Journal of Tropical Resources and Sustainable Science

journal homepage: www.jtrss.com.my

Wound Healing Properties of Ethanolic Extract of *Acrostichum aureum* and *Acrostichum speciosum* Rhizome in Rats

Hendy Putra Herman[1], Deny Susanti[2*], Shahbudin Saad[3], Muhammad Taher[4], Norazsida Ramli[5]

[1]Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia.
[2]Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia.
[3]Institute of Oceanography and Maritime Studies, Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia.
[4]Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia.
[5]Department of Biomedical Science, Kulliyyah of Allied Health Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia

Keywords:
Wound healing
Acrostichum aureum
Acrostichum speciosum
Excisional wound
Rats

⊠ *Corresponding Author:
Deny Susanti
[2]Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia.
Email address:
deny@iium.edu.my

Abstract

This paper presents the assessment of wound healing properties of ethanolic extract of *Acrostichum aureum* and *A. speciosum* in rats. It was carried out on 6 treated groups with 6 rats each group. The excisional wound was made on the dorsal interscapular region of each rat by a 6 mm biopsy punch. T1 and T2 were treated with 5% and 10% *A. aureum* ethanolic extract in aqua cream, respectively. Meanwhile, T3 and T4 were treated with 5% and 10% *A. speciosum* ethanolic extract in aqua cream, respectively. Solcoseryl jelly and aqua cream were used as positive and negative controls. The treatments were applied topically twice daily, wound contraction and period of epithelization were measured every 2 days. The results showed that wound treated with 10% *A. speciosum* (T4) exerted faster wound contraction significantly ($p < 0.05$) compared to the negative control. It was also enhanced epithelization period (9.33 ± 3.20 days) of the wounds significantly ($p < 0.05$). In conclusion, these results strongly suggested the beneficial effects of both plant extracts for enhancing wound healing process.

1. Introduction

Normal wound healing response begins the moment tissue is injured. An injury is defined as an interruption in the continuity of tissues. Thus, the objective of repairing the injury is mainly to reestablish the continuity by proliferation, migration, and differentiation of involved cells (Goss, 1992). The first body response to injury is inflammation which allows control of blood loss and fends off bacterial invasion. It signals the cells necessary for repair and regeneration to come to the site of injury. The second body response is proliferation. In this phase, new tissues are built to fill the gap left by damaged and debrided tissues. There are four crucial events occur; angiogenesis (formation of new blood vessels), granulation tissue formation (proliferation of fibroblasts and scar tissue formation), wound contraction (generation of forces within the wound pull surrounding tissue over the wound that lead to its size reduction), and epithelization (migration and

multiplication of keratinocytes across the wound bed) (Myers, 2004). Lastly, in maturation and remodeling phase, there will be reorganization of scar tissue to reach maximum strength and function. This remodeling phase also involves both the resorption and synthesis of components to form the healed skin.

The use of medicinal plants in treating the wounds is common in many parts of the world including Malaysia. Some of Malaysian medicinal plants have been extensively studied for their wound healing properties such as *Plantago major* (Mahmood and Phipps, 2006), *Rafflesia hasseltii* (Mahmood *et al*., 2009), *Ficus deltoidea* (Mahmood *et al*., 2010a), and *Boesenbergia rotunda* (Mahmood *et al*., 2010b).

Acrostichum spp. is a large ferns under Pteridaceae family which can grow up to 4 m tall with not more than 30 leaflets. *A. aureum* has a blunt leaflet while *A. speciosum* has an elongate-pointed leaflet (Giesen *et al*., 2006). These ferns are found in upper part of the foreshore up to the spring tide and water mark in Malaysia, Indonesia, Thailand, Singapore, and India (Kathiresan and Rajendran, 2005).

Several studies have reported the traditional use of *A. aureum*'s rhizome for wound healing among Malays in Malaysia (Bandaranayake, 1999; Hong and San, 1993; Mannan *et al*., 2008; Hossan *et al*., 2010). This similar practice is also can be found in Vietnam in which the rhizomes also used for wound healing (The Vietnam MAB National Committee, 2000). Furthermore, the use of *A. aureum* for wounds, boils, and rheumatism is also popular in Bangladesh (Pattanaik *et al*., 2008), India (Benjamin and Manickam, 2007), and Yap islands, Micronesia (Defilipps *et al*., 1988).

The research on the finding of the new alternative drugs for wound healing is really promising due to the abundance of medicinal plants used by many communities in Malaysia. Therefore, in this paper, the traditional use of this fern by Malaysian communities for wound healing was supported using rats as *in vivo* model.

