

SCOPING REVIEW: POTENTIAL WOUND HEALING EFFECT OF *EUPHORBIA HIRTA* (ARA TANAH) BASED ON *IN VIVO* MODELS

Terence TYC¹, Norliana MY¹, Raja Nazatul Izni RSS¹, Norman DD¹, and Teh BP¹.

¹Herbal Medicine Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Setia Alam, 40170 Shah Alam, Malaysia

Correspondence:

Terence Tan Yew Chin,
Herbal Medicine Research Centre,
Institute for Medical Research, National Institutes of Health,
Ministry of Health Malaysia,
Setia Alam, 40170 Shah Alam, Malaysia
Email: terencetyc@moh.gov.my

Abstract

Wound management is important as it helps to promote healing without microbial infection. *Euphorbia hirta*, a species of Euphorbiaceae family, also known as *Ara tanah* among Malaysians, is traditionally used to promote wound healing where its poultice is applied to the sores on the legs, bruises and wounds. Several reported pharmacological properties including antioxidant, antimicrobial, antidiabetic, anti-anaphylactic activities have been reported on this plant. The aim of this review was to evaluate the wound healing effects of *E. hirta*. Information involving only *in vivo* studies on wound healing effect of *E. hirta* was searched using electronic databases. The electronic databases include PubMed, Google Scholar, Ovid, CENTRAL, LILACS, and ClinicalTrials.gov from year 1962 to 2020. A total of five out of 70 studies were included and assessment was made. All included articles studied different wound models in rats. All showed significant wound healing activity with different mechanism of action for incision, excision and dead space wound. Quality assessment of the included studies suggested that experimental animal study design can be improved. It can be concluded that *E. hirta* displayed potential as a wound healing agent *in vivo* studies although further research on structure-activity relationship of compounds responsible for the wound healing effect and toxicological studies before it can proceed to clinical studies.

Keywords: *Euphorbia hirta*, Extract, Wounds, Wound Healing, *In Vivo*

Introduction

Wounds are defined as discontinuity of the skin, mucous membrane or tissue caused by physical, chemical or biological insult. Wounds may be classified according to its aetiology, location, type of injury, wound depth or clinical appearance. There are four general categories of wound which include surgical, traumatic, burns and chronic. Wound management is important as it helps to promote healing without microbial infection (1). Prolonged wound care may increase production of exudate and delay wound healing (2).

Wound healing undergoes four different phases: hemostasis phase, inflammatory phase, proliferative phase and maturation phase. During the hemostasis phase, the bleeding stops and the blood is confined within the damaged blood vessel. The blood vessels will then start to grow during the inflammatory phase. After several

days or weeks, the proliferative phase follows where the wound starts to close and contract. Remodeling or maturation of the skin tissue will take several weeks or maybe years depending on the aetiology of the wound (3). An estimated number of 312.9 million surgical operations were performed worldwide in year 2012 (4). The current available treatment options of wounds include different types of dressings such as hydrocolloid, hydrogel, alginate, transparent, collagen, foam and cloth (5).

Euphorbia hirta, known as *Ara tanah* is a plant belonging to the Euphorbiaceae family. The *E. hirta* is a very common weed native to Central America and it has a very large area of distribution (6). Its poultice is applied to the sores on the legs, bruises and also wounds. Its juice has been given to mothers after childbirth to prevent postpartum depression. The decoction of the plant is also reportedly used for sexually transmitted diseases in South America (7).

In herbal medicine, many plants have shown wound healing potential such as yarrow, *Aloe vera*, angelica, oats, neem, rosemary, red sanders and St. John's wort (8). Although there are many pharmacological properties of *E. hirta* reported, there is currently a lack of data on its effect for wound healing. Therefore, the aim of this review is to evaluate *E. hirta*'s wound healing efficacy based on scientific evidences available.

Method

We conducted this review in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (9).

