

Research Article

## Wound healing activity of methanolic extract of *Turbinella pyrum* from Gulf of Mannar, India

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### Abstract

Molluscs are a promising source of bioactive substances and are virtually untapped resources of novel compounds. Many novel metabolites with potent pharmacological properties have been discovered in marine organisms in recent years. The present study aimed to investigate the wound healing activity of a methanolic extract of the marine gastropod *Turbinella pyrum* using an excision and incision wound model. To determine the minimum lethal dose, acute oral toxicity studies were performed as per the Organization for Economic Co-operation and Development (OECD) guidelines. Excision and incision wound models were used to evaluate the wound healing activity of the methanolic extract of *T. pyrum* by preparing a simple ointment base BP at different concentrations using five groups of Wistar albino rats. Group I (control) received a topical application of the simple ointment BP. Group II (Standard) was treated with topical application of 5% framycetin sulphate cream, and Groups III, IV and V were treated with 5%, 10% and 15% methanolic extracts of *T. pyrum*. Student's *t* test was used to analyse the results obtained from the present study, and  $P < 0.05$  was considered significant. In the incision wound model, the epithelialization period was also found to be highly significant ( $P < 0.05$ ) in group V (10.77%) when compared to the standard (12.69%). In the excision wound model, the epithelialization period was also found to be highly significant ( $P < 0.05$ ) in group IV (13.17%) and group V (11.38%) when compared to that of the standard (Group II) (14.56%). This finding justifies that the methanolic extract of *T. pyrum* has properties that render it capable of promoting accelerated wound-healing activity.

**Keywords:** Mollusc, Pharmacology, *Turbinella pyrum*, Wistar albino rats, Wound healing

### INTRODUCTION

The wound is a physical injury, especially when the skin or another external surface is torn, pierced, cut or broken with disruption of anatomical and functional continuity of structures (Srilekha *et al.*, 2018). Dermal wound healing is a physiological process that restores the anatomical structures and function of injured skin (Park *et al.*, 2017). Unhealed wounds constantly produce inflammatory mediators that cause pain and swelling at the wound site. Chronic wounds may even sometimes lead to multiple organ failures or the death of the patient. To restore the integrity and to avoid severe damage to the body, rapid wound healing is required. The present treatment system using cortisones and other anti-

inflammatory drugs may impair healing (Senapati *et al.*, 2011). Drugs of natural origin are an important source for treating many diseases worldwide (Pandima Devi *et al.*, 2003). Natural derivatives play an important role in wound healing, as synthetic drug formulations cause various harmful side effects to human beings (Subalakshmi *et al.*, 2014).

Marine invertebrates offer a rich source of potential drugs with excellent biological activities (Jain *et al.*, 2018). Among invertebrates, molluscs are widely distributed worldwide and have many representatives in marine and estuarine ecosystems (Grasian *et al.*, 2012). The Materia medica of India provides a great deal of information on folklore practices and traditional aspects of therapeutically important natural products

(Yadav *et al.*, 2011). Considering the importance of marine natural products, an attempt was made to investigate the wound healing property of the marine gastropod *Turbinella pyrum* from the Gulf of Mannar coastal region in the present study.

## MATERIALS AND METHODS

The specimens of *Turbinella pyrum* were collected from the Gulf of Mannar coastal region of Thoothukudi during low tides from the sea. In the present study, whole-body tissue extract of *T.pyrum* was used for the wound healing assay. The freshly collected samples were cleaned and washed with fresh seawater to remove all impurities. The shells were removed, and the tissues were then dried in a hot air oven at 60°C for 48 hours. One hundred grams of powder was exhaustively extracted with methanol in a Soxhlet apparatus and concentrated in a rotary vacuum evaporator when 15g of brown sticky mass was obtained. The methanolic extract was incorporated into a simple ointment base. The formula used in the British Pharmacopoeia (BP) was used for the preparation of a simple ointment base that contains woolfat (50g), hard paraffin(50g), white soft paraffin (170g) and cetostearyl alcoholic 1 (50 g). Three formulations of the extract ointment of 5% (W/W), 10% (W/W) and 15% (W/W) were prepared by incorporating 5g, 10 g and 15 g of extract in 100 g of simple ointment base BP, respectively, for topical administration. For faster recovery, the extracts were suspended in 1% carboxymethylcellulose (CMC).

