335-340.