

**Review Article**

**THE POTENTIAL ROLE OF GENUS *TINOSPORA* IN WOUND HEALING: A REVIEW**

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**ABSTRACT**

Genus *Tinospora* has been used in traditional medicine throughout various parts of Asia, particularly in India, China, Thailand, Malaysia and Cambodia. Its ethnomedicinal uses include treatment for fever, cold, oral ulcer, headaches, diarrhoea, diabetes, digestive disorder and rheumatoid arthritis. Some of its ethnomedicinal uses have been justified via scientific studies, include antidiabetic, antioxidant, anti-inflammatory and antimicrobial effects. Recently, several studies have been initiated to investigate the role of *Tinospora* species in wound healing. This comprehensive review used updated information obtained from systematic literature search using PubMed, Scopus, Science Direct and Google Scholar, to assess the wound healing properties of eleven major *Tinospora* species namely, *T. cordifolia*, *T. crispa*, *T. sagittata*, *T. sinensis*, *T. bakis*, *T. craveniana*, *T. tuberculata*, *T. capillipes*, *T. rumphii*, *T. smilacina*, and *T. baenzigeri*. The information obtained revealed that only *T. cordifolia* and *T. crispa* have been reported on its wound-healing abilities. Studies demonstrated that *T. cordifolia* is associated with early re-epithelisation, enhanced tensile strength, increased granulation tissues and increased collagen contents. Whereas *T. crispa* is reported with wound healing activity through the elevated antioxidant enzymes and inflammation phase. Nevertheless, there is limited evidence on phytochemical constituents from plant extracts that contribute to the wound healing process and their underlying mechanisms. This review deduced the potential gaps for future research aiming to study the genus *Tinospora* as a potential wound healing agent.

**Keywords:** *Tinospora*, Wound healing, Wound

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**INTRODUCTION**

A wound is an injury to living tissue by a cut, blow or other impacts, which typically causes the disruption of the normal structure and function of the skin and its architecture [1]. The fundamental response to the injury is wound healing which results in restoration of tissue integrity. Wound healing is a process that consists of four overlapping and continuous phases which are independent of each other. The four phases are coagulation, inflammation, granulation tissue formation and scar tissue formation [1, 2]. For optimal wound healing, the said phases must occur in the proper sequence for a specific duration of time in an optimal condition.

At present, wound management includes medical care such as antibiotic and wound debridement (enzymatic, biologic, surgical and irrigation), topical therapy (growth factors, antiseptic and antimicrobials agents) and wound dressings (film, hydrogel, foam, alginate, hydrocolloids) has been utilised [3]. These measures are meant to improve the wound healing environment and prevent infections [3]. However, there is lacking data to support and justify any of these measures. As such, the need to discover compounds that improve wound healing through intrinsic factors such as oxidative stress and inflammation is crucial.

Unfortunately, drug discovery is a time-consuming and expensive process that involves the identification of new chemical entities (NCEs). NCEs can be discovered through either chemical synthesis (an approach that was found to be less effective in terms of the overall success rate) [4] or by isolating natural compounds. Wound healing activity of plant extracts can be attributed to the antimicrobial, anti-oxidative or free radical scavenging properties and anti-inflammatory activity of the phytoconstituents, as well as mitogenic activity, angiogenesis, collagen production and DNA synthesis, which are induced by their phytoconstituents [5].

Genus *Tinospora* is of the family Menispermaceae. Plants of this family are twinning and woody climbing herbs and in some rare cases, they are erect upright shrubs or small trees or lianas [6]. The leaves of the plants are petiolate, alternate spiral and the flowers are small and dioecious [6]. The genus *Tinospora* comprises of 35

species: 7 found in tropical Africa, 3 in southern Africa, 2 in Madagascar and 23 in the Australia, Asia and Pacific region [6]. The current review was based on the hypothesis that the *Tinospora* family bears a significant amount of bioactive compounds such as alkaloids, phenolic substances and related compounds that possess properties such as antioxidative, anti-inflammatory and mitogenic agent [7]. This review provides an insight into the wound healing studies conducted on the 11 selected *Tinospora* subspecies over the last 10 y and focused on the crude or alcoholic or chemical extracts of the plants. The available research gaps and the potential of the *Tinospora* species as a wound healing agent have been identified.

