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**Original Research Article**

# Evaluation of wound healing activity of *Hydrilla verticillata* (L.F.) Royle collected from unpolluted and polluted water sources

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**A B S T R A C T**

Experiments were carried out to evaluate and compare the wound healing activity of ethanolic whole plant extract of *H. verticillata*, collected from unpolluted and polluted water sources. The result of wound healing activity showed significant curing of wound in both samples from unpolluted and polluted water source than control (Indomethacin) and the induced (carrageenan) treatments. The standard drug (Indomethacin) treated animals showed faster epithelialization of wound ($18^{th}$ day) than the animals treated with ethanol extracts ($21^{st}$ day). There is a significant deviation from ($p_{<0.05)}$ between the *H. verticillata* samples collected from unpolluted and polluted water. At high dose of plant samples from unpolluted water, there was a higher rate of wound healing and reduced the epithelialization period ($15^{th}$ day) but in plant samples from polluted water, the complete wound healing was observed on $18^{th}$ day. While comparing the plant samples from polluted water, the plant sample from unpolluted water showed faster rate of healing ($15^{th}$ day). The time required for complete epithelialization of the wound is an important parameter to assess the wound healing process. In this study, it was noted that the period of complete epithelialization was delayed for three days in polluted plant samples and closure was observed in standard and induced group (low and high dose) within $15^{th}$, $18^{th}$ and $21^{st}$ day respectively.

**Keywords**

Wound healing, *H. verticillata*, Epithelialization, Unpolluted and Polluted water, Indomethacin and Carrageenan, Whole plant ethanolic extract

## Introduction

Wound is a loss or breaking of cellular and anatomic or functional continuity of living tissues. As physical injuries it results in as opening or breaking of the skin. Wound healing is a biological process initiated by scar formation by well-organized biochemical and cellular event leading to the growth and regeneration of the wounded tissue in a special manner. Plant extract are used as a source of medicines to treat different ailments including wound healing processes (Fernandez and Lopez, 2002; Suguna *et al.*, 2002) in traditional medicine throughout world.

Significant effects as wound contracting ability, wound closure, drease in surface

area of wound, tissue regeneration at the wound site in ethanol plant extracts treated rats were observed by several workers (Umachingi *et al.,* 2008; Abdullahi *et al.,* 2011; Alagesaboopathi, 2011; Vijayabaskar *et al.,* 2011). The present study aimed to evaluate the wound healing properties of the whole plant ethanol extracts of *H. verticillata* collected from unpolluted and polluted water sources.

## Materials and Methods

**Plant materials:** *H. verticillata* samples were collected from the unpolluted and polluted water sources of Asaripallam, Nagercoil, Kanyakumari district, Tamilnadu and were authenticated by the Taxonomist of Botanical Survey of India (BSI), Coimbatore.

**Water analysis:** Both unpolluted and polluted water samples were collected separated from a depth of one ft below the surface and kept in one liter prewashed polythene containers separately. Half part of the water samples were analyzed for the physico-chemical parameters within 24 hours of collection and the other half part kept in refrigerator at -4°C with 1 ml conc. $HNO_3$ per 500 ml in order to avoid precipitation and used to analyze the heavy metals (Table 1) (APHA, 1995).

**Preparation of whole plant extract:** Whole plant extract of *H. verticillata* collected from unpolluted and polluted water dried separately were dried under shade and were ground to obtain the smooth powder. Ethanol extracts of the powered material were prepared separately by soaking 20 gm of the material for 72 hrs and after every 24 hrs the mixture were stirred with a sterile glass rod. After the completion of 72 hrs time period the extracts were filtered with Whatmann filter paper no.1 in order to obtain the filtrate. The filtrate was kept in water bath to obtain the crude extracts (Alade and Irobi, 1993) and is used for wound healing assay.

**Assay of wound healing activity**

Wistar albino rats (180–200 gm) were divided into seven groups of each with six animals (n=6). Group-1 served as carrageenan induced control rat; Group- 2 served as carrageenan induced rats administered with indomethacin considered as standard (10 mg/kg/day p.o.). Group-3 and 4 were administered with whole plant ethanolic extract of *H. verticillata* collected from polluted area samples at 200 (low) and 400 (high) mg/kg/day p.o. doses, respectively. Similarly group 5 and group 6 were administered with whole plant ethanolic extract of *H. verticillata* collected from unpolluted area samples at 200 (low) and 400 (high) mg/kg/day p.o. doses, respectively. Wounds were created at the back of each animal Group of 1 to 6. An area of about 1sq cm is marked out. The marked area is excised with sharp knife and scissors under ether anaesthesia (Sainuddin and Haneefa, 2010). The length and breadth of the wounds were measured for 21 days using a vernier caliper (Table 2).

