

Evaluation of Anti-Bacterial and Efficacy of plant extract (*Urtica urens*) on Skin Wound Healing in Rabbit

Ghada A. Taqa¹, Eman A. Mustafa², Siba M. Al-Haliem³

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

Abstract: This study was tested the alcoholic and aqueous extracts of *Urtica urens* for their antimicrobial activities on *Pseudomonas aeruginosa* by using disc diffusion method (in vitro) and was evaluated the efficacy of *Urtica urens* extract ointment 25% to accelerate the repairing of wound healing induced skin wound and contaminated by *Pseudomonas aeruginosa* bacteria using local rabbits as experimental animals (in vivo). The result demonstrated a significant increase in the contract ability which was evaluated by the reducing width of wound daily , it was observed that the contracting ability of the wounds treated by *urtica urens* ointment 25% is similar to that of antibiotic (Gentamycin ointment 0.3%). The closure time of the wounds of both was more or less similar . The present results was concluded that the uses of *urtica urens* extract as 25% ointment in topical skin care , healing wounds and the ability of extract to relive the contaminated wound skin by *Pseudomonus aeruginosa* bacteria , therefore may be used to treat the wound.

Keywords: Anti-Bacterial, Skin care , Wounds healing , *Urtica Urens*.

INTRODUCTION

The consumption of medicinal herbs or herbal preparation is increasing nowadays in order to identify alternative approach to improve the quality of life and maintain a good health with little side effects (Alattin et al., 2007). Traditional plants based medicine systems return to play an important role about health care. In reality, the World Health Organization (WHO) estimates that about 80% of the world's population depended mainly on traditional remedies for their health care (Gottlieb and Kaplan,1993). Herbal medicine has been fulfilled for many hundreds years by substantial proportion of the population of Iraq. The interest in the study of medical plants as a source of pharmacological active compound has increased world wide. *Urtica urens* (dwarf nettle) is plant with stinging hairs belonging to the plant family Urticaceae. *Urtica* seed are widely used in folk medicine in many country of world (Alaattin , 2007) . *Urtica urens* has been indicating the benefits of using the extract of the leaves or other part of the plant for the treatment in different conditions such as diabetes (Roman Ramos et al., 1992, Bijan et al.,2003, Ozkol et al.,2013) as well as others disorder like prostatic hyperplasia (Hirono, et al., 1994), Anti-inflammatory effect (Johnson et al.,2012), Rheumatoid arthritis (Jonathan Treasure, 2003) , Antihypertensive activity (Nandini , 2010) and nowadays used the plant as Anticancer (Timoshenko et al., 1996). Many plant extract have antibacterial effect on gram positive *Streptococcus pyroge*, *Staphylococcus aureus* and *Candida albicans* by inhibiting both proliferation of bacteria and inflammation caused by antigen and gram negative (*Pseudomonas aeruginosa*) (Kamber et al., 2004). *Urtica urens* had been shown possess antibacterial effect against other species of bacteria such as; *Salmonella*, *Shigella flexneri* and *Sh. sonnei* (Rahman, 2010) The current study, we evaluated in vitro antimicrobial activity of different part of *Urtica Urens* growing in Mosul city of Iraq using ethanolic extract and evaluated the wound healing efficacy of this plant and compare the healing rates of wound treated with *Urtica urens* extract or Gentamycin in experimental rabbits.

MATERIALS AND METHODS

1- Plant Collection:

Healthy leaves, stem bark and roots of *Urtica urens* was collected from Mosul City, Iraq, University of Mosul, Iraq. The plant sample were washed by distilled water to remove the dust and other related foreign materials, dried by oven at 40C ,finally a perfect grinding was done by an electrical mill and the dried powder was kept in a sterilized container until use.

2- Preparation of plant extract:

The plant was extracted with two types of solvent which were ethanol and distilled water. Aqueous and ethanolic extracts were prepared from 20g of plant, pulverized by maceration in 200 ml ethanolic or distilled water at room temperature for 24hrs. After filtration, extracted ethanolic substrates were air dried and the aqueous extract dried at 40C in incubator. The powder stored in to a plastic bottle in a freezer to prevent contamination and decomposition of the extract (le Grand et al., 1988, Al-Bayati and Al-Mola 2008).