**Methodology**

**Searching strategies**

Articles were obtained through a systematic literature search in three databases: PubMed, Scopus and Science Direct. Manual search from grey literature, i.e. Google Scholar was also done. The search was conducted for 11 selected *Tinospora* plants: *Tinospora cordifolia*, *Tinospora crispa*, *Tinospora sagittata*, *Tinospora sinensis*, *Tinospora bakis*, *Tinospora craveniana*, *Tinospora tuberculata*, *Tinospora capillipes*, *Tinospora rumphii*, *Tinospora smilacina* and *Tinospora baenzigeri*. The keywords or phrases employed to be used in combination with the plant names were wound healing, wound, ulcer, incision, excision and burn.

**Inclusion and exclusion criteria**

Studies that were conducted to assess the wound healing abilities of the *Tinospora* plant family in relation to the skin and were published from January 2009 until October 2020 were included in this review. Wound healing abilities related to the gastrointestinal system, such as peptic ulcer or oral ulcer, were excluded from the review. The search was limited to articles published in the English language only irrespective of the sample size used and/or duration of follow-up. Congress abstracts were also excluded from the review. All articles that studied the included subspecies of the *Tinospora* family for its wound-healing abilities on an animal model were included so long as the outcomes were clearly documented. Duplicated articles were excluded.

Table 1: Databases used with respective search terms in the literature search

Database and search terms used to extract all available articles	No. of articles retrieved (n)	No. of relevant articles (n)
<b>PubMed</b>		
" <i>tinospora cordifolia</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	506	0
" <i>tinospora crista</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	77	0
" <i>tinospora sinensis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing."	56	0
" <i>tinospora bakis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	14	0
" <i>tinospora craveniana</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	0	0
" <i>tinospora sagittata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	16	0
" <i>tinospora tuberculata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	6	0
" <i>tinospora capillipes</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	4	0
" <i>tinospora smilacina</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	4	0
" <i>tinospora rumphii</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	6	0
" <i>tinospora baenzigeri</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	3	0
<b>Science direct</b>		
" <i>tinospora cordifolia</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	527	0
" <i>tinospora crista</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	109	0
" <i>tinospora sinensis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	74	0
" <i>tinospora bakis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	7	0
" <i>tinospora craveniana</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	2	0
" <i>tinospora sagittata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	15	0
" <i>tinospora tuberculata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	8	0
" <i>tinospora capillipes</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	11	0
" <i>tinospora smilacina</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	7	0
" <i>tinospora rumphii</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	16	0
" <i>tinospora baenzigeri</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	0	0
<b>Scopus</b>		
" <i>tinospora cordifolia</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	999	12
" <i>tinospora crista</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	158	8
" <i>tinospora sinensis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	44	0
" <i>tinospora bakis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	5	0
" <i>tinospora craveniana</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	0	0
" <i>tinospora sagittata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	19	0
" <i>tinospora tuberculata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	8	0
" <i>tinospora capillipes</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	5	0
" <i>tinospora smilacina</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	4	0
" <i>tinospora rumphii</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	6	0
" <i>tinospora baenzigeri</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	4	0
<b>Google scholar</b>		

" <i>tinospora cordifolia</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	7592	56
" <i>tinospora crisa</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	908	16
" <i>tinospora sinensis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	530	1
" <i>tinospora bakis</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	62	0
" <i>tinospora craveniana</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	5	0
" <i>tinospora sagittata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	129	0
" <i>tinospora tuberculata</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	73	0
" <i>tinospora capillipes</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	39	0
" <i>tinospora smilacina</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	54	0
" <i>tinospora rumphii</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	96	0
" <i>tinospora baenzigeri</i> " AND/OR "wound" AND/OR "excision" AND/OR "incision" AND/OR "burn AND/OR "ulcer" AND/OR "wound healing"	7	0

### Data extraction and synthesis

Initial screening of the articles was reviewed by researchers from the title, abstract and full text. Articles that met the predefined inclusion and exclusion criteria were selected and included in the study. Data were then extracted from the articles and tabulated into tables to assemble the appropriate information on study design, intervention and treatment outcomes.

### RESULTS

In the present review, a total of 12213 number of articles were retrieved from different sources related to wound healing abilities of *Tinospora* family (table 1). Of the retrieved articles, 61 articles met the inclusion criteria stated in the methodology (fig. 1). Upon excluding the duplicated articles and 1 congress abstract, 11 of the scientific articles were found to be relevant and data from the articles were included in this article. Based on the retrieved data over the last 10 y, only two of the 11 species of *Tinospora*, namely *Tinospora cordifolia* and *Tinospora crisa* were studied for its wound-healing abilities.