Search strategy

The search to look for related published articles was done using six electronic databases with two sets of keywords (Table 1). Bird's eye view strategy was applied to identify all the wound healing properties of *E. hirta*. Relevant studies were further identified by going through the citations and lists of references in the related articles. All the related articles found in English were included. Two teams consisting of two authors independently extracted the data, which was later counter-checked and verified between teams. Any disagreements were resolved by discussion and consultation with a third party. The search was conducted on 8th September 2020 on the selected databases in which the years searched were by default.

Table 1: Search strategies used

Database	Year of search (by default mode)	Keywords used	Search result
PubMed	1962–2020		54
Google Scholar	2004–2017	(<i>Euphorbia hirta</i> AND wound healing)	10
Ovid	1998–2020	OR	4
CENTRAL	(No hit)	(<i>Euphorbia hirta</i> AND wounds)	0
LILACS	2013		2
ClinicalTrials.gov	(No hit)		0
TOTAL SEARCH =			70

As of 8th September 2020

Selection of samples and intervention

Studies using *E. hirta* extracts with wound healing properties were selected.

Selection of outcomes

Studies reporting on the ability of *E. hirta* to heal incision and excision wounds as well as other types of wound

(e.g. dead space, diabetic and burn) with pharmacological mechanism.

Selection of study model

We included *in vivo* and human study models that evaluated the effectiveness of *E. hirta* for wound healing.

Data extraction

Two authors independently assessed the titles and abstracts of a set of articles, based on the following criteria: Inclusion criteria: (i) Original research that presents *E. hirta* wound healing efficacy (ii) *In vivo* /animal models papers (iii) Clinical papers (if any) (iv) Full-text articles are written in English (v) No limitations on years of study or publication; and exclusion criteria: (i) *In vitro* /in silico / modeling papers (ii) Not primary papers (e.g. reviews) (iii) Safety assessment papers. Full texts were subsequently screened and any disagreements in the assessment were resolved by discussion leading to a consensus. The included articles were manually reviewed and extracted into the data extraction table. From each full text paper, data was extracted according to the type of wound, mode of administration, formulation, significant value and findings by different authors. Articles that did not meet the criteria above were excluded.

Quality assessment of included studies

The risk of bias (RoB) tool for animal intervention studies, i.e. SYRCLE's RoB tool was used to assess the risk of bias of all included studies (10). Two independent authors performed quality assessment of all included studies. Disagreements were resolved by discussion.

Results

Our search produced a total of 70 related articles (Table 1). Out of this, a total of five articles were included for evaluation upon meeting the inclusion criteria (11–15) (Figure 1).

All five articles which involved animal studies are as shown in Table 2 and Table 3. In terms of mode of administration, we found that *E. hirta* was given orally (20%) with dexamethasone administered intramuscularly and povidone given topically in three different assays (11). In the other two studies (40%), *E. hirta* was administered both topically and orally to two different groups (12, 14). In the remaining two studies (40%), topical intervention of *E. hirta* alone and compared with nitrofurazone and gentamicin sulfate was administered respectively (13, 15). The outcome measures include period of epithelization (11, 12), percentage or rate of wound contraction and percentage of wound closure (12–15) and breaking strength (11).