3- Bacterial cultures:

Ps.aeruginosa was local isolated from wounds. The isolates were identified according to colonial microscopic, morphology and biochemical tests (Konenman et al.,1997).

4- Antibacterial susceptibility test:

The disc diffusion method (Bauer et al.,1966) was used to screen the antimicrobial activity .In vitro antimicrobial activity was screened by using Muller Hinton agar, 0.1% inoculums (0.5 Mcfarland Standard) suspension was swabbed uniformly and the inoculum was allowed to dry (5 minutes).

The different concentration of test extract (12.5, 25, 50, 100) mg/ml were loaded on 6mm sterile individual discs .The loaded disc was placed on the surface of medium and the extracts was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37⁰C for 24hrs.At the end of incubation inhibition zones formed around the disc were measured with transparent ruler in millimeter. Negative controls were maintained where pure solvent (D.W,Dimethyl Sulfoxide DMSO) we used instead of the extract for positive Control Gentamycin (10mg/disc) was used. These studies were performed in triplicate.

5- Preparation of bacterial broth:

Ps.aeruginosa was inoculated in nutrient broth for 18 hrs.at 37C, then bacterial growth was harvested by centrifugation (5000g/min) for 20 minutes(Abdul-Baki, 2001). The resultant pellet was washed twice in phosphate-buffered saline solution(Potoxn and Brown,1996), the bacterial cells were enumerated with haemocytometer and diluted in P.B.S.solution to 10⁷ cells/ml.

6- Experimental animals

In this study, male local rabbits aged (5-6) months and weighing (1.5-1.75) Kg were obtained from local market. The dorsal aspects of all rabbits were clipped and prepared for a septic surgery. All rabbits were anaesthetized with intramuscular administration of 10 mg \ kg Xylazine hydrochloride (Interchemie, Holland) and 50mg/kg ketamine hydrochloride (Holden Netherland, India). On each animal, tow cranially and two caudally located square 4cm Full – Thickness wound were created using a surgery scalp. After induced the wound, its contaminated by psudomomous bacteria . These wounds sites were allocated into four groups , and treated according to the following :

- 1- group 1: Normal saline +Normal saline
- 2- group 2: Psudomonus + Normal saline
- 3- group 3: Pseudomonas + Gentamycin 0.3%
- 4- group 4: Pseudomonas + Urtica Urens extract ointment 25%

These application were repeated every day , and wound area were measured in 1st , 3rd , 7th ,12th and 15th days of the experimental procedure. The degree of healing was expressed as the wound contraction ratio (WCR) :

$$WCR = \frac{(A_0 - A_t)}{A_0} * 100$$

Where A₀ and A_t are respectively the initial area and the wound area after the application of the treatment (Noorbala, 2009)

Observation of the wound

The contract ability and closure time

The control ability can be define as the ability of the wound to become narrower than the beginning area ; Throughout the observation of the periphery around the wound look like normal appearance .

Observation of the tissue type:

This refers to the types of the tissue that are present in the wound bed and recorded according to the following Score:

- 4: Necrotic tissue.
- 3: Slough.
- 2: Granulation tissue.
- 1: Epithelial tissue.
- 0: Closed the wound (wound completely covered with epithelium)

Statistical analysis

The data were expressed as mean ± SD , difference between three experimental groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test. The level of significance was at $p < 0.05$.

RESULTS

Our study focused on the effect of alcoholic extract of *Urtica urens* against *Ps.aeruginosa* (in vitro). (Figure1) showed that (25mg/ml) concentration of the extract was the best one against the test microorganism with inhibition zone diameter (22mm), while the 100mg/ml and 12.5mg/ml concentration did not inhibit the growth of this bacteria .