### Experimental animals

Adult Wistar albino rats weighing approximately 150 - 180 g maintained at SB College of Pharmacy Animal House, Sivakasi, were used for the present study with prior approval of the Institutional Animal Ethics Committee. The rats were housed in standard environmental conditions and fed standard food and water ad libitum during the whole period of the experiment. Excision and incision wound models were used to evaluate the wound healing activity of the methanolic extract of *T. pyrum*.

### Grouping of animals

Thirty animals were divided into five groups, each group consisting of 6 rats viz. Group I was treated with a simple ointment base and served as a control. Group II was treated with 5% standard drug, i.e., Framycetin sulphate cream. Group III was treated with 5% methanolic extract ointment. Group IV was treated with 10% methanolic extract ointment. Group V was treated with 15% methanolic extract ointment.

### Experimental models used:

#### Excision wound model

All the rats were afflicted with an excision wound as

described by Morton and Malone (1972) under light ether anaesthesia. Superficially, a single circular wound of 500 mm<sup>2</sup> was made on the rat depilated ethanol-sterilized dorsal thoracic region. Group 1 animals were topically treated with the plain ointment base as a placebo control. The animals of group II were topically treated with standard drug, i.e., Framycetin sulphate cream. Groups III, IV and V were topically treated with 5%, 10% and 15% of the methanolic extract ointment, respectively. Once-daily, the ointment was applied topically with a fine brush until the wound was completely healed. Wound contraction was monitored planimetrically by tracing the wound margin on transparent paper every other day and retracing the wound margin on millimeter-scale graph paper. Wound contraction, which contributes to wound closure or reduction in the wound area, was expressed as a percentage reduction of the original wound area (500 mm<sup>2</sup>). The percentage wound contraction was determined using the following formula: Percentage wound contraction = Healed area / Total wound area

....Eq. 1

Healed area = Original wound area – Recent wound area

The wound margins were traced and measured to calculate the nonhealed area, which was subtracted from the original wound area to obtain the healed area.

### Incision wound model

In this model, 6cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of all the rats as described by Ehrlich and Hunk (1969). The wound was then enclosed with interrupted sutures 1cm apart. The animals were randomized into all five groups (n=6/group). Group I (Ointment control) received an atypical application of the simple ointment BP; Group II (Standard drug) was treated with a topical application of 5% Framycetin sulphate cream; Groups III, IV and V were treated with 5%, 10% and 15% methanolic extracts of *T.pyrum*. Each treatment outlined here was utilized each day for eight consecutive days after wound infliction.

### Statistical analysis

The results, expressed as the mean ± SE, were evaluated using the t test. Values of P < 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

The effect of the methanolic extract of *T.pyrum* on the contraction of excision wounds is presented in Table 1. Topical application of the methanolic extract ointment of *T.pyrum* showed a significant dose-dependent effect on the healing process. It was noted that the wound contracting ability of extract-treated groups viz. Group

**Table 1.** Effect of the methanolic extract of *T. pyrum* on the contraction of excision wounds

Groups	Epithelialization period	Percentage of wound contraction in post wounding days						
		2	4	6	8	10	12	14
I (Control)	18.61±0.12	16.60±0.23	21.04±0.17	29.53±0.14	39.56±0.03	48.22±0.81	53.08±0.92	71.09±0.12
II (Standard)	14.56±0.03	21.22±0.67	43.27±0.88	58.91±0.78	69.44±0.90	76.33±0.14	82.44±0.43	90.01±0.07
III 5% methanolic extract of <i>T.pyrum</i>	16.01±0.82	20.26±0.13	40.11±0.36	49.33±0.46	58.71±0.79	66.27±0.55	70.09±0.08	89.44±0.26
IV 10% methanolic extract of <i>T.pyrum</i>	13.17±0.03*	26.66±0.43	42.78±0.33	50.55±0.78	70.82±0.93	86.34±0.66	90.01±0.38	95.28±0.23*
V 15% methanolic extract of <i>T.pyrum</i>	11.38±0.08*	29.55±0.17	51.15±0.88	67.77±0.76	80.89±0.57	90.01±0.89	93.88±0.21	97.44±0.22*

IV (10% W/W) and Group V (15% W/W) were found to be highly significant from the 8<sup>th</sup> to 14<sup>th</sup> day when compared to that of the standard (Group II), framycetin sulphate (12% W/W). The epithelialization period was also found to be highly significant ( $P<0.05$ ) in Group IV (13.17%  $\pm$  0.03) and group V (11.38%  $\pm$  0.08) when compared to that of the standard (Group II) (14.56%  $\pm$  0.03).