## Results and Discussion

Evaluation of the wound healing activity of the whole plant ethanol extracts of *H. verticillata* collected from unpolluted and polluted waters was carried out and the data are depicted in Table 2 and Figure 1. The result of wound healing activity showed significant curing of wound in both unpolluted and polluted than control (Indomethacin) and the induced (carrageenan) treatments. The standard drug (Indomethacin) treated animals showed faster epithelialization of wound ($18^{th}$ day)

than the animals treated with ethanol extracts (21st day). There is a significant deviation from ($P<0.05$) between the *H. verticillata* samples collected from unpolluted and polluted water. At high dose the plant samples from unpolluted water, there was a higher rate of wound healing and reduced epithelialization period (15th day) as compared to plant samples from polluted water in which the complete wound healing was observed on 18th day. The time required for complete epithelialization of the wound is an important parameter to assess the wound healing process. In this study, it was noted that the period of complete epithelialization was delayed for three days in polluted plant samples than in unpolluted plant samples. Wound closure was observed on 18th day in Group-2 standard animals treated with carrageenan and in *H. verticillata* (extract-400 mg) from unpolluted water source on 21st day in all treatment groups except group -1 (carrageenan only) treated animals and in *H. verticillata* (extract 200 mg) from polluted water source (Table 2)

The natural phases of wound healing include hemostasis, inflammation, proliferation and remodeling. Each step of wound healing is distinct, overlapping the following one, involving a series of interactions between varieties of cell classes (Clark, 1991). The success of wound healing depends on sufficient nutrients being supplied to the wound site; moreover, the objective of wound healing is to heal the wound as quickly as possible with minimal pain and scarring to the patient (Meyer and Ingold, 1998).

Phytochemical studies on *H. verticillata* revealed that the whole plant sample is rich in flavonoids. Flavonoids have therapeutic uses due to their flavonoids and their derivatives are known to decrease lipid peroxidation by improving vascularity and preventing or slowing down the progress of cell necrosis. Flavonoids are also known to endorse wound healing processes primarily owing to their antimicrobial and astringent properties, which appear to be responsible for wound contraction and elevated rate of epithelialization (Pesin *et al.,* 2011; Tsuchiya *et al.,* 1996). Several reports revealed the anti-inflammatory, antifungal, antioxidant and wound healing properties (Santos and Mello, 2004; Zuanazzi and Montanha, 2004; Nayak *et al.,* 2009; Okuda, 2012) of flavonoids found in plants. Many substances like tissue extracts (Udupa *et al.,* 2005), vitamins and minerals and a number of plant products (Dahanukar *et al.,* 2000) possess pro-healing effects.

A number of secondary metabolites or active compounds isolated from plants have been demonstrated in animal models (*in vivo*) as active principles responsible for facilitating healing of wounds. In the present study, it was noted that the *H. verticillata* possesses tannin, phenols, flavonoids and these compounds might have great contribution in the healing process as reported by many workers (Chaudhari and Mengi, 2006; Mann *et al.,* 2007; Abdullahi *et al.,* 2011).

The free radicals and oxidative reaction product produce tissue damage and play a major role in the aggravation of tissue damage during wound healing (Sonel, 1997). Several antioxidants like tannin, phenols and flavonoids have been reported to quench oxidative damage to the tissue (Taczolowski, 1992; Sieradzki *et al.,* 1998). *H. verticillata* showed significant wound healing activity which could be attributed to these properties.

Thus the present study indicate that the whole plant ethanol extract of *H. verticillata* have potent wound healing property and it

may be either due to their individual or additive effect that fastens the process of wound healing. Among whole plant extract of *H. verticillata* the plant sample from unpolluted water source was found to possess better wound healing property as compared to the plant sample collected from polluted water sample. Moerover there was a faster epithelialization observed in high dose (400mg/kg bw.) than low dose (200mg/kg bw.) and treatment with whole plant extract.