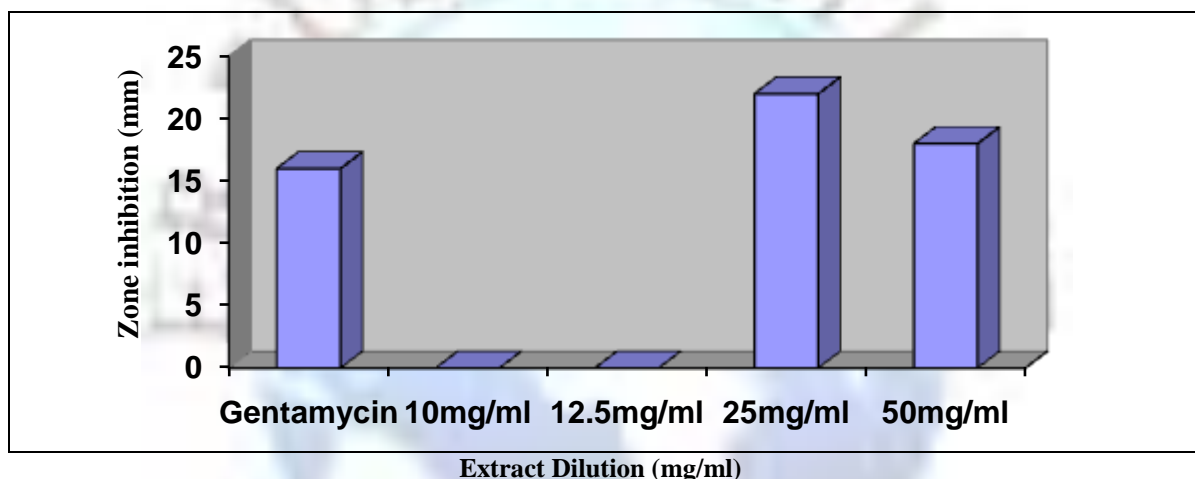


Figure 1: Antimicrobial effect (in vitro) of alcoholic extracts of *Urtica urens* (10,12.5,25,50 mg/ml) on *Ps. aeruginosa* compared with (Gentamycin 10mg/disc)

In the present study no died any rabbits during the experimental procedure. In our study we found all wound were completely healing at sixteen day except group of *pseudomonas* was completely healing at seventeen day. Observation the wound healing found that the mean wound contraction area in *urtica urens* plant significantly difference after 2 , 3 days (1.02 ± 0.10), (0.99 ± 0.08) cm^2 respectively after induced the wound in comparison with control (1.92 ± 0.31), (1.39 ± 0.19) cm^2 and *Ps.aeruginosa* group (1.64 ± 0.19), (1.30 ± 0.11) cm^2 respectively at the same days (Figure 2,3)

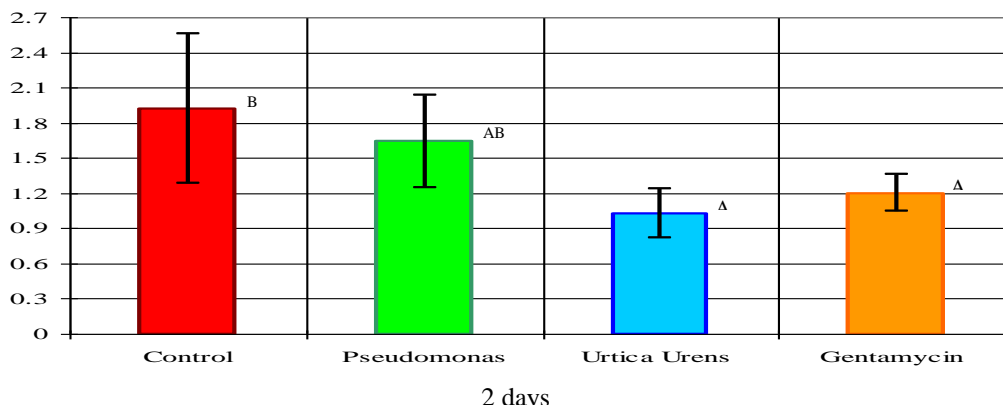


Fig (2): Effect of *Urtica Urens* ointment (25%, Topically) , Gentamycin (0.3%, Topically) and *Ps.aeruginosa* on wound contraction area in rabbits after two days.