#### *Tinospora cordifolia*

Based on the search done with the aforementioned methodology, eight papers reported on the wound healing properties of *T.*

*cordifolia* (table 2). Studies have been done on topical treatment [8-11] with doses ranging from 1% to 10%, in either cream or ointment bases, as well as oral treatment [12-15] with doses ranging from 200 mg/kg to 600 mg/kg.

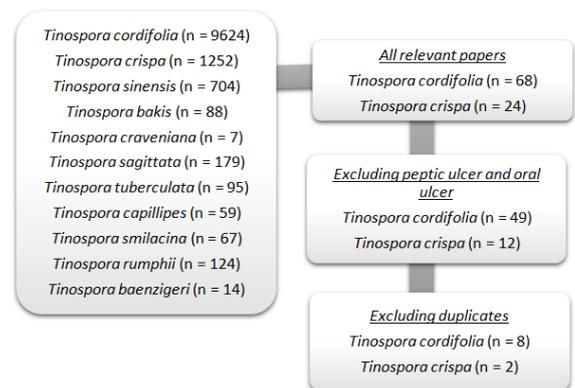


Fig. 1: Flow chart of the article screening phase based on the inclusion and exclusion criteria

Table 2: Summary of wound healing studies involving *Tinospora cordifolia* conducted between 2009 to 2020

Part of plant	Solvent/extraction method	Wound model and animal used	Treatment	Findings	Reference
Leaf	Methanol, Maceration	Excision and incision wound model on male Sprague Dawley rats	5% ointment (base: Vaseline), BD, 28 d	Early reepithelisation and faster wound closure is possibly associated with increased keratinocyte proliferation and migration to the wound surface. An increase in tensile strength was also noted. Proliferation of fibrous connective tissue and angiogenesis was noted. Polymorph nuclear lymphocytes were detected on day 21 of the treatment.	[8]
Leaf	Petroleum ether, ethanol, water, Soxhlet extraction	Dead space, excision and incision wound model on either sex of albino Wistar rats	200 mg/kg, OD, 16 d	Complete epithelisation and percentage of wound closure were the highest in the ethanol extract-treated group. Increased tensile strength noted in the ethanol extract-treated group, followed by the aqueous treated group, but was less in the petroleum ether extract-treated group. Ethanol and aqueous extracts shown increased regenerated tissue, epithelialization, fibroblasts and revascularisation.	[12]
-	-	Resutured incision wound, dead space wound on male	200 mg/kg (base: 2% gum acacia) OD, 10 d	Significant increase in tensile strength in resutured incision wound. Histology of TC treated dead space wound shown an increase in the number of fibroblasts, thick bundles of collagen tissue	[13]

		Wistar rats		deposition with lesser macrophages. Significant increase in dry weight of granulation tissue and tensile strength in TC treated rats. Higher hydroxyproline content in the TC treated in comparison to control group rats.	
-	-	Excision wound model on male Wistar rats	1% TC cream (base: cold cream), OD, 16 d	Mean percentage of wound contraction and rate of epithelisation of TC treated group higher than the control group. Enhanced wound contraction as denoted by stellate-shaped scars, whereas the control group had oval-shaped scars with regular margins.	[9]
-	-	Burn wound model on male Wistar rats	2% ointment (base: petroleum jelly), OD until complete healing	Mean epithelisation time and percentage of wound contraction was significantly more in TC treated group in comparison to the control group	[10]
Root	Methanol, Soxhlet extraction	Excision and incision wound model on male and female albino rats	10% ointment (base: petroleum jelly), OD, 12 d	Significant wound healing activity. A higher percentage of wound closure in comparison to standard. Increased tensile strength.	[11]
Stem	Methanol, Soxhlet extraction	Excision wound, resutured incision wound and dead space wound on male Wistar rats	250 mg/kg OD, 10 d	Rate of wound contraction not significantly different from the control group until day 12. Enhanced wound contraction as denoted by stellate-shaped scars in comparison to oval-shaped scars of control groups. The TC-treated group had an increased amount of granulation tissue and collagen content.	[14]
Stem	Methanol, Soxhlet extraction	Excision wound model on alloxan induced diabetic white rabbits	600 mg/2 ml/kg, OD, 28 d	Methanolic extract of TC enhances the rate of wound healing. However, the wound healing ability of the combination of topical insulin and TC extract was more significant than the extract alone.	[15]

TC: *T. cordifolia*.