**Table.1** Analysis of physico-chemical properties and heavy metal analysis of water samples collected from unpolluted and polluted water sources of *Hydrilla verticillata*

| **Parameters analysed** | **Water sample from UPWS of *H. verticillata*** | **Water sample from PWS of *H. verticillata*** | **WHO permissible limit** |
|---|---|---|---|
| 1. pH | 7.20 | 8.60 | **6.5-8.5** |
| 2.Dissolved Oxygen | 2.40 | 9.20 | **4.00** |
| 3. BOD | 0.80 | 13.50 | **3.00** |
| 4. COD | 0.12 | 2.30 | **6.00** |
| 5. Total alkalinity | 1.53 | 2.03 | **200.00** |
| 6. Total hardness | 62.00 | 66.00 | **300.00** |
| 7. Phosphate | 0.24 | 0.10 | **500** |
| 8. Ammonia | 1.21 | 3.10 | **5.00** |
| 9. Calcium | 5.61 | 16.83 | **75.00** |
| 10. Nitrate | 1.72 | 4.30 | **45.00** |
| 11. Potassium | 0.62 | 0.21 | **30.00** |
| 12. Chloride | 23.83 | 26.10 | **250.00** |
| 13. Sulphate | 22.96 | 15.10 | **200.00** |
| 14. Magnesium | 0.89 | 1.26 | **30.00** |
| 15. Nitrite | 0.62 | 1.09 | **40.00** |
| **Heavy metals analysed** | | | |
| 1. Arsenic (As) | 84.20 | 419.40 | **100.00** |
| 2. Cadmium (Cd) | 0.01 | 8.11 | **0.20** |
| 3. Chromium (Cr) | 21.40 | 72.10 | **100.00** |
| 4. Copper (Cu) | 1.51 | 52.10 | **5.00** |
| 5. Ferrous (Fe) | 99.21 | 812.10 | **200.00** |
| 6. Lead (Pb) | 13.00 | 204.21 | **25.00** |
| 7. Manganese (Mn) | 3.00 | 26.20 | **5.00** |
| 8. Nickel (Ni) | 12.00 | 81.40 | **25.00** |
| 9. Silver(Ag) | NP | 9.21 | **0.20** |
| 10. Zinc | 9.20 | 169.20 | **30.00** |

UPWS-Unpolluted Water Source; PWS-Polluted Water Source; UPSS-Unpolluted Soil Source; PSS-Polluted Soil Source; NP- Not Present; All the parameters are expressed in mg/l except pH

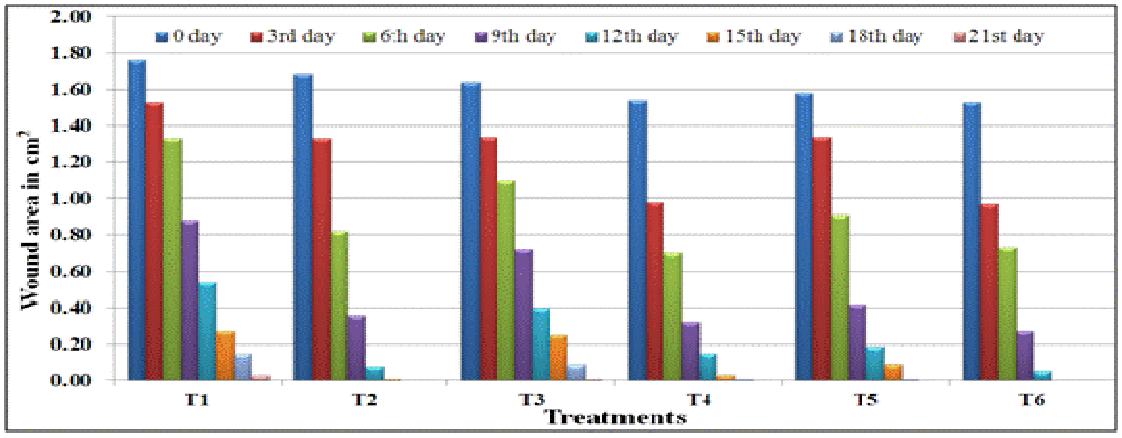
**Table.2** Effects of ethanol extract of *Hydrilla verticillata*, whole plant samples collected from unpolluted and polluted water sources, on wound healing activity in rats