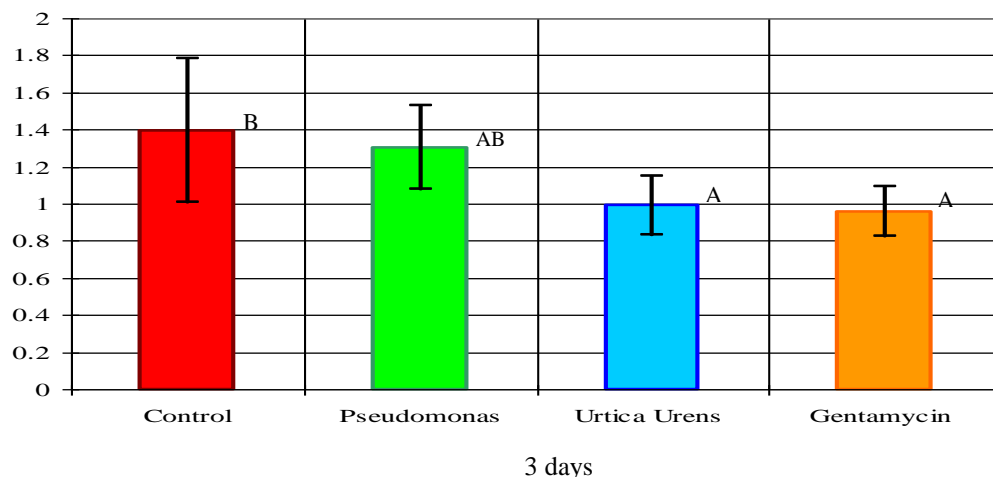


Fig (3): Effect of Urtica Urens ointment (25%, Topically) , Gentamycin (0.3%, Topically) and Ps.aeruginosa on wound contraction area in rabbits after Three days of induced wound.

Topically applied of urtica urens plant on induced infected wound accelerate the wound contraction area and decrease the size at 9th ,12th , 15th days (0.46±0.24),(0.08±0.05),(0.03±0.03) cm² in comparison with the same days of Ps.aeruginosa group (0.96±0.19),(0.74±0.23),(0.48±0.28) cm² but the effect of the plant were like the effect of gentamycin (0.57±0.18),(0.25±0.16),(0.04±0.04) cm² respectively (Table 1) .

Table 1: Effect of Urtica Urens ointment (25%, Topically) , Gentamycin (0.3%, Topically) and Ps.aeruginosa on wound healing (cm²)

Groups Days	Control (cm ²)	Pseudomonas (cm ²)	Gentamycin (cm ²)	Urtica plant (cm ²)
2 nd *	1.92±0.31	1.64±0.19	1.20±0.07	1.02±0.10
3 rd a	1.39±0.19	1.30±0.11	0.95±0.06	0.99±0.08
7 th b	1.25±0.19 *	1.1±0.11	0.92±0.08	0.98±0.12
9 th c	0.78±0.12 *a	0.96±0.19*	0.57±0.18*ab	0.46±0.24*a
12 th d	0.46±0.09 *ab	0.74±0.23*	0.25±0.16*abc	0.08±0.05*ab
15 th e	0.07±0.03 *abc	0.48±0.28*ab	0.04±0.04*abc	0.03±0.03*abc

The best effect about accelerated the wound healing were we found in the 12th days in Urtica Urens plant group and gentamycin group. (Figure 4)