Almost every study used the excision and incision wound model [8, 9, 11-14]. Only two studies were done on the dead space wound model [12, 13], one on the burn wound model [10] and one on the diabetic wound model [15]. All the studies indicated that *T. cordifolia* extracts have positive effects on wound healing in terms of the wound contraction rate, tensile strength and histopathology of the skin. Two studies included the estimation of hydroxyproline, a basic constituent of collagen and an important constituent of extracellular matrix healing [12, 13].

Based on the parameters observed, the use of *T. cordifolia* is found to hasten wound healing [8, 11, 12, 14, 15]; early re-epithelisation which is associated with increased keratinocyte proliferation and migration to wound surface [8], increased tensile strength [8, 11-13]. *T. cordifolia*-treated groups also exhibited an increased amount of granulation tissue which indicates higher protein content [13, 14] and increased collagen content which is indicated by the increased amount of hydroxyproline [12, 13].

The studies also investigated the histology of granulation tissue and wound site to further strengthen the claims of *T. cordifolia*'s wound healing potential. The most commonly used stain was haematoxylin and eosin [8, 11, 13-15]. One study employed van Gieson's staining [14]. The histology results shown that topical treatment of *T. cordifolia* enables the epidermis to be fully formed within 12 d of treatment with 10% ointment once daily [11]. Furthermore, it also enhanced collagen formation [11]. Barua *et al.* applied the same model with different dose (i.e. 5% ointment twice daily) and shown that there was proliferation of fibrous connective tissue with increase angiogenesis and increased collagen maturation by cross linking on day 21 of treatment [8]. However, the study also reported that there was still polymorph nuclear lymphocyte infiltration at the wound site of the *T. cordifolia*-treated group, indicating that wound healing was not complete yet [8].

Orally treated animals also shown positive wound healing activities. Van Gieson staining of granulation tissues of rats treated with the ethanol extract of *T. cordifolia* depicted an increase in collagen formation and lesser macrophages [12]. Similarly, methanol extract-treated rats also reported positive results [14] on haematoxylin and eosin staining with an increase in granulation tissue and increased cell proliferation with lymphocytes and macrophages, whereas Van Gieson staining shown a marked increase in collagen content in comparison to the control group. In a different study, methanol extract-treated rats had a scanty amount of granulation tissues with a maximum amount of fibrocollagen tissue in comparison to control, which had abundant granulation tissue and scanty fibrocollagen tissue [13]. 28-day old diabetic wounds from white rabbits did not have properly formed epidermis and had mild tissue reactions with few fibrous and collagen tissues. Scanning Electron Microscopy study of diabetic wounds from white rabbits treated with 600 mg/kg/day methanolic extract of *T. cordifolia* illustrated that on day 14, there was irregularly arranged collagenous tissue along with numerous newly formed blood vessels in the wound samples. On day 28, although the collagenous tissue became denser and was arranged in more regular form, the epithelial layer was not completely formed and angiogenesis was reduced but still present [15].

#### *Tinospora crispa*

In the last 10 y, only three papers have reported on the wound healing ability of *T. crispa* (table 3). Two papers reported on the wound healing ability of the excision wound model [16, 17]. All two papers reported that *T. crispa* extract has positive wound healing properties. The studies conducted assessed the wound healing ability of the herb as a topical treatment: 5% ointment OD [17] or 100 mg/ml or 200 mg/ml intrasite gel BD [16].

Table 3: Wound healing studies involving *Tinospora crispa* conducted between 2009 to 2020

Part of plant	Solvent/extraction method	Model	Treatment	Findings	Reference
Stem	Methanol and chloroform, Maceration	Excision wound model on albino rats of either sex	5% ointment (base: simple ointment), OD, 17 d	Better wound healing activity was observed in the methanolic fraction in comparison to the chloroform fraction. Accelerated wound closure may be associated with increased keratinocyte proliferation and their migration to the surface.	[17]
Stem	95% ethanol, maceration	Excision wound on female Sprague Dawley rats	100 mg/ml and 200 mg/ml (base: 2% gum acacia), BD, 15 d	Significant wound healing action is seen through the improved activity of endogenous antioxidants: SOD and CAT that prevent free-radical mediated tissue injury. Rapid movement to the proliferative phase and shortened inflammatory phase helped enhance wound contraction.	[16]

SOD: superoxide dismutase; CAT: catalase.

