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Santalaxme Vijendren

shrewdyclawz_santa@yahoo.com, shrewdyclawz_santa@yahoo.com

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Assessment of *In-vitro* Wound Healing Activity of the *Tinospora crispa* Extracts

Santalaxme Vijendren, Shankar Jothi, Kushha Rajandran, Vignesh Muruganandham

Masterskill University College of Health Sciences, Selangor, Malaysia

E-mail: shrewdyclawz_santa@yahoo.com

Abstract - Wound healing is a complex and dynamic process which follows the normal physiology trajectory through the phases of homeostasis, inflammation, granulation and maturation. Malaysia has a rich collection of plant based healing. A large number of plants are used for the treatment of cuts and wounds by folklore traditions in Malaysia. *Tinospora crispa* Miers. (Menispermaceae) is popular in Asian countries for its miracle of curing diseases. *T. crispa* differs slightly from *T. cordifolia* which is well distributed in India. Considerable researches have reported the activity of this plant possessing anti-malarial, diabetes treatment etc. In Malaysia, it is used traditionally for wound healing. Hence, the present study was aimed to evaluate its scientific validity. Stems of the plant were air dried after reduced into smaller size. Dried stems were then crushed into coarse powder. Then it was introduced into methanol for extraction by cold maceration technique. The extract was filtered after 7 days and fractionated with addition of chloroform. The methanol and chloroform fractions were made to evaporate until concentrated and formulated into ointments. Albino rats were separated into four groups of six rats in each group. All four groups were divided and served as a control, methanol fraction, chloroform fraction and standard drug (Betadine) respectively. The methanol extract and chloroform extract were investigated for the evaluation of its healing efficiency on excision wound model in Albino rats. Wound closure in percentage was used to evaluate the effect on wound healing. The methanol fraction and chloroform fraction showed a significant wound healing activity which was well comparable with the standard drug used. Methanol fraction ointment showed greater activity than chloroform fraction. This study indicated that the methanol fraction ointment possesses wound healing property which substantiates the folklore claim.

Keywords: *Tinospora crispa*, wound healing, cold maceration, methanol and chloroform extract, Albino rats

I. INTRODUCTION

Wound is the interruption of the continuity in tissue resulting from the opening or break of the skin. The healing of wound is essential in order for the restoration of the tissue continuity and disturbed skin status [1]. Wound healing is a complex and dynamic process with the parallel changes of wound environment with the individual's health status. The knowledge of the physiology of the cutaneous wound healing is trajectory through the phases of inflammation, formation of granulation tissue, and extracellular matrix remodeling [2]. Cutaneous wounds heal by primary union or secondary union whereby secondary healing involves more extensive scarring and wound contraction. Appropriate treatment and care for the wound will not only promote healing but save the hospitalization cost and the patient from other complications such as amputation. Many different methods of treatment are being introduced currently for wound healing yet the outcome is still far from optimal. This condition indicates the need for our field to introduce efficient and

safe means of wound healing agent. In recent days many traditional wound care agents are being practiced yet they lack scientific evaluation to substantiate the folklore claims. *Tinospora crispa* is one very valuable example of such agents which has not received any establishment as a wound healing agent with valid scientific data. *T. crispa* is well known for its medicinal properties which cured many disorders and diseases. It is well distributed in Asian countries such as Malaysia, Thailand, Indonesia and the Philippines and differs slightly from *T. cordifolia* which is well distributed in India. The leaves, roots, stems, all have their own miracle in curing diseases. Anti-malarial [3] study was done using this plant in the chloroform extract having better activity when compared to methanol extract. This plant was also found to possess potent cardiovascular activity [5], antioxidant activity and anti-inflammatory [6] activity. Traditionally in Thailand, the decoction of stems, leaves, and roots of *T. crispa* were used to treat fever, diabetes [4], cholera, rheumatism and snake bites. The people of the Philippines and Malaysia take this plant as a universal medicine to treat many digestion

related problems. Chinese medicine techniques were incorporated by the Vietnamese to treat diseases as claimed earlier. After realizing how much good *T. crispa* has done to our community, it was decided to scientifically prove the folklore claim of wound healing activity.

II. MATERIAL AND METHODS

Plant material and chemicals

The sample was collected from Pahang and Kelantan, states of Malaysia in the month of March. The plant was selected as *Tinospora crispa* on basis of morphological characteristics and authenticated by Dr. Anbu Jeba Sunilson, pharmacognosist from Masterskill University College of Health Sciences. A voucher specimen has been deposited in the herbarium, School of Pharmacy, MUCH (MUCHH/G-8/012/2009) for further reference. Methanol, Chloroform, Wool Fat, Hard Paraffin, Cetostearyl Alcohol and White Soft Paraffin were purchased from Sigma Aldrich Chemical (Malaysia), Povidone-Iodine (Betadine). All other reagents used in the study were of high purity analytical grade.

