Research Article

# Wound Healing Activity of the Methanolic Extract of *Urena lobata* Linn

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**ABSTRACT**

To screen the wound healing activity of the methanolic extract of *Urena lobata* L.(MEUL) in excision, incision, burn wound and dead space on albino rats. Preliminary Phytochemical investigation of reveled the presence of flavonoids, and tannins. Two doses (200mg/kg, 400mg/kg) were selected based on the acute toxicity studies; there was no mortality up to 4000mg/kg. The MEUL exhibited significant wound healing activity in excision, incision, burn, and dead space wound model, which is comparable to the marketed Povidone-Iodine formulation. A significant decrease in the period of epitheliazation and wound contraction as compared with control. The wet and dry tissue weight is significantly increased in MEUL (400mg/kg) compared to MEUL (200mg/kg) and control. The Hydroxyproline content was significantly more in 400mg/kg compared with 200mg/kg. From these results obtained the study can be concluded that the methanolic extract of *Urena lobata* has significant wound healing potential.

**Keywords:** *Urena lobata* Linn, Wound healing activity, Methanolic extract.

## INTRODUCTION

Wound is a physical trauma where the skin is torn, cut burn or punctured .Wound often possesses problems in clinical practice. A lot of research has been envisaged to develop the better healing agents. The rapidity of wound healing depends to a considerable extent on the contraction that begins a few days after injury and continues for several weeks[1, 2]. World Health Organization (WHO) has been promoting traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries. Eight percent of the world's population relies on medicinal plants for their primary healthcare. WHO also recognized the importance of traditional medicine and has treated strategies, guidelines and standard for botanical medicines[3,4].Wound healing is a complex phenomenon, involving a number of well-orchestrated processes, including regeneration of parenchyma cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of ECM (extracellular matrix) proteins, remodeling of connective tissue (C.T) and parenchymatous components and collagenisation and acquisition of wound strength[5]. In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases[6].Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diaorrhorea, scabies, venereal disease, ulcers, snake bite[7]. Many Ayurvedic herbal plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way. The healing process can be physically monitored by assessing the rate of contraction of the wound.

*Urena lobata* Linn of Malvaceae family is of medicinal value .It is a shrub of 60-250cm or more height and basal diameter of 7 cm[8].This medicinal plant is useful in many diseases, in the form various extracts of leaves and roots. Traditionally the plant being used as diuretic, febrifuge and rheumatism .It is useful for wounds, toothache, gonorrhea and for food for animals as well humans[9]. Aerial parts of *Urena lobata* contain mangeferin and quercetin and from roots imperatonin and furcoumarin were isolated[10].Thus popularity of natural drugs all over the world in recent years is an indication of significant contribution of pharmacognosy in modern medicine. The present work intends to

study wound healing activity of the methanolic extract of *Urena lobata*.

## MATERIALS AND METHODS

### Collection and authentification of the herb

The plant material of *Urena lobata* Linn were collected from the Herbal Garden Division of Kerala Ayurveda Ltd, Aluva, India and authentified. A specimen voucher was deposited in college herbarium for future references. Methanol, sodium alginate, normal saline, anesthetic ether, HCl, NaoH, Standard hydroxy proline solution, $CuSO_4$, $H_2O_2$, Para Di methyl Amino Benzaldehyde, n-propanol, $H_2SO_4$.

### Plant Extraction

The coarsely powdered (3.0 kg) whole plant of *Urena lobata* was added to a 20 L round bottom flask which was fitted with a reflux condenser. To the above settings, 15 L of Methanol was added and the mixture refluxed at 65 °C for about 1 h. Finally the mixture was filtered and the extract was collected. The extraction process was repeated with 15 L of Methanol and combined all the extracts. The water is then evaporated under reduced pressure in a Buchi Rotary Evaporator at 95 °C, to obtain 105 g of powder extract. The percentage yield obtained was 4.5.

### Experimental Animals

Albino wistar rats weighing 200-220 gm and Albino mice 20-30 gm was procured from Biogen, Bangalore, India. They were maintained in the animal house of Gautham College of Pharmacy. Animals were maintained under controlled condition of temperature at 27±2 °C and 12 h light-dark cycles. They were housed in polypropylene cages and had a free access to standard pellets (Amruth) and water *ad libitum.* All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of pharmacy, Bangalore (REF-IAEC/08/06/2012) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

### Acute Toxicity Studies ($LD_{50}$)

Organisation for Economic Co-operation and Development (OECD) Guideline on acute oral toxicity, environmental health and safety monograph series on testing and adjustments no: - 423 [11.]