Only one study reported on the wound contraction rate of *T. crispa*. Vijendren and colleagues reported that *T. crispa* extract hastened wound contraction and the rate was consistent with the Betadine-treated group [17]. Besides that, the study demonstrated that methanol fraction (100%) had a higher wound contraction percentage than chloroform fraction (96.46%) [17]. In term of histopathology, the haematoxylin and eosin-stained granulation tissue of the rat treated with 100 mg/ml and 200 mg/ml for 15 d demonstrated comparatively less inflammatory cells and more collagen, fibroblast and blood proliferating capillaries in comparison to the control group. Scabs were also noted in the slides [16]. For the wound healing ability of *T. crispa*, one study has conducted biochemical analysis of the skin wound site. Biochemical analysis of antioxidant enzymes shown elevated superoxide dismutase (SOD) level, catalase (CAT) level and significantly lowered malondialdehyde level among *T. crispa*-treated rats, in comparison to the vehicle treated group. As for the levels of inflammatory cytokines, the study demonstrated significantly elevated TGF- $\beta$ 1 in the treated group compared to vehicle control and intrasite gel-treated group and significantly lowered TGF- $\alpha$  in treated group compared to vehicle control group [16].

## DISCUSSION

There are many factors which may affect wound healing through one or more phases of the wound healing process, thus causing improper or impaired wound healing. Such wounds frequently enter a state of pathologic inflammation due to an uncoordinated wound healing process. These factors can be categorised into local and systemic factors. Local factors include oxygenation, infection, foreign body, and venous insufficiency directly influence the characteristics of the wound. On the other hand, systemic factors are those factors that associated overall health or disease state of the individual that affect the ability of the wound to heal, such as age, gender, stress, ischaemia, diseases, obesity, the use of medication, alcoholism, smoking, nutrition, and immunocompromised conditions [18]. Plants have immense potential when it comes to the management and treatment of wounds. In the past, many plants were known to be used by tribal and traditional medicine practitioners for the treatment of wounds. The natural agents induce wound healing through mechanisms such as antimicrobial, anti-inflammatory, and antioxidant activities. As phytomedicine is not only affordable but also safe, it has been a great interest to scientists to discover new effective drugs [19].

*T. cordifolia* (Menispermaceae) or more commonly known as "Guduchi" in Sanskrit is a large, perennial, deciduous climbing shrub of weak and fleshy stem with greenish-yellow typical flowers that are commonly found at higher altitudes [5, 20]. In Ayurveda, *T. cordifolia* is known for its immense application in the treatment of many diseases. Today, it is clinically used by many patients as it is available as either a single herbal drug or in formulations [21]. Traditionally, the leaves and stems of *T. cordifolia* are used to treat rheumatism, fever, jaundice, cough, eye disease and diabetes [22-25], whereas the root is used to treat vomiting [22]. It is also known to

be used to treat skin diseases and to be an anti-poison and antibacterial [25]. Scientifically, *T. cordifolia* has been demonstrated to have anti-cancer activity [26-28], the ability to enhance cognition and memory, anti-inflammatory and wound healing activity [10, 14, 29], anti-malaria activity [30] and antifungal [31].

Over recent years, the wound healing ability of *T. cordifolia* has been explored. Studies have been demonstrated the potential wound healing ability of *T. cordifolia* on different wound models with different formulations and dosages, either in topical (1% to 10%) or oral (200 mg/kg to 600 mg/kg) route of administration. The dosage used was within the safe dose range. Acute toxicity tests conducted in different studies shown that no toxicity nor death was noted at doses above 3 g/kg of ethanolic and aqueous extracts [32-34] and 5 g/kg of methanolic *T. cordifolia* extract [8]. Additionally, up to 200 mg/kg of chloroformic extract of *T. cordifolia* did not show visible signs of toxicity in short term treatment [34]. Long term treatment (60 d) of 400 mg/kg *T. cordifolia* aqueous extract shown no effect on red blood cell count, white blood cell count, haemoglobin levels and haematocrit levels [33]. A clinical study reported that when *T. cordifolia* was administered at a dose of 500 mg/day for 21 d in healthy individuals, it did not exert any remarkable adverse effects on the cardiovascular system, central nervous system, renal system and gastrointestinal system [20].

In term of wound healing model, wound healing activities of *T. cordifolia* was demonstrated to be potent in normal wounds and it was not as potent in diabetic wounds [15]. It was found to hasten wound healing via early reepithelisation, increased tensile strength, increased granulation tissue and collagen content in normal wound models [8, 11-14]. In the diabetic wound model, the epidermis layer was not properly formed for a 28-day old diabetic wound with few fibrous and collagen tissues [15]. This shown that, though oral *T. cordifolia* may not be an effective treatment on its own for diabetic wounds, however, when used together with topical insulin, it may synergistically help to improve diabetic wound healing [15].