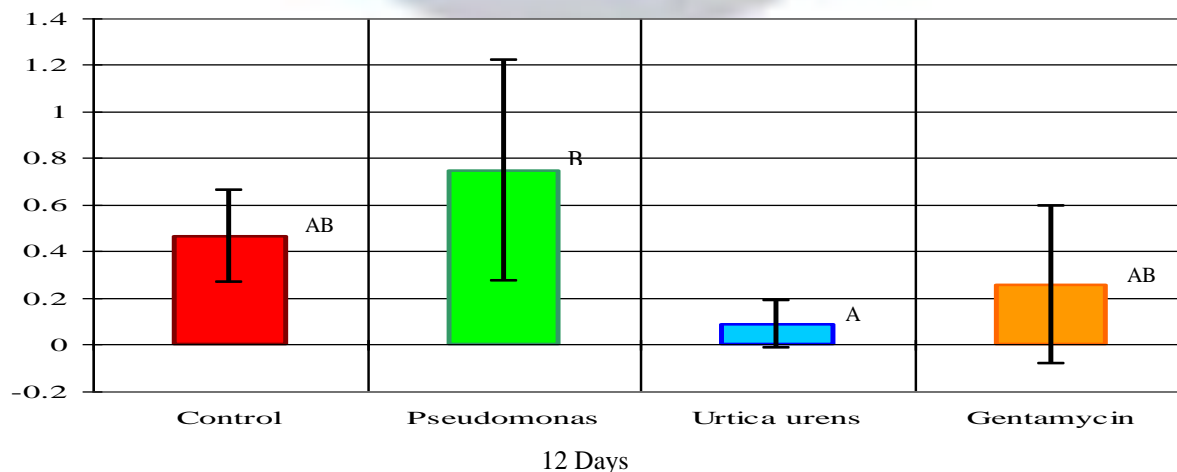


Figure 4: Effect of urtica urens ointment (25%, Topically) , Gentamycin (0.3%,Topically) and Ps.aeruginosa on wound healing after twelve days

The wound contraction ratio (WCR) of *Urtica Urens* plant group were (74.5), (88.5), (98),(99.25)% higher than gentamycin group(70),(85.75),(93.75),(99)%, *Ps.aeruginosa* group (59), (76), (81.5),(88)% and control (74), (52),(80.5), (98.5)% respectively at 1,9, 12,15 days . (Table 2).

Table 2: Effect of *Urtica Urens* ointment (25%, Topically) , Gentamycin (0.3%, Topically) and *Ps.aeruginosa* on Wound contraction ratio(cm²).

Group	Wound contraction ratio (WCR)%					
	2 nd	3 rd	7 th	9 th	12 th	12 th
Control	52.0%	65.25%	68.75%	80.0%	88.5%	98.5%
<i>Ps.aeruginosa</i>	59.0%	68.0%	72.5%	76.0%	81.5%	88.0%
Gentamycin	70.0%	76.0%	77.0%	87.75%	93.75%	99.0%
<i>Urtica urens</i>	74.5%	75.25%	75.25%	88.5%	98.0%	99.25%

Observation of the tissue type that present in the wound were we found that score of *Urtica* plant decline from 2nd day of wound healing and converting to Granulation tissue immediately without formation slough or Necrotic tissue in comparison with others all groups in the same day. In *Urtica urens* plant group fast closed the wound same as the control group. While in gentamycin group the types of tissue formation started from necrotic tissue , slough , and epithelial tissue then closed the wound but in *Ps.aeruginosa* group the types of tissue formation started from necrotic tissue , slough , granulation tissue and epithelial tissue then closed the wound and completely covered with epithelium at 17th days. (Figure 5)

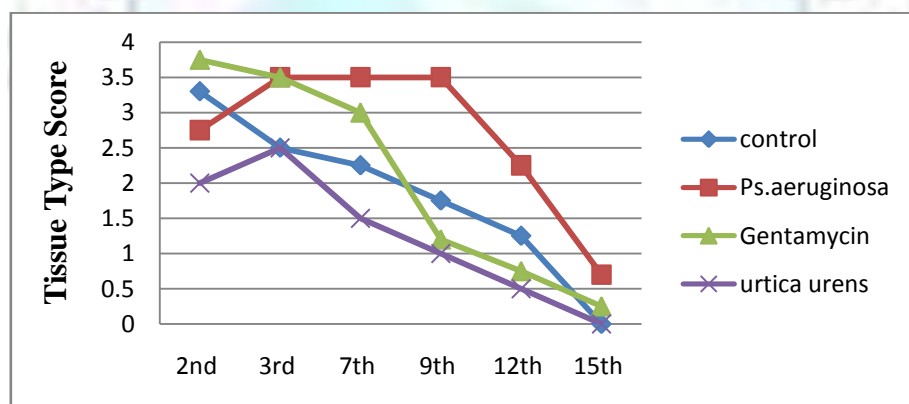


Figure 5: Effect of *urtica urens* ointment (25%, Topically) , Gentamycin (0.3%, Topically) and *Ps.aeruginosa* on the tissue type Score during wound healing

DISCUSSION

Recent studies evaluated the antibacterial effect of alcoholic extract of *Urtica urens* against many of gram positive and gram negative bacteria: *Staph.aureus*, *Ecoli* and *Pr.mirabilis*), (Abdul-Baki,2001, Mustafa et al.,2003) Gulcin et al Showed that all concentration of water extract of nettle (*Urtica dioica*) possessed noticeable antimicrobial activity against gram positive and gram negative bacteria: (*Staph.aureus*, *Str.pneumoniae*, *Staph.epidermidis*, *Pr.mirabilis*,*Citrobacter koseri*, *Enterobacter aerogenes*, *Micrococcus luteus*, and the yeast *Candida albicans*, WEN (water extract of nettle) exhibited antimicrobial activity against all microorganisms but have no detected against *Ps.aeruginosa*.