Preparation of Extract

The stems of *T. crispa* were air dried in the shade with the precaution of contamination from dust. This method needed to make sure that the stems were dried well, so that there will be no moisture content which may interfere the extraction process. The dried stems were then crushed with the help of mortar and pestle until reduced into coarse powder form. The coarsely crushed stems were extracted with methanol by cold maceration technique for 7 days. 60 g of coarse powder was transferred into a volumetric flask, 500 ml of methanol added and sealed with parafilm. After 7 days of maceration, the extracts were filtered. The further process of fractionation of extract was carried out with 60 ml of the methanol extract poured into a separating funnel followed by 60 ml of chloroform. A little amount of distilled water was added into the separating funnel containing two other solvents to increase the polarity of the two solvents since both are miscible with each other. Chloroform fraction settled at the bottom of the separating funnel, whereas, methanol and distilled water fraction at the top of chloroform fraction. Then, the usual procedure to operate the separating funnel was followed to separate the two fractions into separate beakers. The end products from the fractionation process, chloroform fraction and methanol and distilled water fraction in separate beakers were concentrated using a water bath.

III. RESULTS

Preparation of Ointment

Simple ointment was made as per calculation for both the fractions together. The ingredients used were wool fat, hard paraffin, cetostearyl alcohol and white soft paraffin. All the ingredients were melted using a china dish over the water bath then stirred until cooled down which resulted in a homogenous product. Then, 95% of simple ointment was mixed to 5% of extract to formulate ointment from both fractions separately. The formulation was stored in well tight container and kept in cool and dry place.

Experimental Animal

Albino rats of either sex (weighing 150 to 200 g) were selected for wound healing activity. Mice were divided into four groups, each group having six animals. Group I was assigned as control. Group II received the standard drug (Betadine). Group III and IV received ointment of methanol fraction and chloroform fraction of stems of *T. crispa* respectively. The mice were fed on standard pellet diet and water ad libitum.

In vitro Wound Healing Activity

The wound site was prepared following the excision wound model [11]. The mice were lightly anaesthetized with ether. The surgical interventions were carried out under sterile conditions. A wound was made on shaved skin of dorsal thoracic region of each mouse with maximum 200 mm of wound size. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced. The extract ointments were applied once daily to treat (group III and group IV) animals. Betadine was applied in the same way to serve as standard (group II). Group I animals were left untreated to serve the control group. The measurement of wound area was done by using vernier calipers on 3rd, 5th, 7th, 9th, 11th, 15th and 17th post wounding day till the wound was completely healed. The percentage of wound contraction (wound healing) area was calculated by using the formula:

Percentage of Wound contraction =

$$\frac{\text{Initial wound size} - \text{specific day wound size} \times 100}{\text{Initial wound size}}$$

In the excision wound model, the extract formulation treated animals showed high percentage closure of wound area which is similar to the values of standard drug treated group. The rate of wound contraction was less in control group of animals. This indicated the effect of plant on promoting healing of excision wound. The results of excision wound studies were shown in table 1 and figure 3.


Fig 1: 1st day of excision (Group III)

Fig 2: 15th day of excision (Group III)

Treatment group	Wound closure (mm ²)								
	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
Control	200± 10.10 (0%)	196.2± 8.1 (1.9%)	192.3± 5.3 (3.6%)	180± 5.1 (10%)	131± 3.2 (34.5%)	116± 4.6 (42%)	102± 3.7 (49%)	98± 4.1 (51%)	72± 2.9 (64%)
<i>T.crispa</i> chloroform fraction 100mg/kg	160.8± 10.6 (0%)	158.2± 4.6 (1.6%)	142.3± 6.4 (11.5%)	96.2± 3.5 (40.2%)	75.3± 2.3 (53.2%)	33.4± 3.5 (79.2%)	21.4± 2.4 (86.7%)	13.3± 1.2 (92%)	5.7± 0.6 (96.4%)
<i>T.crispa</i> methanol fraction 100mg/kg	158.7± 8.6 (0%)	134.1± 5.3 (15.5%)	112.3± 4.2 (29.2%)	61.2± 2.4 (61.4%)	31.4± 4.6 (80.2%)	15.7± 1.4 (90.1%)	8.3± 1.1 (95%)	1.7± 0.3 (99%)	- (100%)
Standard drug (Betadine)	134.6± 4.2 (0%)	130.2± 3.6 (3.3%)	125.3± 4.8 (6.9%)	82.3± 5.6 (39%)	67.1± 3.3 (50.2%)	27.3± 2.6 (80%)	16.2± 2.2 (88%)	8.7± 1.3 (94%)	2.7± 0.6 (98%)