2. Materials and Methods

Plant materials

The rhizome samples of both *A. aureum* and *A. speciosum* were collected from Matang mangroves, Perak on February 2011. The plants were identified by Assoc. Prof. Dr. Shahbudin Saad from Institute of Oceanography and Maritime Studies, Kulliyyah of Science with herbarium specimens were deposited in Herbarium Kulliyyah of Pharmacy with voucher specimen of MT1011-9 (*A. aureum*) and MT1011-10 (*A. speciosum*).

Preparation of the extracts

The rhizomes of the plants were dried at warm room at 30 °C for 7 days. Then, it was ground to a fine powder in a mechanical grinder and approximately 200 g was weighed accurately on a digital balance. Each of the species was extracted using Soxhlet with ethanol (90%) and evaporated using rotary evaporator yielded the crude ethanol (EtOH) extracts of *A. aureum* (22.5 g; 11.3%) and *A. speciosum* (17.6 g; 8.8%) in which all of them were dark brown sticky liquids.

Animal models

Thirty six male, Sprague-dawley rats (110 to 240 g) were purchased from animal lab, Kulliyyah of Science, International Islamic University Malaysia, Kuantan, Pahang. The animals were left for 3 days at room conditions for acclimatization. They were maintained on standard pellet diet and water *ad libitum* throughout the experiment. A minimum of six animals were used in each group. Throughout the experiments, animals were processed as suggested by international ethical guidelines for the care of laboratory animals and this study was approved by IIUM Research Ethics Committee (IREC) Meeting No 3/2011 on 5th December 2011.

Wound preparation

This model was used to monitor wound contraction and period of epithelization. Each group of animals (six animals in each) was anaesthetized by chloroform in open mask method. The dorsal hairs of the rats were shaved. Then, the circular wound was created on the dorsal interscapular region of each animal by excising the skin with a 6 mm biopsy punch; wounds were left open. All wounds were treated with the extracts and controls topically twice a day till the wound was completely healed. The wounding day is considered as day 0. The progressive changes in wound area were monitored by tracing with transparent graph paper having a millimeter scale that was measured by a caliber with an accuracy of 1/20 mm. Apart from that, the wound area were

monitored and photographically documented by a camera on day 0, 1, 3, 5, 7, 9, 11, and 13.

Topical wound application

Aqua cream produced by UPHA Pharmaceutical Manufacturing (M) Sdn. Bhd, contains emulsifying wax 8 % w/w, white soft paraffin 12 % w/w and liquid paraffin 8 % w/w and used as a negative control. Each extract was homogenized with 1 g of aqua cream according to the dose chosen. Wounds in group T1 was treated topically with 5 % *A. aureum* , T2 with 10 % *A. aureum*, T3 with 5% *A. speciosum* and T4 with 10 % *A. speciosum.* Meanwhile, Solcoseryl jelly produced by Legacy Pharmaceuticals Switzerland GmBH, contains 4.15 mg haemodialysate from calves' blood, 1.73 mg methyl parahydroxybenzoate and 0.27 mg propyl parahydroxybenzoate and used as a positive control.

The wound was observed daily until complete wound healing occured. The wound contraction was evaluated as follow:

$$\text{Wound contraction (\%)} = \frac{(WDo-WDt)}{WDo} \times 100$$

Where:
WDo = The wound diameter on day zero
WDt = The wound diameter on day t

Statistical analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS) version 16. Data are expressed as the mean ± S.E.M. Significant differences between the treated groups and the control was determined by the One way ANOVA test, at a level of p-value < 0.05 is considered statistically significant.

3. Results and Discussions

Wound contraction was calculated as percentage of the reduction in wounded area. It was measured every two days until they were healed completely on day 13. Based on the results (Table 1), there were significant reductions in wound contraction percentage on day 7 especially for dose 10% *A. speciosum* (T4) compared to the negative and positive controls. On day 9, the extract further improved the wound contraction (77.83%) compared to the negative control (57.00%). Then, dose of 5% *A. aureum* (T1) and 5% *A. speciosum* (T3) also showed significant effect on wound contraction (81.67%) compared to the negative control (63.83%) on day 11. Thus, it could be summarized that topical application of both extracts has shown significant effect in wound healing process. Table 2 shows the wound healing process in different treatment groups on day 5, 9, and 13.

For epithelization period, it was measured as number of days required for the falling of the eschars (dead-tissue remnants) without any residual raw wound. From our results (Table 3), the group that shown significant effect on epithelization period was T4 (9 days) that treated with 10% *A. speciosum* compared to the negative control (14 days). This was very important as faster epithelization period assisted by the treatment of the plant extract showed that the plant possessed the ability to be alternative medicine for the wound healing treatment.