### Animal

Female Albino mice of weighing 20-30 gm were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing.

### Procedure

Methanolic extract of *Urena lobata* was dissolved in saline/water. The doses were selected according to the OECD guidelines 423. The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female mice (each set of 3 mice's) were used for this experiment. First set of animals were divided into three groups, each of three in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 3000 mg/kg was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 1-2 h. All animals were observed for clinical signs during the first 30 min and then at approximately 4h after administration of extracts on day 0 and once daily during 1 to 14 days.

### Drug Formulations

#### For Topical Application

10 & 20 gm of extract incorporated with 90 & 80 gm of 2% sodium alginate.[12.]

#### For Oral Administration

The extract is dissolved in normal saline

### Wound Healing Activity

Wound healing activity was studied using four models viz. excision, incision, burn wound and dead space wound model.

### Excision Wound Model

All animals in each group were anaesthetized by the open mask method with anesthetic ether. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 $mm^2$. Hemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Animals are divided into four groups of each with 6 animals. Group-I as control, Group-II consider as standard and treated with 5% w/w Povidone-iodine ointment, Group-III and group-IV are MEUL treated group and applied gel 10% and 20% respectively. Drugs

were topically applied once a day till complete of epitheliazation, starting from day of excision. The progressive changes in wound area were monitored plan metrically by tracing on the 1mm$^2$ wound healing and subsequently on alternate days i.e. 2$^{nd}$, 4th, 6$^{th}$, 8$^{th}$, 10$^{th}$, 12$^{th}$, 14$^{th}$, 16$^{th}$, 18$^{th}$, 20$^{th}$ and 22$^{nd}$ days post wounding. Number of days required for falling of scab without any residual raw wound, gave the period of epitheliazation [13, 14.]

$$\text{Percentage Wound Closure} = \frac{\text{(Initial area of Wound - N}^{th}\text{day area of wound)}}{\text{(Initial area of Wounds)}} \times 100$$

**Incision Wound Model**

Animals were grouped which is divided into four groups same as followed in excision wound model. The incision wound model was studied under light ether anesthesia the animal was secured to operation table in its natural position. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back. After complete haemostasis, the wound was closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0th day to 9$^{th}$ post-wounding day. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. All the sutures were removed on the 9$^{th}$ post wounding day. On 10$^{th}$ day the tensile strength was measured by continuous constant water supply technique [15, 16].

**Measurement of Wound Breaking Strength of Incised Wounds**

Measurement of wound breaking strength was performed following lee with certain modifications. A board was placed on the table, on which the anaesthetized animal was made to lie on its abdomen. Two clamps were clamped on either sides of healed wound at a distance 0.5 cm. the left clamp was fastened tightly to stand by means of thread. The right clamp was connected to a leak proof polythene container through a pulley, by means of a thread. A reservoir containing water was placed at a suitable height and connected to a polythene bag by means of a rubber tube. The position of the board was adjusted so that, the polythene bag was hanging freely. Water was added to polythene bag rapidly at constant rate from the reservoir until the wound opened. Amount of water in polythene bag was measured (in ml) and was considered as tensile strength of the wound.

**Burn Wound Model**

All animals in each group were anaesthetized by the open mask method with anesthetic ether. The hair on the back was clipped with electric clippers. Burn wounds were created by pouring hot molten wax at 80°C into a metal cylinder placed on the back of the rat. The metal cylinder has 100 mm$^2$ area of circular openings and capacity of to hold 4.0 gm of wax. On solidification of wax (8 min), the metal cylinder with wax adhered to the skin was removed, which left distinctly demarked circular wounds of 100 mm$^2$. After this each animal was placed in a separate cage for full recovery from anaesthesia before being returned to holding rooms[17,18.] The following parameters were studied[19].

**Epitheliazation Period**

It was monitored by noting the number of days required for eschar to fall away, leaving no raw wound behind.

**Wound Contraction**

It was assessed by noting the progressive changes in wound area plan metrically, excluding the day of the wounding. The size of the wounds was traced on a transparent paper every two days, throughout the monitoring period. The tracing was then transferred to 1 mm2 graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, as 100%, by using the following equation:

$$\text{Percentage Wound Closure} = \frac{\text{(Initial area of Wound –N}^{th}\text{ day area of wound)}}{\text{(Initial area of Wounds)}} \times 100$$

**Dead Space Wound Model**

The animals were divided into four groups of 6 each. Group-I animals served as control and provided with plain drinking water. Group-II animals were treated with 5% w/w Povidone-iodine ointment. Group-III and Group-IV were treated with methanolic extract of 200mg/kg, 400mg/kg respectively. On the 10$^{th}$ post wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anaesthesia. The wet weight of the granulation tissue was noted. These granulation tissues were dried at 60 °C for 12 h, weighed and recorded the dry weight. To the dried tissue 5 ml of 6N HCl was added and kept at 110°C for 24 h. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline. Additional piece of wet granulation tissue was preserved in 10% formalin for histological studies[20, 21.]