On the other hand, the method of extraction and solvent used may influence the wound healing ability of *T. cordifolia*. Rani and colleagues reported that the ethanolic extract of *T. cordifolia* leaves and stem had the highest percentage of wound contraction, rate of epithelisation, highest collagen content and tensile strength in comparison to aqueous and petroleum ether extracts. This might be attributed to phytochemical content in ethanolic extracts of the leaf, which contains alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, phenols, tannins and terpenoids, whereas the stem contains the same compounds except for carbohydrates and cardiac glycosides [35]. Methanol is the common solvent used in the extraction of phytochemicals from *T. cordifolia*. However, as studies have indicated that its ethanolic extract is known to have higher concentrations of high levels of phenols and flavonoids [36-38], a study to compare the wound healing ability of methanolic and ethanolic extract of *T. cordifolia* would be helpful to understand which fraction of extract has better wound healing properties.

*T. crispata* (Menispermaceae) is an herbaceous woody vine with shiny green leaves that grows in the tropical and subtropical regions of Southeast Asia: India, Malaysia, Philippines, Indonesia, Thailand and Vietnam [39]. It is commonly known as "Patawali" or "Seruntum" in Malaysia, "Brotawali" or "Andawali" in Indonesia, "Makabuhay" in the Philippines and "Boraphet" in Thailand [39]. It is a popular herb used traditional medicine in the treatment of diabetes mellitus in Malaysia, Thailand and the Martinique Island [39]. It is also used as anti-pyretic, anti-inflammatory and anti-malarial in Thai traditional medicine [40]. In Chinese traditional medicine, the herb is also used as treatment for fever and scabies, tropical ulcer related disorders, fractures and septicaemia [40]. Besides this, the herb is also used for the treatment of rheumatism, hypertension, tuberculosis and diarrhoea [39]. Oral administration of the decoction of the stem of the herb is claimed to possess anti-malarial and anti-helminthic properties [40]. The poultice of fresh leaves, on the other hand, is reported to be used for the treatment of wounds and itches [40]. Scientifically, many of the ethnopharmacological claims of *T. crispata* have been proven. However, its claim as a wound healing agent has not been extensively studied nor reported.

Similarly, the potential wound healing ability of *T. crispata* has been studied on different wound models with different formulations and dosages. Studies have been demonstrated the potential wound healing ability of *T. crispata* on different wound models with different formulations and dosages, either in topical (5% ointment; 100 mg/ml and 200 mg/ml intrasite gel) or oral (250 mg/kg) route of administration. Acute toxicity test of *T. crispata* done in the past shown that there was no death nor toxic signs up to the dose of 2 g/kg [16]. Other acute toxicity studies shown that, at the highest dose of 4 g/kg of *T. crispata* ethanolic extract, there were no toxic signs [39, 41]. However, chronic toxicity study of 6 mo shown that the administration of ethanolic extract of *T. crispata* at a dose of 9.26 g/kg/day to rats causes renal and hepatic toxicity [39]. A more recent study revealed that the ethanolic extract of *T. crispata* contained certain hepatotoxins which potentiated thioacetamide-induced hepatotoxicity in rats when 100 and 200 mg/kg of the extract was administered for 8 w [42].

As for *T. crispata*, the wound healing studies conducted used only the stem of the plant but studied the effects of extracts from three different solvents: ethanol, methanol and chloroform. Methanolic extract was reported to have better wound healing properties than chloroform extract [17]. Unfortunately, there are no reported studies conducted to compare the efficacy between the ethanolic and methanolic extracts. Methanolic stem extracts of *T. crispata* are known to contain alkaloids, steroids, saponosides, flavonoids, tannins, phenols and terpenoids [43]. The information on the phytochemicals in ethanol extracts on the other hand had not been analysed yet.

The enhanced wound healing potency of the plant extracts may be attributed to its antioxidative action. Oxidative stress occurs when there is an imbalance of free radicals and antioxidants in the body. At a high concentration, reactive oxygen species induces severe tissue damage and even leads to neoplastic transformation, this further impedes wound healing by causing damage to cellular membranes, DNA, proteins, as well as lipids. High oxidative stress would therefore cause damage to the growing tissue at the repair site. Antioxidants act as oxygen scavengers by reacting with free radicals and chelating catalytic metals. Besides from this, lipid peroxidation levels would be reduced following by the reduced oxidative stress. Reduced lipid peroxidation levels increase the viability of collagen fibrils by increasing the strength of collagen fibres, improving the circulation, preventing cell damage, and promoting DNA synthesis. Therefore, by reducing oxidative stress, reepithelisation time is expected to be reduced [19].