The effect of the methanolic extract of *T.pyrum* on the contraction of incision wounds is presented in Table 2. In the incision wound model, the extract-treated groups, even at a very low dose, produced a significant increase in wound contraction when compared with the standard. A highly significant result was noticed in group V (15% W/W) on day 16. It showed 92.52% wound contraction when compared with the standard (Group II)(88.12%). The epithelialization period was also found to be highly significant ( $P<0.05$ ) in group V (10.77  $\pm$  0.17) when compared to the standard (12.69  $\pm$  0.22).

Topical application of the methanolic extract of *T.pyrum* showed a significant dose-dependent effect on the healing process. In both excision and incision wound models, animals treated with the methanolic extract of *T. pyrum viz.* Group III, Group IV and Group V showed better and faster healing than the standard framycetin sulphate cream (Group II) and control(Group I) groups. Different studies also support the wound healing activity on molluscs. Subavathy *et al.* (2018) analysed the methanolic extract of *Tonna dolium*, which revealed that the wound contraction and epithelialization period was higher in the methanolic extract ointment of *T. dolium* than in the standard (povidone iodine cream) in both the excision and incision wound models. Similar observations have been reported by Grasian *et al.* (2012) on *C. moneta*. The lipid extract from *Mytilus galloprovincialis* and *Rapana venosa* observed by Badiu *et al.* (2008) was found to be even more efficient in healing induced skin burns in Wistar albino rats. In the present study, the wound-healing property of *T. pyrum* may be attributed to some bioactive compounds present in the methanolic extract of *T. pyrum*, which may be the primary cause for the quicker wound healing process. These bioactive compounds need to be explored.

## Conclusion

*T. pyrum* is a divine conch, a marine gastropod mainly found in the Indian Ocean. The results of the present study emphasized that the wound healing activity of the methanolic extract of *T. pyrum* increased the wound healing activity of Wistar albino rats. This, in turn, implies that some bioactive components present in the marine gastropod *T. pyrum* are responsible for this re-

**Table 2.** Effect of the methanolic extract of *T.pyrum* on incision wounds

Groups	Wound area (mm <sup>2</sup> ) and % of wound contraction given in parenthesis				
	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	Epithelization period (%)
I (Control)	332.11±0.26 (20.11)	308.07±0.87 (22.86)	219.11±0.97 (52.13)	152.88±0.86 (60.77)	22.34±0.11
II (Standard)	212.66±0.88 (47.13)	149.09±0.17 (64.17)	108.78±0.62 (80.78)	52.22±0.79 (88.12)	12.69±0.22
III 5% methanolic extract of <i>T.pyrum</i>	282.87±0.66 (40.66)	213.89±0.52 (42.86)	103.01±0.17 (60.52)	49.87±0.89 (89.56)	14.41±0.56
IV 10% methanolic extract of <i>T.pyrum</i>	230.66±0.46 (52.88)	182.11±0.71 (56.14)	96.33±0.88 (73.21)	38.45±0.67 (90.17)	15.78±0.48
V 15% methanolic extract of <i>T.pyrum</i>	209.11±0.01 (49.78)*	170.01±0.86 (61.83)	75.28±0.46 (82.52)	30.11±0.32 (92.52)	10.77±0.17*

Values expressed as the mean ± SE, n = 6 animals in each group; \*highly significant (P <0.05) compared to control and standard groups

markable wound healing activity. It is suggested for treating various types of wounds in human beings as a clinical trial. Further research on the clinical side and mechanism of action is needed to evaluate the bioactive component present in *T. pyrum* to pave a better way to explore a cost-effective pharmaceutical ointment for fast and better-wound healing.

### Conflict of interest

The author declares that they have no conflict of interests.

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