**Hydroxyproline Estimation**

Samples of varying concentrations were taken for analysis. Hydroxyl proline was oxidized by adding 1 ml of chloramines-T to each tube. The contents were mixed thoroughly by shaking and allowed to stand for 20 min at room temperature. Adding 1 ml of 70% perchloric acid to each tube then destroyed the chloramines-T. The contents were mixed and allowed to stand for 5 minutes. Finally 1 ml of Para Di methyl Amino Benzaldehyde (PDAB) solution was added and the mixture was shaken well. The color developed was read spectrophotometrically at 557 nm. The collagen content was then calculated by multiplying the hydroxyproline content by the factor 7.46 and was expressed as mg/100mg of dry weight of the sample.

**Histopathological Study**

The granulation tissues were obtained on day 10 from the test and control group animals for the histological study. For the better appreciation of collagen deposition Van Geison stain was used which stain the fibres pink.

**Statistical Analysis**

The relative wound area results were compared using one way analysis of variance (ANOVA) followed by Dunnet's tests. *P*-values less than 0.05 were considered as indicative of significance[22.]

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening of methanolic extract *Urena lobata* showed the presence of flavonoids and tannins. A significant decrease in period of epitheliazation was observed after 10% and 20% MEUL Treatment with Povidone-Iodine also significantly reduced period of epitheliazation as compared with control group. At the same time 10% and 20% MEUL and Povidone-Iodine also decreased the wound contraction (50%) as compared with control showed in (**Table 1 & 2).**

**Table 1: Effect of MEUL on Excision Wound (% Wound Closure)**

| Day | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 2 | 13.74% | 24.09% | 27.98% | 27.38% |
| 4 | 26.03% | 36.00% | 42.25% | 40.03% |
| 6 | 41.78% | 47.28% | 63.17% | 62.72% |
| 8 | 52.80% | 71.73% | 79.91% | 79.53% |
| 10 | 69.50% | 88.97% | 91.51% | 88.61% |
| 12 | 83.97% | 91.64% | 93.25% | 93.38% |
| 14 | 84.5% | 94.83% | 94.59% | 96.63% |
| 16 | 88.58% | 96.97% | 97.04% | 98.55% |
| 18 | 93.62% | 98.31% | 100% | 100% |
| 20 | 95.26% | 100% | 100% | 100% |

**Table 2: Effect of MEUL on Excision Wound [Wound area mm$^2$] and period of Epitheliazation in Days**

| Groups | Treatment | Wound contraction on | | | | | | | | | | | Epitheliazation time (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 21 | |
| Group I -- | - | 385.0 ± 6.19 | 330.0± 5.77 | 260± 7.30 | 210.8± 8.20 | 38.5± 12.07 | 96.5± 5.22 | 69± 13.12 | 50.83± 12.67 | 28.33± 9. 7 | 10.5±4.7 | 00 | 9.167±0.40 |
| GroupII | 5%w/w Povidone-Iodine | 356.7 ±10.5 | 356.7±10.5* | 287± 8.80 | 213.7±2.66** | 125±6.19*** | 48.67 ±5.01*** | 36.5± 4.63*** | 22.67±4.31** | 13.17±2.37** | 3.66±1.68** | 00** | 8.33±0.21 |
| GroupIII | 10%MEUL | 316.7 ±4.21*** | 231.7±13.02 | 161.7 ±13.27*** | 88.33±11.08*** | 37.33± 9.26*** | 26.67 ±7.72*** | 19.83 ±6.86*** | 8.66±2.91*** | 00* | 00* | 00 | 8.33±0.21 |
| GroupIV | 20%MEUL | 321± 5.31*** | 263.8±8.44*** | 164.8 ±9.44*** | 90.17±9.57*** | 37.83± 5.9*** | 29.17 ±4.48*** | 12.33 ±2.89*** | 4.16±1.61*** | 00** | 00* | 00 | 7.33±0.21*** |

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, **represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

In incision wound model the breaking strength of wound on 10$^{th}$ day was significantly increase in all treatment groups when compared to control **Table 3.**

**Table 3: Effect of MEUL on breaking strength (g) in incision wound model**

| Groups | Treatment | Wound Breaking Strength(g) |
|---|---|---|
| Group I | -- | 268.0±7.5 |
| Group II | 5%w/w Povidone-Iodine | 368.9±14.51*** |
| Group III | 10%MEUL | 417.9±12.23*** |
| Group IV | 20%MEUL | 483.8±11.0*** |

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet'Stest where * represents significant at < 0.05, **represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

Table 4: Shows the effect of the methanolic extract of *Urena lobata* on percentage wound closure in burn wound model. Burn wound model showed both 10% and 20% MEUL gel applied topically shorten the period of epitheliazation significantly demonstrated in Povidone iodine ointment also applied topically shortens the period of epitheliazation and all three also decreased the wound contraction (50%) significantly when compared with control Table 5.