| Duration of wound healing | | Induced – (Carrageenan) control-1 | Standard – (Indomethacin) conrol-II | Wound healing activity (wound closure rate) of ethanol extracts (in mm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | *H. verticillata* whole plant sample from UPWS | | *H. verticillata* whole plant sample from PWS | |
| | | | | Low dose (200mg/kg bw) | High dose (400mg/kg bw) | Low dose (200mg/kg bw) | High dose (400mg/kg bw) |
| **0-Day (Initial)** | **Length** | 13.52 ±0.43[a] | 13.55 ±0.29[a] | 12.60 ±0.17[c] | 12.37 ±0.22[e] | 12.76 ±0.14[b] | 12.45 ±0.26[d] |
| | **Breadth** | 13.05 ±0.61[a] | 12.43 ±0.41[d] | 12.55 ±0.24[c] | 12.37 ±0.40[d] | 12.85 ±0.18[b] | 12.38 ±0.32[d] |
| **3rd Day** | **Length** | 12.71 ±0.43[a] | 11.74 ±0.98[b] | 11.52 ±0.29[d] | 10.24 ±0.52[f] | 11.62 ±0.24[c] | 10.34 ±0.46[e] |
| | **Breadth** | 12.00 ±0.37[a] | 11.35 ±0.31[d] | 11.64 ±0.30[b] | 9.46 ±0.24[f] | 11.54 ±0.19[c] | 9.50 ±0.54[e] |
| **6th Day** | **Length** | 11.60 ±0.27[a] | 9.01 ±0.89[d] | 9.59 ±0.37[c] | 8.51 ±0.26[e] | 10.59 ±0.27[b] | 8.42 ±0.31[f] |
| | **Breadth** | 11.48 ±0.14[a] | 9.13 ±0.71[d] | 9.51 ±0.11[c] | 8.56 ±0.28[e] | 10.41 ±0.25[b] | 8.27 ±0.22[f] |
| **9th day** | **Length** | 9.42 ±0.38[a] | 6.17 ±0.49[d] | 6.68 ±0.16[c] | 5.27 ±0.40[f] | 8.68 ±0.30[b] | 5.97 ±0.42[e] |
| | **Breadth** | 9.35 ±017[a] | 5.76 ±0.94[d] | 6.24 ±0.27[c] | 5.21 ±0.66[f] | 8.24 ±0.22[b] | 5.31 ±0.56[e] |
| **12th day** | **Length** | 7.41 ±0.32[a] | 2.45 ±0.15[e] | 4.26 ±0.06[c] | 2.18 ±0.18[f] | 6.26 ±0.10[b] | 4.01 ±0.42[d] |
| | **Breadth** | 7.24 ±0.24[a] | 3.43 ±0.20[e] | 4.36 ±0.11[c] | 2.38 ±0.34[f] | 6.36 ±0.14[b] | 3.72 ±0.26[d] |
| **15th Day** | **Length** | 5.26 ±0.18[a] | 0.95 ±0.11[e] | 2.97 ±0.12[c] | 0.64 ±0.11[f] | 4.97. ±0.18[b] | 1.84 ±0.19[d] |
| | **Breadth** | 5.15 ±0.09[a] | 0.95 ±0.10[c] | 2.95 ±0.16[c] | 0.65 ±0.11[f] | 4.95 ±0.16[b] | 1.89 ±0.20[d] |
| **18th day** | **Length** | 3.98 ±0.17[a] | 0.00 0.00[d] | 0.96 ±0.25[c] | 0.00 0.00[d] | 2.94 ±0.15[b] | 0.92 ±0.12[c] |
| | **Breadth** | 3.86 ±0.21[a] | 0.00 0.00[d] | 0.96 ±0.36[c] | 0.00 0.00[d] | 2.96 ±0.24[b] | 0.94 ±0.15[c] |
| **21st Day** | **Length** | 1.64 ±0.21[a] | 0.00 0.00[c] | 0.00 0.00[c] | 0.00 0.00[c] | 1.01 ±0.08[b] | 0.00 0.00[c] |
| | **Breadth** | 1.72 ±0.17[a] | 0.00 0.00[c] | 0.00 0.00[c] | 0.00 0.00[c] | 0.99 ±0.12[b] | 0.00 0.00[c] |

| Three-way ANOVA | CD 5% | CD 1% | Sign. |
|---|---|---|---|
| Between period of observation –Po | 0.098 | 0.127 | * |
| Between Wound size –Ws | 0.049 | 0.063 | * |
| Between Treatments –Tr | 0.075 | 0.105 | * |
| Po x Ws | 0.129 | 0.177 | * |
| Po x Tr | 0.220 | 0.297 | * |
| Ws x Tr | 0.115 | 0.148 | * |
| Po x Ws x Tr | 0.312 | 0.412 | * |

Low dose -200 mg/kg body weight; High dose – 400 mg/kg body weight; UPWS-Unpolluted Water Source; PWS-Polluted Water Source; Alphabets indicate the critical difference (CD) comparison at 5% level between treatments.

**Figure.1** Effects of whole plant ethanol extract of *Hydrilla verticillata*, collected from unpolluted and polluted water sources, on wound healing activity in rats

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