The aim of this work was to evaluate wound healing properties of *A. aureum* and *A. speciosum* rhizome extracts because there is lack of published report on medicinal use of *Acrostichum spp.* in wound healing. Based on our results, wound contraction was statistically significant for group of T1 (5% *A. aureum*), T3 (5% *A. speciosum*), and T4 (10% *A. speciosum*) compared to the negative control on the day 11. Epithelization period was reduced in all treatment groups compared to the negative control (14.00 ±1.10 days). The fastest epithelization period among four treatment groups are T4 (9.33 ± 3.20 days) and T2 (11.66 ± 3.72 days) that treated with 10% *A. speciosum* and 10% *A. aureum*, respectively. It means that higher dose is needed to enhance wound healing process. Thus, it was essential to test the extract using low and high dose in this study to compare the effectiveness for wound healing treatment.

Table 1: Effects of the ethanolic extracs of *A. aureum* and *A. speciosum* on circular excisional model.

Day	Percentage wound contraction ±S.E.M. (%)					
	Negative control	T1	T2	T3	T4	Positive control
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	1.50±0.20	7.00±0.38	5.50±0.52	5.50±0.41	11.17±0.52	16.67±0.00*
5	13.83±0.82	20.83±0.61	26.33±1.24	26.33±0.97	43.00±1.36	36.17±1.72
7	33.33±0.95	61.17±0.41*	61.17±1.21*	61.17±0.52*	66.67±1.26*	63.83±0.98*
9	57.00±0.66	70.83±0.42	69.50±0.75	69.50±0.26	77.83±1.03*	86.17±0.75*
11	63.83±0.52	81.67±0.41*	77.83±0.75	81.67±0.26*	84.67±0.4*	91.67±0.45*
13	77.83±0.61	84.67±0.38	82.00±0.86	83.33±0.55	87.50±0.61	98.67±0.20*

S.E.M.: standard error means. Extracts and the reference material were compared to vehicle group.
* $p < 0.05$; Negative control: aqua cream; Positive control: solcoseryl jelly
T1: 5% *A. aureum*; T2: 10% *A. aureum*, T3: 5% *A. speciosum*; T4: 10% *A. speciosum*

Table 2: Wound healing process in different treatment groups on day 5, 9, and 13

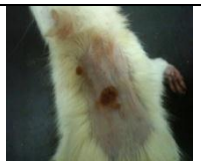
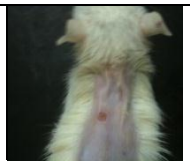
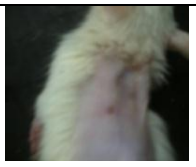
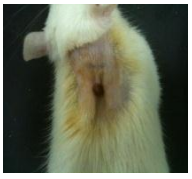
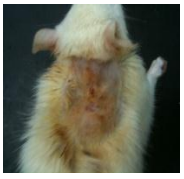
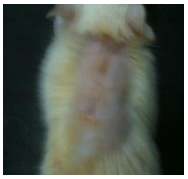
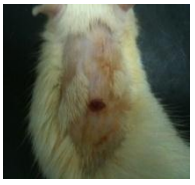
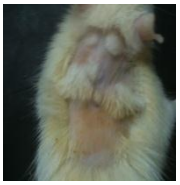
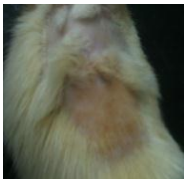
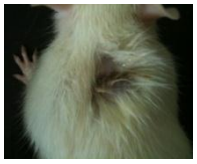
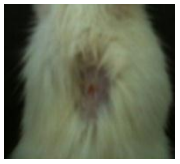
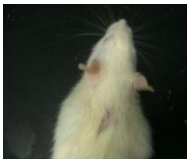
Treatment	Day 5	Day 9	Day 13
Aqua cream			
10% *A. aureum*			
10% *A. speciosum*			
Solcoseryl Jelly			

Table 3: Epithelization period of the ethanolic extracs of *A. aureum* and *A. speciosum* on circular excisional model.