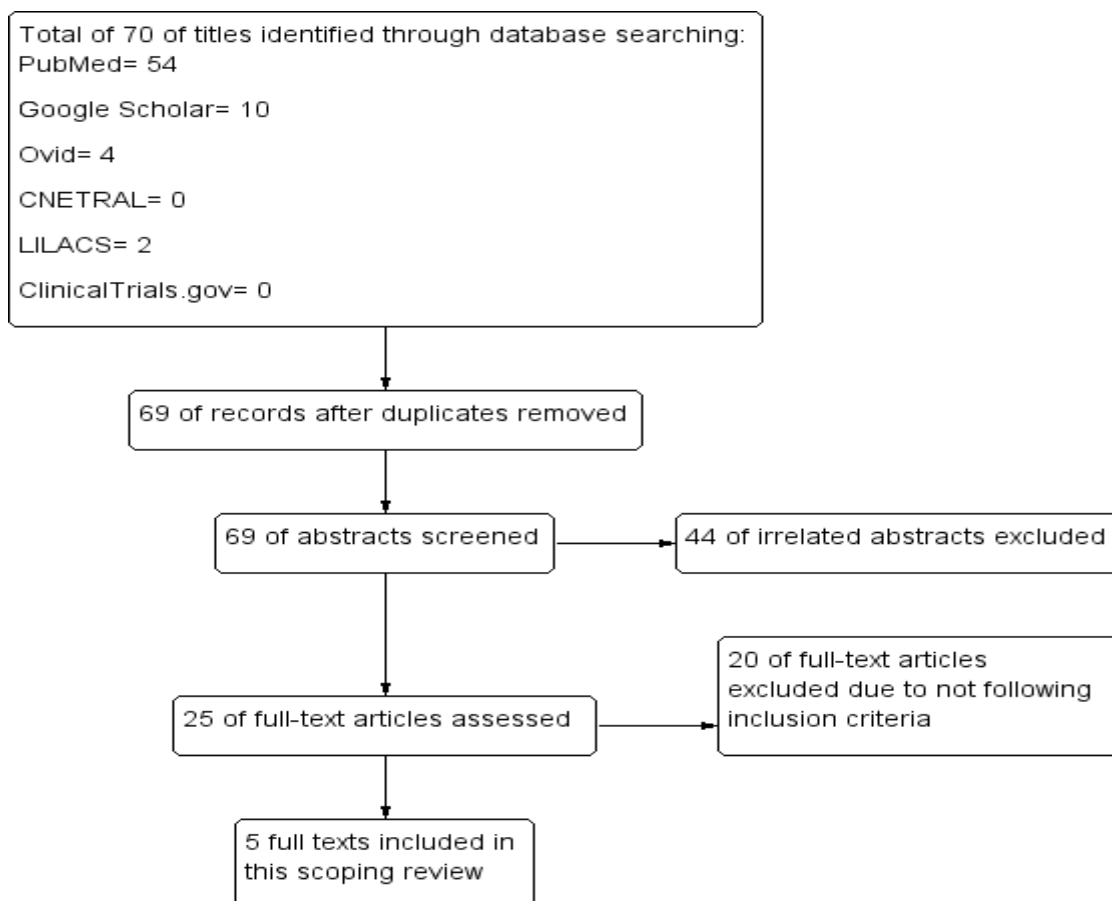


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flowchart that shows the review work to investigate the association of *E. hirta* (*Ara tanah*) plant and wound healing

Table 2: Part I - Data extraction table

Author, Year	Type of animal	Type of extraction	Plant part
Bigoniya, 2013	Male Wistar rats	Total flavonoid fraction	Whole plant
Ganju, 2013	Male albino rats	Ethanollic extract	Whole plant
Jaiprakash, 2006	Male Wistar albino rats	Ethanollic extract	Whole plant
Tuhin, 2017	Female Swiss albino rats	Ethanollic extract	Leaves
Upadhyay, 2014	Male Wistar & Swiss albino rats	Methanollic extract	Leaves

Total flavonoid fraction from methanol extract of *E. hirta* whole plant (600 mg/kg body weight, once daily for at least seven days) administered orally to male Wistar rats significantly ($p < 0.001$) increased the wound breaking strength, granulation tissue weight and hydroxyproline content (11).

Dried ethanolic extract of *E. hirta* whole plant (200 mg/kg body weight, once daily for at least 11 days) administered orally to male Albino rats significantly ($p < 0.01$) increased tensile strength, white blood cell count, wound contraction, hydroxyproline, hexosamine, & protein content and wet & dry granulation weight compared to 2% tragacanth suspension. Another formulation using the same extract in 10% w/w ointment showed significant ($p < 0.01$) increased tensile strength, white blood cell count, wound contraction, hydroxyproline, hexosamine and protein content compared to hydrophilic ointment (12).

Ethanolic extract of *E. hirta* whole plant (2% w/w ointment, once daily for 16 days) applied topically to male Wistar albino rats significantly ($p < 0.001$) increased wound closure (88%) compared to 0.2% w/w nitrofurazone ointment (94.67%) on the 16th day post-burn wound (13).