*T. cordifolia* was reported to possess strong free radical scavenging properties against superoxide anions ( $O_2^-$ ), nitric oxide radicals, hydroxyl radicals (OH) and peroxy nitrite anion (ONOO<sup>-</sup>) [44]. A study comparing the antioxidative potential of different *T. cordifolia* stem and leaf extracts with methanol, acetone, water, chloroform, ethyl acetate and hexane, reported that among leaf extracts, methanol shown the highest inhibition (70.25%), whereas amongst stem extracts, ethyl acetate shown the highest inhibition (85.45%) of carbonyl modification of protein (PCO) [45]. The assessment of

PCO is a widely used marker for oxidative protein modification and it is a sensitive early marker of oxidative stress to tissues as compared with lipid peroxidation [46]. Another study indicated that the ethanol extract of *T. cordifolia* stem which contains substantial amounts of phenols also has marked antioxidant activity [47]. The antioxidative activity of *T. cordifolia* is credited to its alkaloids and diterpenoid lactones such (-)epicatechin, tinosporin, isocolumbin and palmatine [48,49]. Consistently, *T. crispata* also possesses antioxidative activity. The antioxidative potency of aqueous extract of *T. crispata* stem is equivalent to establish antioxidants such as butylhydroxy toluene and vitamin C [50]. It was reported that methanol extract of the stem has the strongest antioxidant potential between aqueous, methanol and chloroform extracts of *T. crispata* stem, which potentially due to the phenolic compounds presented in the extract [51]. Besides this, it is known that the antioxidant and free-radical scavenging potency of *N-trans-feruloyltyramine*, *N-cis-feruloyltyramine* and secoisolariciresinol which were isolated from the dichloromethane extract of *T. crispata* is higher than of butylhydroxy toluene [52].

In the case of chronic wounds, due to a myriad of factors which can delay wound healing—chronic diseases, diabetes, vascular insufficiency, malnutrition, ageing, and local factors, the damaged tissue is detained in a prolonged and heightened inflammatory state [53]. While inflammation is essential in wound healing for clearing the debris and infection, prolonged inflammation can result in delayed wound healing and increased scarring [54]. During the inflammatory phase, macrophages secrete effector molecules such as nitric oxide (NO), prostaglandin (PG)<sub>E2</sub>, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , and IL-6 [55]. NO, which is released by the inducible nitric oxide synthase (iNOS), functions as an antimicrobial agent and a regulator of inflammatory reactions [56]. TNF- $\alpha$  is known to promote tissue fibrosis and also synthesis of collagenases and adhesion molecule. Whereas IL-6 recruits inflammatory cells, especially neutrophils. The crosstalk between iNOS and TNF- $\alpha$  regulates the secretion of another proinflammatory cytokine, PGE<sub>2</sub>, which exerts vasodilation, pain, and cellular proliferation at the site of inflammation [57,58]. The persistent upregulation of these cytokines prolongs inflammation, hence, agents that downregulate their production are valuable in the treatment of chronic inflammation.

In the case of *T. cordifolia*, chloroform extract was reported significantly lowered levels of inflammatory cytokines such as PGE<sub>2</sub>, IL-1 $\beta$  and IL-6. The study also proved that chloroform extract of *T. cordifolia* downregulated both bacterial lipopolysaccharide (LPS) induced transcription and translation of proinflammatory biomarkers such as TNF- $\alpha$ , COX-2 and iNOS dose-dependently, whereas COX-1 gene expression was observed to be similar to that of normal control [59]. The study indicated that the possible phytochemicals responsible for this action could be stigmasterol and  $\beta$ -sitosterol [59]. Furthermore, the aqueous extract of *T. cordifolia* was also reported to be significantly reduced the production of NO and downregulated gene expression of TNF- $\alpha$  and COX-2. Besides this, the extract also inhibited the gene expression of toll-like receptor 4 [60], which is an important regulator of wound inflammation [61]. However, the aqueous extract of *T. cordifolia* only significantly inhibited IL- $\beta$  at higher concentrations of 250–500  $\mu$ g/ml. The study also reported that tinosporoside significantly reduced NO production [60]. Besides from this, the anti-inflammatory activity of *T. cordifolia* is also credited to its alkaloids: berberine, jatrorrhizine, magnoflorine, palmatine and isocolumbin, glycosides; cordifolioside A, cordioside and syringin, steroids; ecdysterone diterpenoid lactones; furanolactone, tinosporin, tinosporide, jateorine, columbin and clerodane derivatives [15, 48]. In *T. crispata*, cordioside, quercetin, paullinic acid, and boldine, are the phytochemicals known to possess anti-inflammatory activity [62]. The latter phytochemicals improved intracellular expression of cytokines such as INF- $\gamma$ , IL-6, and IL-8 [62]. Tinocrisposide, a compound isolated from *T. crispata*, on the other hand, was reported to inhibit the formation of NO in LPS induced RAW 264.7 cells in a dose-dependent manner [63]. However, there is limited studies conducted to assess wound healing ability of these phytochemicals independently.