Table 1: Wound closure (mm²) with percentage in parenthesis according to treatment group

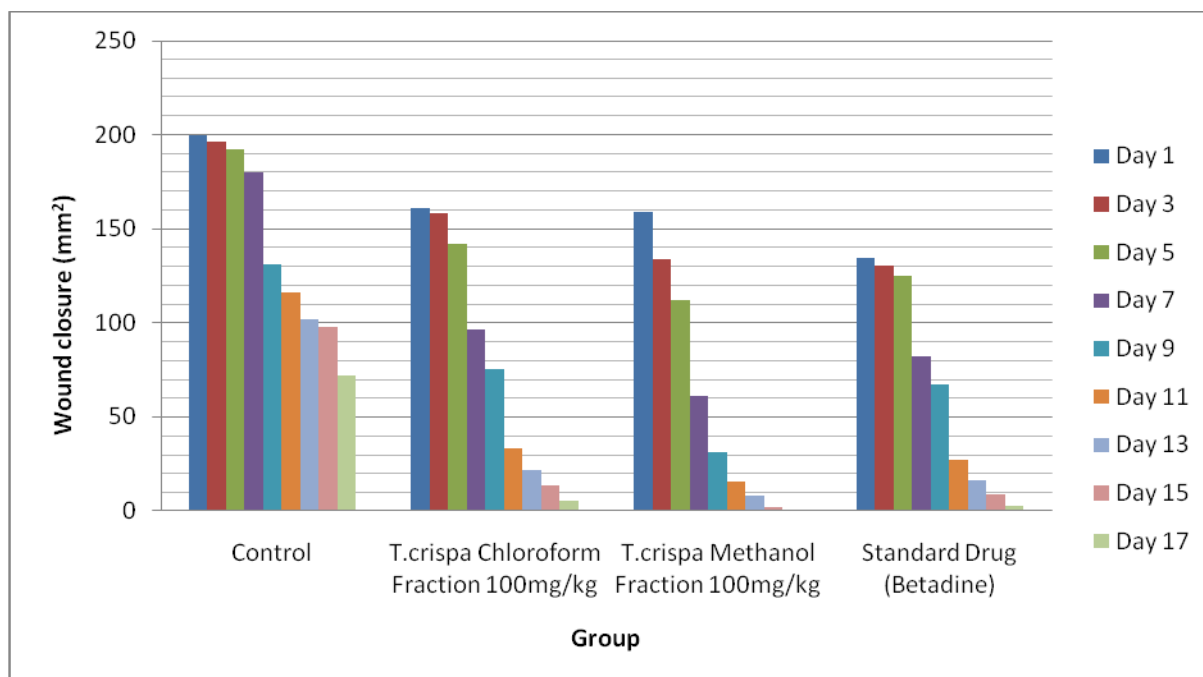


Fig 3: Comparison of wound healing activity with treatment groups

IV. DISCUSSION

Wound healing is the effort of tissues to restore normal function and structure after injury. This process is necessary to reform barriers to fluid loss and infection, limit further entry of foreign organisms and material and restores the mechanical integrity of the injured system. Wound healing process is orchestrated by the carefully regulated release of cytokines. Normal wound healing follows a predictable pattern that can be divided into overlapping phases; homeostasis and inflammation, proliferation and maturation and remodeling. The remodeling of the extracellular matrix process is under the responsibility of the fibroblasts. The faster wound closure in *T.crispa* treated wounds might also be associated with the increased keratinocyte proliferation and their migration to the surface. In the present study, the wound healing activity of the methanol and chloroform extracts of stems of *T.crispa* was evaluated using excision wound model in Albino mice. In the excision wound model, the percentage of closure of wound increased significantly from day '0' till 17th day which was 96.46% in case of *T.crispa* chloroform fraction treated group. In *T.crispa* methanol fraction treated group, the wound closure percentage was 100% in comparison to the standard group (98%) and control group (64%) on 17th day post wounding

(Table 1). Complete wound healing took place on 17th day itself in *T.crispa* methanol fraction treated group, whereas in the *T.crispa* chloroform fraction, standard and control group, wound persisted beyond 17th day indicating better wound healing activity of the plant's methanol fraction extract.

V. CONCLUSION

The present study showed that *T.crispa* having good wound healing activity comparable to the standard drug that is used in this study. Further studies should be done to study on toxicity and clinical studies in order to develop a future drug containing *T.crispa* as potent wound healing agents.

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VII. DECLARATION OF INTEREST

The authors report no conflicts of interest.

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