Its noted from the result(Fig1) that this plant did not affect in high concentration (100mg/ml) but it was the best act inhibitory at the concentration of (25mg/ml) , this may be due to the inability of the extract to penetrate the cell wall of the bacterium at high concentration and the inhibitory effect of *Urtica urens* extract may be due to the presence of

lectins, glycosides and tanins components.(Mossa et al.,1987).Other words the cell walls of the bacteria are not allowed the high concentration of this extract to pass through it (Abdul-Baki, 2001).

Urtica urens plant have been used in medicine for long years in folkloric and herbal medicine.Traditional used as a nourishing and blood cleanser (Nandini, 2010) or alternative agent for treatment of many disease such as arthritis and for treatment of symptom of benign prostatic hyperplasia (Jonathan , 2003).

Wound healing is a complex process consisting of many step starting by inflammation , granulation tissue formation ,angiogenesis , re-epithelization and wound contraction (Kotade and Asad , 2008). In the present study the Wound contraction ratio (WCR) of both treated animals groups of urtica urens and gentamycin groups were similar ,faster repairing the wounded than control and Ps.aeruginosa group. However , the contraction of the wound was the same in both treated groups from the second day. The action of plant may be occur because urtica urens plant possess potent anti-inflammatory activity (Johnson et al.,2012), antimicrobial (Rahman et al., 2010) and antineoplastic activity (Timoshenko et al., 1996). These different therapeutic action of urtica urens, especially the antimicrobial activity acceptable with our results here which concluded that topically dressing of urtica urens ointment have antimicrobial activity and the action same as therapeutic effect of application Gentamycin antibiotic in the present study.

In this experiment it well observed that the topical medication (urtica urens ointment) should be produced a specific desired effect during the wound stage healing this result is in agreed with previous study suggested that the topical ointment decrease the wound healing time (Kilic et al., 2002). It was also found that extract has a therapeutic effect on wound healing by enhance granulation tissue formation, Riehemann et al., 1999, Chrubasik et al., 1997 reported that urtica urens possess a significant anti inflammatory properties therefore it accelerate the wound healing.

To undertake the role of urtica urens extract in the treatment of skin wound it was shown that the immune response was enhanced, this may be due to presence of glycosides which have high intracellular killing activity and chemotaxis activity, this result is in agreement by other study by Basaran et al.,1996 about urtica urens who was supported patients suffering neutrophil function deficiency and chronic granulomatous disease because this plant stimulate the division and proliferation of lymphoid cells and have anticarcinogenic effects (Timoshenko et al.1996, Harput et al.,2005).

Urtica urens plant contains proteins which may stimulate the dermal fibroblast . Phan et al., used extract of plant contain protein to treat soft tissue wound and skin infection in order to demonstrate whether the extract increased expression of several component of adhesion complex and fibronectin by human keratocytes, they showed that the extract stimulated expression of many proteins which are essential to stabilize epithelium. By this effect urtica urens may increase the healing wound in present study. It was indicated that the urtica urens extract had antioxidant activity (Rahman et al., 2010, Körpe et al., 2013) and exerted a therapeutic action in different studies ; preserving liver cirrhosis and fibrosis protect them against oxidative stress (Alaattin, 2007). The beneficial effect of the use such plant might be related to their cytoprotective and antioxidative actions (Rahman, 2010, Namazi, et al., 2012).

CONCLUSION

Our data revealed that plant extracts Urtica Urens extract ointment 25% could be considered a suitable alternative to topical antibiotic used to treat skin wound healing and to control bacterial contaminated the wound by Pseudomonas aeruginosa .

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