**Table 4: Effect of MEUL on Burn Wound (% Wound Closure)**

| Days | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| 0 | 0% | 0% | 0% | 0% |
| 1 | 0% | 0% | 0% | 0% |
| 3 | 24.44% | 20.82% | 23.24% | 22.54% |
| 5 | 33.45% | 44.13% | 49.97% | 40.12% |
| 7 | 46.86% | 56.50% | 63.85% | 53.46% |
| 9 | 60.97% | 70.24% | 72.97% | 65.94% |
| 11 | 72.71% | 79.77% | 83.85% | 81.92% |
| 13 | 84.87% | 90.10% | 92.18% | 90.41% |
| 15 | 92.22% | 96.18% | 96.43% | 95.79% |
| 17 | 100% | 100% | 100% | 100% |
| 19 | 100% | 100% | 100% | 100% |

**Table 5: Effect of MEUL on period of Epitheliazaton and wound contraction in Burn wound model**

| Groups | Treatment | Contraction of Wound on Different Days (sq.mm) | | | | | | | | | | Epitheliazaton time (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 3rd | 5th | 7th | 9th | 11th | 13th | 15th | 17th | 19th | |
| Group I - - | - | 74.67± 1.406*** | 94.67±1 .838*** | 67.83±1. 579*** | 64.83± 1.579 | 38.83± 1.376*** | 50.00± 0.574*** | 22.83±0 .7923*** | 10.83±0.47 73*** | 7.167 ±0.30 7*** | 3.833 ±0.30 73*** | 9.00±0.2236*** |
| GroupII | 5%w/w Povidone-Iodine | 74.33± 1.64 | 59.83± 1.44 | 1.33± 1.56*** | 32.17± 1.49 | 22.00± 1.31** | 15.00± 1.55** | 7.333± 0.33*** | 2.833±0. 30*** | 00 | 00 | 6.66±0.21* |
| GroupIII | 10%MEUL | 0.35± 0.76 | 54.00± 1.36 | 35.17± 1.24*** | 26.00± 0.36*** | 19.00± 0.73*** | 11.33± 0.61*** | 5.500± 0.22*** | 2.500±0. 22*** | 00 | 00 | 5.5±0.22*** |
| GroupIV | 20%MEUL | 1.00± 0.68 | 55.00± 1.46 | 42.50± 0.71** | 33.00± 0.77** | 24.17± 1.35 *** | 13.17± 0.600*** | 6.833± 0.90*** | 3.000±0. 63*** | 00 | 00 | 5.66±0.21*** |

9Values are mean ± SEM (n=6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, **represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

**Dead Space Wound Model**

The wet and dry tissue weight is significantly increased in MEUL (400mg/kg) compared to (200mg/kg) MEUL and compared with control. The hydroxyproline content was significantly more in 400mg/kg compared with 200mg/kg **Table 6.**

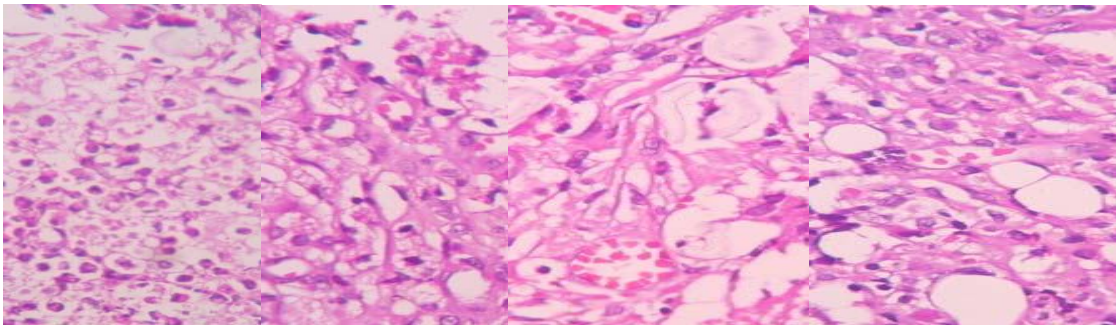
**Table 6: Effect of AESE on Wet, Dry Tissue Weight and Hydroxyproline**

