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The Comparison of Antibacterial Efficacy, Cell Proliferation, and Wound Healing Properties in Extracts Derived from Leaves and Inflorescences of Hang Kra Rog Phu Phan ST1 (*Cannabis sativa* L.)

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Abstract

The therapeutic potential of *Cannabis sativa* extracts in wound healing applications has garnered significant interest due to their diverse pharmacological properties. This study aimed to comparatively evaluate the antibacterial properties and wound-healing activity of ethanolic extracts derived from *C. sativa* leaves and inflorescences of the Hang Kra Rog Phu Phan ST1 cultivar. The extracts were prepared using ethanol, and their antibacterial properties, cytotoxicity and ability to promote cell proliferation and migration were investigated. The antibacterial activity was assessed against *Staphylococcus epidermidis* TISTR 518, *Klebsiella pneumoniae* TISTR 1383, *Pseudomonas aeruginosa* TISTR 2370, *Staphylococcus aureus* TISTR 1466 and *Bacillus cereus* TISTR 2373 using the disk diffusion method and broth microdilution assay. Cell viability and proliferation were evaluated using the MTT assay on human fibroblasts in serum-supplemented and serum-depleted media. The scratch wound healing assay was employed to quantify cell migration and wound closure. The results demonstrated that both leaf and inflorescence extracts exhibited antibacterial activity, with the inflorescence extract showing higher efficacy against *S. aureus*. Furthermore, the extracts enhanced fibroblast proliferation and migration in a concentration-dependent manner. Notably, the leaf extract outperformed the inflorescence extract in promoting fibroblast proliferation and migration, particularly at earlier time points. These findings suggest that *C. sativa* extracts, particularly from the leaves, possess wound-healing properties and could be developed as novel therapeutic agents for promoting efficient wound repair and managing chronic non-healing wounds.

Keywords: Wound healing, *Cannabis sativa*, Hang Kra Rog Phu Phan, Anti-bacterial, Human fibroblast, Proliferation, Cell migration

Introduction

The skin is the body's first line of defense against various external insults, including mechanical damage, microbiological invasion, extreme heat, UV rays and environmental toxins. Its complex structure and multifaceted functions are crucial for maintaining homeostasis and safeguarding internal tissue from external threats. In the event of injury, the skin initiates a coordinated series of biological processes

collectively known as the wound-healing cascade. These processes involve inflammation, proliferation and tissue remodeling, orchestrated by various cell types and molecular mediators. Proper wound healing is essential for restoring tissue integrity and function, minimizing the risk of infections and preventing further damage [1]. Proper wound healing is crucial for restoring tissue integrity and function, minimizing infection risks and preventing further damage. However, chronic non-healing wounds remain a significant clinical challenge, necessitating the exploration of novel therapeutic approaches to promote efficient wound healing and mitigate associated complications.

Natural products have emerged as promising candidates for wound management, owing to their diverse pharmacological properties and potential to modulate key cellular processes involved in tissue repair [2-4]. *Cannabis sativa* L. (*C. sativa*) boasts a rich history of medicinal use due to its diverse pharmacological profile. This profile encompasses a range of therapeutic effects, including anti-inflammatory, antioxidant, antibacterial, analgesic, anticancer and anxiolytic properties [5]. The cutaneous endocannabinoid system (ECS) plays a crucial role in maintaining skin homeostasis and regulating various skin functions such as sebaceous gland activity, inflammation, immune response and the wound healing process, which responds to cannabinoid compounds. Recent research has delved into the potential of *C. sativa* extracts and their key components, cannabinoids such as cannabidiol (CBD) and tetrahydrocannabinol (THC), for wound healing applications [4,6,7]. The anti-inflammatory attributes of cannabinoids have been documented *in vitro* and in animal models. [8,9]. Moreover, both CBD and THC have been found to enhance cell viability in a dose-dependent manner in healthy and senescence-induced fibroblasts. Specifically, CBD fosters proliferation by elevating the expression of cyclin D1 and Ki-67, while also stimulating cell migration through the activation of the p38 MAPK and ERK1/2 pathway. Moreover, *C. sativa* is known to be a rich reservoir of various phytochemicals, including cannabinoids, terpenoids, alkaloids, flavonoids, peptides, tannins and phenolic compounds. Extracts obtained from different parts of the cannabis plant have shown strong antioxidant and antimicrobial properties [10-13]. Numerous studies have proven that these extracts can effectively neutralize free radicals and inhibit the growth of a broad spectrum of microorganisms, including bacteria, fungi and viruses. The results suggest that the unique phytochemical composition of *C. sativa* contributes to its exceptional bioactivities, positioning it as a promising candidate for various therapeutic and industrial applications.