Epithelization period ± S. E. M. (days)					
Negative control	T1	T2	T3	T4	Positive control
14.00 ± 1.10	12. 33± 1.63	11.66 ± 3.72	12.33 ± 2.07	9.33 ± 3.20*	9.00 ± 1.26*

S.E.M.: standard error means. Extracts and the reference material were compared to vehicle group.
* $p < 0.05$; Negative control: aqua cream; Positive control: solcoseryl jelly
T1: 5% *A. aureum*; T2: 10% *A. aureum*, T3: 5% *A. speciosum*; T4: 10% *A. speciosum*

Furthermore, there was a reduced visible scar area observed in both groups. Therefore, the results and the observation in this study indicated that ethanolic extract of *A. speciosum* and *A. aureum* rhizome may significantly stimulate wound contraction as well as epithelization.

The effectiveness of the plant extract to enhance wound healing process might be associated antibacterial activity of this plant that reported by Lai *et al*. (2009). And the mechanism of antiseptic in the wound treatment is suggested by minimizing bacterial infection that can faster wound healing process. Thus, it could be concluded that ethanolic extract of *A. aureum* and *A. speciosum* could enhance wound healing process by inhibiting bacterial growth on the wound environment (Thomas, 2012*)*.

Ethanol was chosen as the solvent for extraction as it can extract a wide variety of compounds in medicinal plants (Purohit and Vyas, 2004) and alcoholic solvents efficiently penetrate the cell membranes, permitting the extraction of high amounts of endocellular components including phytochemicals produced in the plants (Silva *et al*., 1998). Phytochemicals present in both *A. speciosum* and *A. aureum* extracts might also responsible for wound healing properties.

In a phytochemical screening conducted by Hemayet (2012) on *A. aureum*'s rhizome, it showed that ethanolic extract of this plant contains tannins, flavonoids, saponins, and reducing sugars. Furthermore, some bioactive compounds have been isolated from *A. aureum*'s stems, leaves, and rhizomes such as amino acids, condensed and hydrolysable tannins, diterpenes, flavonoids, hydrocarbons, sesquiterpene, steroids, sugars, triterpenes. Plants contain tannins, saponin, flavonoids and alkaloids shown the antimicrobial activity (Bandaranyake, 2002). Thus, tannins, saponins and flavonoids in these plants might responsible in the enhancing wound healing process. Flavonoids can reduce lipid peroxidation by improving vascularity and slowing the onset of cell necrosis (Getie *et al*., 2002). Thus, flavonoids in *A. speciosum* and *A. aureum* could enhance the wound healing process by increasing the strength of collagen fibers, circulation, preventing the cell damage, and promoting the DNA synthesis (Getie *et al*., 2002). In addition, tannins also play an essential role in wound healing process, where tannins will precipitate the proteinaceous matter and act as astringents drawing tissues and contracting them. These properties are used in treating inflamed mucous membranes characteristics of coughs, colds, alleviating intestinal infections and bathing wounds. As a topical application, tannins can be used to stop bleeding, reduce inflammation, and heal the wounds (Ong, 2004).

Although rats are not ideal for studying the efficacy of therapy on wound healing in humans due to the differences in the skin structure, they still can be used as a model for wound healing because laboratory rats are inbred. Thus, there is a little variation in their wound healing due to genetic differences. Apart from that, rats will most likely continue to be the most often used model because of their low cost, ease of handling, and nondemanding care (Mast, 1992). Lastly, there were numerous rats and mice studies of wound healing using Malaysian medicinal plants that proven effective and reliable (Mahmood and Phipps, 2006; Mahmood *et al.*, 2009; Mahmood *et al.*, 2010a; Mahmood *et al*., 2010b). For a long period of time, animal studies will continue to serve a major part in the discovery of new novel drugs from plants for wound healing treatment because of the complexity of wound healing mechanism itself (Hau, 2008).

4. Conclusions

In conclusion, this study has shown that ethanolic extract of *A. speciosum* and *A. aureum* rhizome possessed wound healing properties. Wounds treated with 10% *A. speciosum* (T4) exerted faster wound contraction significantly (p <0.05) compared to the negative control. It could accelerate wound contraction and epithelization period (9.33 ± 3.20 days) significantly (p <0.05) compared to the negative and positive controls. Therefore, further studies with purified constituents are needed to understand the complete mechanism of wound healing activities in order to develop a novel plant-based ointment for wound healing treatment.

5. Acknowledgements

The authors would like to thank the Ministry of Higher Education Malaysia for the financial funding through FRGS11-003-0151 grant and Jabatan Hutan Taiping for the permission of sample collection.

6. References

Bandaranayake, W. M., (1999). Economic, traditional and medicinal uses of mangroves.AIMS, 24-26.

Bandaranayake, W. M., (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management, 421–452.

Benjamin, A. & Manickam, V. S., (2007). Medicinal pteridophytes from the Western Ghats. Indian J. Traditional Knowledge, 6 (4), 611-618.