The common parameters analysed in wound healing studies for these plants mostly are physical parameters such as wound contraction rate,

tensile strength and histopathology. In terms of biochemical parameters studied, *T. crisper* has more biochemical parameters studied compared to *T. cordifolia*. Thus far, the biochemical parameters studied have been the estimation of hydroxyproline, SOD levels and CAT levels. However, many other parameters that can be studied to understand the underlying mechanisms by which the extract improves wound healing. These includes the estimation of hexosamine [64] and expression of matrix metalloproteins (MMPs) such as MMP-2, MMP-8 and MMP-9 [65]. Increased hexosamine content reflects the stabilisation of collagen molecules by enhancing the electrostatic and ionic interaction [64]. MMPs play an important role in regulating extracellular matrix degradation and deposition which is essential for wound reepithelialisation. However, for successful wound healing, timed expression and activation of MMPs in response to wounding is important [65]. By studying the expression of MMPs, we can better understand the wound healing mechanism of the extract or phytochemical. More molecular studies on the mechanisms affected by the extract or phytochemicals in wound healing is needed in the future research.

#### Research gaps in study

Out of the 11 species from the *Tinospora* genus, only two species, notably *T. cordifolia* and *T. crisper* have been explored on their wound healing abilities. However, in comparison to *T. cordifolia*, there are very scarce and limited articles on wound healing abilities of *T. crisper*. The effect of other species in wound healing remains unknown. Based on the literature, positive results have been reported regardless of the part of plants or solvent used for extraction in both species. *T. crisper* studies did not address the superiority in wound healing ability between the different types of solvent used for extraction or different parts of the plant used. Furthermore, although various phytochemicals of *Tinospora* species had been reported to possess antioxidants and anti-inflammatory effects, there are limited studies conducted to assess the wound healing ability of these phytochemicals independently. Hence, the exact phytochemical constituents in the plant extracts that contribute to hastened wound healing is unclear. This inevitably also entails that the underlying molecular mechanisms of these extracts or phytochemicals in wound healing have not been elucidated. More research is needed to elucidate the molecular mechanism of action of the extracts or phytochemicals responsible in improving wound healing process.

#### CONCLUSION

Based on the literature review, in the family of *Tinospora*, two out of 11 of selected species have been studied for its wound healing abilities. Scientific research has shown that both *T. cordifolia* and *T. crisper* possess significant wound healing abilities regardless of the part of the plants, extraction methods and wound models used. Although various phytochemicals of *Tinospora* species had been reported to possess antioxidants and anti-inflammatory effects, there are limited studies conducted to assess the wound healing ability of these phytochemicals independently. Inevitably, the underlying molecular mechanisms of these extracts or phytochemicals in wound healing have not elucidated yet. Therefore, more in-depth research is required in the future to elucidate the phytochemicals responsible and its mechanism of action on chronic and acute wound healing.

#### ABBREVIATIONS

NCE: new chemical entities, TC: *Tinospora cordifolia*, SOD: superoxide dismutase, CAT: catalase, PCO: carbonyl modification of protein, NO: nitric oxide, PGE2: prostaglandin E2, TNF- $\alpha$ : tumour necrosis factor- $\alpha$ , IL-1 $\beta$ : interleukin-1 $\beta$ , IL-6: interleukin-6, iNOS: inducible nitric oxide synthase, LPS: lipopolysaccharide, MMP: matrix metalloproteins

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#### AUTHORS CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content

of this article will be borne by them. MF, YHY and NKS developed the idea to review the topic. YHY and NKS coordinated the research activities. MF performed the literature search, data analysis and manuscript preparation. YHY and NKS reviewed the literature review. All authors read and approved the manuscript.

#### CONFLICT OF INTERESTS

Declared none

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