Ethanolic extracts of *E. hirta* leaves administered orally (200 mg/kg & 400 mg/kg body weight, once daily for 16 days) significantly ($p < 0.05$, $p = 0.01$) reduced wound area on day 16th compared to untreated group while topical administration (5% & 10% w/w, once daily for 16 days) reduced wound area on day 16th when compared to the untreated group. However, the reduction in wound area was not significant with topical administration (14).

Table 3: Part II - Data extraction table

Author, Year	Type of wound	Mode of administration	Formulation	P-value	Finding(s)
Bigoniya, 2013	Incision	Oral	400 & 600 mg/kg BW	$p < 0.05$	tensile strength
	Excision		400 & 600 mg/kg BW	$p < 0.05$	wound area & epithelization period
	Dead space		400 & 600 mg/kg BW	$p < 0.05$	dry weight of granulation tissue, hydroxyproline content, catalase activity, superoxide dismutase, & total protein
Ganju, 2013	Incision	Oral	200 mg/kg BW	$p < 0.01$	tensile strength & white blood cell count.
	Excision		200 mg/kg BW	$p < 0.01$	wound contraction, hydroxyproline, hexosamine, & protein content.
	Dead space		200 mg/kg BW	$p < 0.01$	wet granulation weight, dry granulation weight, protein, hydroxyproline, & hexosamine content
	Incision	Topical	10% w/w ointment	$p < 0.01$	tensile strength & white blood cell count
	Excision		10% w/w ointment	$p < 0.01$	wound contraction, hydroxyproline, hexosamine, & protein content
Dead space	10% w/w ointment	$p < 0.01$	wet granulation weight, dry granulation weight, protein, hydroxyproline, & hexosamine content		
Jaiprakash, 2006	Excision (burn)	Topical	2% w/w ointment	$p < 0.05$	wound closure
Tuhin, 2017	Excision (diabetic)	Oral	200 & 400 mg/kg BW	$p < 0.05$	wound area on day 16th
	Excision (diabetic)	Topical	5% & 10% w/w ointment	$p > 0.05$	wound area on day 16th but not significant
Upadhyay, 2014	Excision	Topical	2.5% hydrogel	$p < 0.05$	wound contraction & hydroxyproline content. Significantly protein expression of granulation tissue compared to gentamicin sulphate.

BW = body weight; w/w = weight/weight

Methanolic extract of *E. hirta* leaves administered topically (2.5% hydrogel, once daily for 14 days) significantly ($p < 0.05$) increased wound contraction and hydroxyproline content compared to untreated & vehicle group. The topical application also increased protein expression of granulation tissue compared to gentamicin sulphate (15).

Figure 2 shows the results of the risk of bias assessment of the five studies included in this review. Based on this assessment, four (80%) of the studies stated that they have baseline characteristics. Since the backgrounds of animals were essentially homogenous, most of the studies did not describe the method of randomization. Besides, none of the studies mentioned whether the allocation was adequately concealed. As shown clearly in this figure, many items were scored as 'unclear' indicating that reporting and presumably experimental design of these animal studies can be improved.

Discussion

In wound research, incision wound is inflicted to determine the wound tensile strength, while excision wound is inflicted to determine rate of contraction, period of epithelization and histology of skin and finally dead space wound is used for evaluation of granulation tissue free radicals, antioxidants, acute inflammatory markers, connective tissue markers and deep connective tissue histology (16). Based on the findings from five animal studies, oral administration of *E. hirta* (200 mg/kg body weight for ethanolic extract; 400–600 mg/kg for total flavonoid fraction) showed significant effect on incision, excision, dead space and diabetic wound. On the other hand, the topical formulation of 2–10% ointment and 2.5% hydrogel demonstrated significant effect on incision, excision, dead space and burn wound.