Defilipps, R. A., Maina, S. L., & Pray, L. A., (1988). The Palauan and Yap medicinal plant studies of Masayoshi Okabe 1941-1943. Atoll Research Bulletin, 317, 1-25.

Getie, M., Gebre, M. T., Reitz, R., & Neubert, R H., (2002). Evaluation of the release profile of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Spindaceae). Pharmazie, 57, 320-22.

Giesen, W., Wulffraat, S., Zieren, M., & Scholten, L., (2006). Mangrove guidebook in Southeast Asia. FAO & Wetlands International, 264-267.

Goss, R. J., (1992). Regeneration versus repairs, in: Cohen, K.,Diegelmann, R. F. & Lindblad, W. J. Wound healing: biochemical & clinical aspects. W. B. Saunders Company, Philadelphia.

Hau, J., (2008). Animals model for human disease, in: P.Michael Conn (Ed.), Sourcebook of models for Biomedical research. Humana Press, New Jersey.

Hemayet, H., Ismet, A. J., Ishrat, N., Md. Musfizur, H., Sariful Islam, H., & Sk. Moniruzzaman., (2012). Antidiarrhoeal activity and total tannin content from the root of *Acrostichum aureum* (Linn.). Int. J. Pharmascholars, 1 (1), 81-85.

Hong, P. H. & San, H. T., (1993) Mangroves of Vietnam. IUCN, 13-16.

Hossan, M. S., Hanif, A., Agarwala, B. Sarwar, M. S., Karim, M., Rahman, M. T., & Rahmatullah, M., (2010). Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. Ethnobotany Research & Pub., 8, 61-74.

Kathiresan, K. & Rajendran, N., (2005). Mangrove ecosystems of the Indian Ocean region. Indian J. Mar. Sci., 34 (31), 104-113.

Lai, H. Y., Lim. Y. Y., & Tan, S. P., (2009). Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Biosci Biotechnol Biochem., 73 (6), 1362-1366.

Mahmood, A. A., & Phipps, M. E., (2006). Wound Healing activities of *Plantago major* leaf extracts in rats. Intl. J. Tropical Med., 1 (1): 33-35.

Mahmood, A. A., Khaled, A. A., Hapipah, M. A., Suzita, M.N., & Salmah, I., (2009). Wound healing activities of *Rafflesia hasseltii* extract in rats. J. Clin. Biochem. Nutr., 45, 304- 308.

Mahmood, A. A., Khaled, A. A., Faisal, M. A., & Mazin, M., (2010a). Role of *Ficus deltoidea* extract in the enhancement of wound healing in experimental rats. Biomedical Research 21 (3), 241-245.

Mahmood, A. A., Abdalbasit, A. M., Siddig, I. A., Salmah, I., & Fouad, A. B., (2010b). Potential activity of ethanolic extract of *Boesenbergia rotunda* (L.) rhizomes extract in accelerating wound in rats. J. Med. Plant. Res., 4 (15), 1570-1576.

Mannan, M. M., Maridass, M., & Victor, B. (2008). A review on the potential uses of ferns. Ethnobotanical leaflets, 12, 281-285.

Mast, B. A., (1992) The skin, in: Cohen, I. K., Diegelmann, R. F., Lindblad, W. J., Wound healing: biochemical & clinical aspects. W. B. Saunders Co, Philadephia.

Myers, B. A., (2004). Wound management: principles and practice. Pearson Education Inc., 201-205.

Ong, H. C., (2004). Tumbuhan liar: khasiat ubatan & kegunaan lain. Utusan Publications & Distributors Sdn. Bhd., Kuala Lumpur:

Pattanaik, C., Reddy, C. S., Dhal, N. K., & Das, R., (2008). Utilisation of mangrove forests in Bhitarkanika wildlife sanctuary. Indian J. Tropical Knowledge, 7 (4), 598-603.

Purohit, S. S., & Vyas, S. P., (2004). Medicinal plant cultivation: A scientific approach. Agrobios, 51-60.

Silva, G. L., Lee, I. S. & Kinghorn, A. D., (1998). Special problems with the extraction of plants, in: Cannell, R. J. P. Methods in Biotechology. Humana Press Inc, Totowa.

The Vietnam MAB National Committee, (2000). Evaluation of the Mangrove Ecosystem in Can Gio Mangrove Biosphere Reserve, Vietnam. Hanoi.

Thomas, T., (2012). *In vitro* evaluation of antibacterial activity of *Acrostichum aureum* Linn.Indian J. Nat. Prod. Resour., 3(1), 135-138.