While the flower clusters of *C. sativa* have been the primary focus of research, the leaves and stems of the plant may also hold unique phytochemical compositions with potential wound-healing capabilities. Prior studies have shown that extracts from the flower clusters can enhance the cell viability of fibroblasts and keratinocytes, which are vital for wound healing [14,15]. However, a thorough assessment of the wound-healing potential of *C. sativa* leaf extracts in comparison to flower cluster extracts is yet to be conducted. Hang Kra Rog Phu Phan ST1 has been officially recognized and registered by the Rajamangala University of Technology Isan, Thailand, and is 1 of 4 cultivars classified by the Thai government. The flower clusters of this cannabis variety are large and densely packed, bearing a resemblance to a triangular, bushy shape akin to a squirrel's tail. Contrary to the strong scent often associated with certain cannabis strains, this cultivar gives off a pleasant, fruity aroma reminiscent of ripe mangoes. Importantly, this variety is recognized for producing a significant amount of THC [16]. Nonetheless, the data on the potential applicability of this cultivar is sparse. As such, this study aims to comparatively evaluate the wound-healing activity of ethanolic extracts derived from *C. sativa* leaves and flower clusters. In particular, we examine their antibacterial properties, cytotoxicity and ability to promote cell proliferation and migration. By shedding light on the potential wound-healing properties of these extracts, this research could result in novel therapeutic approaches for promoting efficient wound healing and managing chronic non-healing wounds.

Materials and methods

Plant extract preparation

The dried leaves and inflorescences of Hang Kra Rog Phu Phan ST1 were kindly provided by the RMUTI Cannabis and Herbs Institute (Sakon Nakhon, Thailand). Harvesting of the plants occurred at 190 days post-cultivation. Following harvest, the leaves and inflorescences were carefully collected and subjected to shade drying. Subsequently, the samples underwent a fine-grinding process. They subjected to extraction using absolute ethanol at a ratio of 1:10. The resultant suspension was then agitated in an orbital shaker operating at 120 rpm at ambient temperature for 72 h. Following this extraction period, the suspension was filtered through a No. 1 Whatman filter. The resulting extracts were then concentrated using a rotary evaporator and subsequently stored at 4 °C until required for further experimentation.

Cell culture

The human fibroblast BJ cell line, procured from the American Type Culture Collection (ATCC, CRL-2522), was cultured in Eagle's Minimum Essential Medium (EMEM) (ATCC, USA). This medium was supplemented with 10 % (v/v) fetal bovine serum (Gibco, USA) and 1 % (v/v) penicillin/streptomycin (Gibco, USA), and the cells were maintained under standard cell culture conditions at 37 °C within a humidified atmosphere containing 5 % CO_2. For certain experimental techniques that necessitated serum deprivation, the cells were cultivated in a serum-free culture medium.

Cell viability assay

The influence of extracts obtained from leaves and inflorescences on cell viability was evaluated using the MTT assay. BJ cells were planted at a density of 5×10^3 cells/well in a 96-well plate and left to incubate overnight in a complete medium. Following this, cells were subjected to a range of concentrations of either leaf or inflorescence extract, ranging from 0.390625 to 200 µg/mL, achieved by a 2-fold dilution of the extracts in either a complete medium or a serum-deprived medium. Each well was administered 100 µL of the treatment solutions. The cells were subsequently incubated under standard conditions of 5 % CO_2 at 37 °C for 24 and 48 h. In accordance with the manufacturer's protocol, 10 µL of a 5 mg/mL solution of MTT (Tokyo Chemical Industry, Japan) was added to each well and further incubated under 5 % CO_2 at 37 °C for 2 to 4 h. The presence of a purple precipitate, indicative of cellular activity, was assessed microscopically. Following this, the treatment medium was carefully removed, and 200 µL of dimethyl sulfoxide (DMSO) was introduced to each well to dissolve the formazan crystals. Absorbance readings at 570 nm were then taken using a microplate reader (SPECTROstar Nano microplate reader; BMG LABTECH, Germany) as presented in **Figure 1**. The experimental procedure was carried out in triplicate and repeated on 3 separate instances.