| Groups | Treatment | Wet Tissue weight (g) | Dry Tissue weight (g) | Concentration of hydroxyproline (µg/g) |
|---|---|---|---|---|
| Control | - | 57.56 ± 5.53 | 35.41 ± 5.23 | 0.2925±0.006292 |
| Group I | Standard Povidone-Iodine | 74.51 ± 8.316 | 51.47 ± 7.17 | 0.2055±0.00637** |
| Group III | 10%MEUL | 70.60 ± 4.847 | 48.06 ± 4.875 | 5.623±0.02428*** |
| Group IV | 20%MEUL | 87.85±6.714** | 64.30 ± 6.002** | 6.190±0.009129*** |

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents Significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.00.

**Histopathology Report**

Histopathology of dead apace wound on 10th day cotton pellets shown in **Fig 1.** Group I, photo shows abundant areas of necrosis, giant cells, damaged vascular spaces, Group II, photo shows proliferating vascular spaces. Group III & IV treated with 200 and 400mg/kg extract, it shows Granulation tissue formation, chronic inflammatory infiltration, Macrophages.



**Group I** **Group II** **Group III** **Group IV**

## DISCUSSION

The present study was undertaken to evaluate whether *Urena lobata* methanolic extract could promote wound healing in experimentally produced wound in rats. The preliminary phytochemical analysis of methanolic extract of *Urena lobata* revealed the presence of flavonoids, tannins. The study was designed to investigate the influence of the MEUL on three main phases of wound healing, namely collagenation, wound contraction and epitheliazation. In the present study the extract applied topically and given orally promoted the breaking strength and the wound contraction and period of epitheliazation. Collagenation, wound contraction and epitheliazation are crucial phase of wound healing. The phase inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually lead to either promotion or depression of the collagenation phase of healing. Growth hormone promotes the healing process by enhancing epithelial cell proliferation and cell collagen formation. Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue, cartilage. Collage is synthesized by a complex biochemical mechanism of ribosome. The collagen synthesis is stimulated by various growth factors. Growth hormone is also known to promote the proliferation of fibroblasts, and fibroblast proliferation from the granulation tissue. In the dead space wound model *Urena lobata* treatment increased granuloma tissue weight. Hence it can assume the pro-healing activity of *Urena lobata* could be due to the direct or indirect influence on growth hormone release. Before screening the test for wound healing activity, the MEUL was subjected to the acute toxicity studies. The methanolic extract of *Urena lobata* was found to be non toxic at 4000 mg/kg. Hence, the 200 and 400 mg/kg dose were selected for the further study. In excision wound model the methanolic extract of *Urena lobata* showed faster healing compared with control groups and wound contraction rate is faster it may be due to stimulation of interlukin-8 and inflammatory α-chemokine which effects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. The epitheliazation period is decreases may be due to enhancing collagen synthesis [23]. In incision model, the wound breaking strength is increased. Increase in tensile strength may be due to increase in collagen concentration and stabilization of the fibres [24]. In burn wound model, the wound contraction is faster and the epitheliazation period is increased. In burn wound trace elements plays an important role. It is possibly due to contribution of Zn & Cu in the inflammatory process [25]. In dead space wound model, the hydroxyproline content is increased it is due to increase in total protein and total collagen content reflected by hydroxyproline content of granulation tissue and increases the angiogenesis. There is increasing the wet and dry weight of the granulation tissue indicating the presence of higher collagen content [26]. Flavonoids are known to reduce lipid per-oxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence any drug that inhibits lipid per-oxidation is believed to increase the viability of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis [23]. The enhanced wound healing may be due to free radical scavenging action and the antibacterial property [9] of the phytoconstituents present in it which either due to their individual or additive effect fastens the process of wound healing. This could be the reason for pro-healing activity of *Urena lobata*. This enhanced wound contraction effect of *Urena lobata* and epitheliazation could possibly be made use of clinically in healing of open wounds. *Urena lobata* was shown more potent than Povidone –Iodine in excision, incision, burn and dead space wound.

## CONCLUSION

The present study was to evaluate the wound healing activity of then methanolic extract of *Urena lobata* and it was confirmed by its effect on excision, incision, burn and dead space wound model. The methanolic extract showed remarkable wound healing activity and further studies are warranted with purified constituents to comprehend the complete mechanism of wound healing activity of *Urena lobata* Linn.

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