

## **The Effect of Tannin Extracts from some plants in treatment of wounds infected by staphylococcus aureus experimentally in mice**

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### **Abstract**

In this study the efficacy of tannin extracts of *Lawsania* sp. Leaves and *punica grantium* peeling of fruits against *staphylococcus aureus* skin infection were assessed in vivo. Cell suspension of *staphylococcus aureus* ( $1 \times 10^8$  cell per ml) were used to infect wounds on the shoulder of (16) laboratory (20-25) gm mice, the mice were divided into four groups two control groups, Negative (treated With Vaseline) and positive (treated by tetracycline), and two test groups that treated by ointment prepared from tannin extracts of *Lawsania* sp. Leaves (group 3) and *punica grantium* peeling of fruits (group 4). The status of wounds and the time of healing and recovery of was studied and compared with each other. Both the extracts tested exhibited effective antibacterial properties and has potential therapeutic value for treatment of dermatophysis since it lead to complete recovery and healing of wounds within three days of treatment only.

**Key words:** *Lawsania* sp. Leaves, peeling of fruits of *punica grantium*, tannin extract, skin infection, in vivo.

### **Introduction**

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substrates that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs (Sofowora, 1982). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 1999). *Lawsania* sp. Leaves contain high amounts of a special tannic acid known as henna tannic acid (Hatton, 1999) and the peeling of fruits of *punica grantium* are rich in, the pomegranate tannin, punicalagin. (Gill et al., 2001). Tannins have been defined as polyphenols of high molecular weight which are water soluble and capable of precipitating

proteins (scalbert, 1991) they have been reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* (Akiyama,2001). while many invitro studies demonstrated that the extracts of *Lawsania sp.* Leaves and *punica grantium* peeling of fruits, have high Antimicrobial activities (Al-tikrity,1997; Ballal,2001; Abdul,1997; Bhurameswarik,2001; Gill et.al.,2001) non are developed for treatment of infectious diseases in vivo. so this study is aimed at investigating the Antibacterial activity of the extracts of, *Lawsania sp.* Leafs, and *punica grantium* peeling of fruits in treatment of wounds infected with *s. aureus* experimentally in mice.

## Methodology

**Plant extracts:** the method of (Taylor,2002) was used to obtain the tannin extract,50gm of *Lawsania sp.* Leafs, and 50gm of *punica grantium* peeling of fruits were washed by water and dried for 48h at room temperature, then crushed and extracted 3X with 1L Dimethylene chloride (1h with stirring) to remove pigments, lipids and non-polar material, then air dried in hood overnight to be extracted in the second day 3X with 800ml of 70% aq.Acetone (1h with stirring) and the extract was rotary evaporated under vacuum to remove Acetone.

**Bacterial strain :** a *S. aureus* strain was clinical isolated from Al-Qadisiah general Hospital. the isolate was grown in tryptic soy broth at 37 °C overnight, following incubation, the bacterial cells were harvested by centrifuging at 6000 r.p.m. for 10m, then resuspended in sterile Normal saline solution and centrifuged again, the process was repeated three times and then the washed bacteria were resuspended to be used in the following steps

**Laboratory animals:** All together (40) white laboratory female mice of (20 -25 gm) weight were gone under this study, the hair on the shoulder region was shaved and the location was disinfected by 70% ethanol. Several wounds were made on the skin by using a sterile blade and a swab was taken from the suspension of *s.aureus* ( $1 \times 10^{-7}$  cell per ml) to infect the wounds. The mice then divided in four groups as below: - Group1: negative control include 4 mice treated by Vaseline only. -Group2: positive control include 4 mice under daily treatment by tetracycline. -Group3: include 4 mice under daily treatment by *Lawsania sp.* Ointment, the base for this ointment was Vaseline with the concentration of 50% of *Lawsania sp.* Leafs extract. Group4: include 4 mice under daily treatment by *punica grantium* ointment, the base for this ointment was Vaseline with the concentration of 50% *punica grantium* peeling of fruits extract.

The treatment was carried out twice a day (12h between each treatment) and the mice were checked every day for the intensity of the wound and to record the time of wound healing and recovery.

## Results and Discussion

The wounds was inflamented on the second day of *s.aureus* infection in all mice ,many dermal changes were noticed like wound lesion, disorientation and the presence of local pus .(fig1) The findings from this investigation is summarized in table(1) indicated that the mice which were treated with the ointments prepared from *Lawsania sp.* Leafs extract (group 3) and the ointment prepared from *punica grantium* peeling of fruits extract (group4) , Take three days to recovering and healing the wounds, while it take seven days in the mice which were treated by Vaseline only (group1), by comparing the activity of both extract ointments with tetracycline activity (group2); which take two days of treatment to wound healing and complete recovery ; the extract ointment have considerable efficacy in controlling dermal infections .

Table (1): Treatment periods (days) of staphylococcal wound infections with \*Vaseline, \*\*Tetracycline, \*\*\* *Lawsonia sp* leaf extract.Ointment and \*\*\*\* *Punica grantium* peeling of fruits ointment,in mice

| Groups             | Treatment periods (days) |
|--------------------|--------------------------|
| negative control*  | 7                        |
| Positive control** | 2                        |
| L. l.Ex.Oint***    | 3                        |
| P. p.f.Ex Oint**** | 3                        |

Fig(1): Different skin lesions due to *s.aureus* after 24h of infection in mice. This study also indicated that both the Extracts of plant studied have a similar effect to the effect of tetracycline ointment against *s.aureus* skin infection (Fig 2). that may be due to the presence of tannins that play a very important role in antimicrobial activity in most plant Extracts , the antimicrobial mechanisms of tannins can be summarized as follows : (i)the astringent property of the tannin may induce complexation with enzymes or substrates . (ii)tannins toxicity can be related to its action on the membranes of microorganisms . (iii) Complexation of metal ions by (tannins may account for tannin toxicity. (Chung,et.al.,1998

Fig(2):-wounds status after 7 days of treatment :- A- Negative control, treated by Vaseline only. B- Positive control, treatment by tetracycline. C- Test group treatment by *Lawsania* sp. Ointment. D- Test group treatment by *punica grantium* ointment

According to these findings, the ointment of both *Lawsania* sp. Leaf's extract and *punica grantium* peeling of fruits extract have effective antibacterial properties and potential therapeutic value which could be developed for treatment of dermatophytosis

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