	Selection bias - Sequence generation	Selection bias - Baseline characteristics	Selection bias - Allocation	Performance bias - Random housing	Performance bias - Blinding	Detection bias - Random outcome assessment	Detection bias - Blinding	Attrition - Incomplete outcome data	Reporting bias - Selective outcome reporting	Other bias
Bigoniya 2013	?	+	?	?	?	?	?	+	+	?
Ganju 2013	?	?	?	?	?	?	?	?	-	?
Jaipakrash 2006	?	+	?	?	?	?	?	?	+	?
Tuhin 2017	?	+	?	?	?	?	?	+	-	+
Upadhyay 2014	+	+	?	?	?	?	?	?	+	+

Figure 2: Risk of bias assessment of included studies using SYRCLE tool

The qualitative screening of phytochemical compounds in *E. hirta* showed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids and phenolic compounds (17). Flavonoids are believed to possess potent antioxidant and free radical scavenging effect. The potential scavenging effect might play a role in enhancing antioxidant enzyme level in granuloma tissues as well as contributing towards supplementary anti-inflammatory, reduction in macrophage and granulocyte infiltration, cartilage degeneration and antimicrobial effects (11). This is further supported by reports of plants such as *Buddleja globosa*, *Moringa oleifera*, *Butea monosperma*, *Parapiptadenia rigida* and *Ononis spinosa* that contain flavonoids demonstrating wound healing activity (18–22).

Other major constituents of *E. hirta* such as triterpenoids and alkaloids should also be studied for its wound healing effect (13). This is supported by a few reports including a systematic review reporting on articles of only animal studies which concluded that triterpenoids improve wound closure for all types of wounds (23). Another animal study

utilising total alkaloidal extract of *Alstonia boonie* root bark at different formulation strengths showed significant ($p < 0.05$) wound healing activity with increased rate of wound contraction and reduction in the period of epithelialisation (24).

It is also found that oral consumption *E. hirta* is effective in diabetic wound healing as the ethanolic extract also significantly decrease blood glucose levels after 16 days. Well controlled blood sugar is important in maintaining and improving wound healing (13). This observation is further supported by the antidiabetic effect of the orally administered ethanol extract of *E. hirta* leaves (300 mg/kg body weight/rat/day) in diabetic animal models. In streptozotocin-induced male albino Wistar rats, this extract significantly ($p < 0.05$) decreased blood glucose level at 60 min when compared with untreated diabetic rats. These results were comparable with gliclazide, an oral hypoglycaemic drug which is commonly and currently used in patients with diabetes (25). Other potential underlying mechanisms of the wound healing properties

of *E. hirta* include increased fibroblast proliferation and its role in regulating the expression of bFGF, COL3A1 and Smad family proteins during the wound repair process in granulation tissue (15).

The main objective of this review is to study the potential wound healing effect of *E. hirta*. However, since no human trials were found, only animal studies were included. Only articles in English language are included as there is a language proficiency limitation. The limitation observed from the available scientific evidence is that there is not much data on the exact compound(s) of this plant that may be responsible for the wound healing activity. Identifying the pharmacologically active compound in this case is important to determine and establish the structure-activity relationship of the healing effects and its mechanisms of action. Safety data on *E. hirta* is limited but acute toxicity of methanolic extract of *E. hirta* has shown median lethal dose (LD₅₀) of 5000 mg/kg body weight. Subchronic toxicity (90 days) of the same extract showed no significant toxicity ($p > 0.05$) upon administration of 50 mg/kg, 250 mg/kg, and 1000 mg/kg body weight per day (26). Therefore, further *in vitro* and *in vivo* toxicological studies should be conducted on different extracts and formulations of the plant before it can be tested in humans.

Conclusion

In conclusion, different extracts and formulations of leaves and whole plant of *E. hirta* exhibited significant wound healing activities. However, most available evidences are based on animal studies which require further investigations to determine the phytochemicals involved (flavonoids, triterpenoids and alkaloids) and their potential underlying mechanism of actions and along with safety assessment. These data are essential prior to proceeding towards human trial before it can be extensively applied in clinical settings.

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Competing interests

The authors declare that they have no competing interests.

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