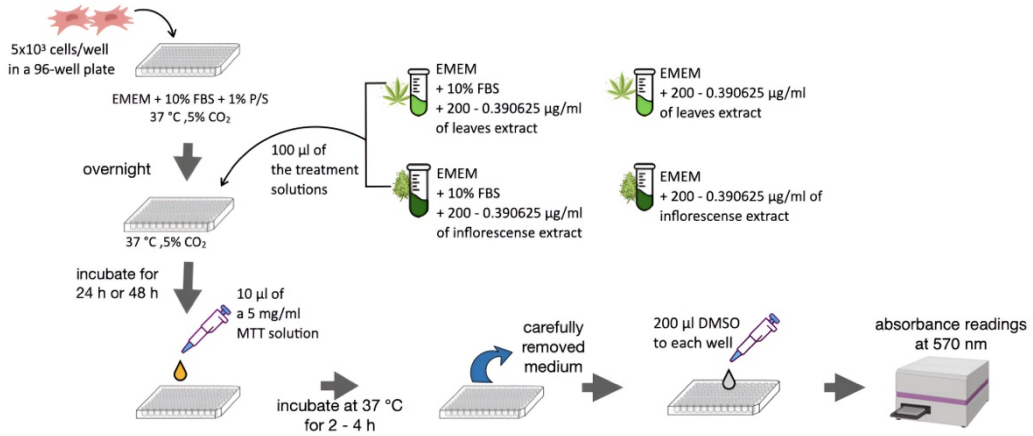

Figure 1 A schematic representation of the cell viability assay.

Cell migration assay

The potential for cell migration and wound healing was assessed using a scratch wound healing assay. In brief, BJ cells were planted in 24-well plates at a density of 1.0×10^5 cells per well and subsequently cultured in Eagle's Minimum Essential Medium (EMEM) enriched with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37 °C in a 5 % CO_2 environment. After a 24-hour incubation period, a scratch was created across the confluent monolayer using a SPLscar Scratcher (SPL Life Sciences, Korea). Following the scratch, the culture medium was replaced with fresh medium to eliminate any cellular debris. For the treatment, cells were exposed to a serum-deprived medium supplemented with either leaf or inflorescence extract, with concentrations ranging from 0.390625 to 50 µg/mL, determined to be non-toxic. Cells that were scratched but untreated served as the negative control in the experiment. The dynamics of cell migration were observed at 6 and 12 h post-treatment using an inverted light microscope (Eclipse TE2000-S, Nikon, Japan). The assay was carried out in triplicate, with 3 images taken from each treatment condition. A quantitative analysis of wound closure was conducted using Image J software. The percentage of wound closure was calculated using the following formula [17] :

$$\text{Percentage of wound closure} = \frac{\text{wound area (0 h)} - \text{wound area (t h)}}{\text{wound area (0 h)}} \times 100\ \%$$

Bacterial preparation

The bacterial strains *Staphylococcus epidermidis* TISTR 518, *Klebsiella pneumoniae* TISTR 1383, *Pseudomonas aeruginosa* TISTR 2370, *Staphylococcus aureus* TISTR 1466 and *Bacillus cereus* TISTR 2373 were procured from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR). A single colony from each of the tested bacteria was meticulously selected and transferred into a 10 mL tube containing nutrient broth (NB). This tube was subsequently incubated at a temperature of 37 °C with continuous agitation for a period of 18 h. Prior to their application, the concentration of the bacteria was adjusted to an optical density (OD600) of 0.1 [18,19].

***In vitro* antibacterial activity determination**

The antagonistic attributes of each extract were assessed using the disk diffusion method. Bacterial cultures, which had been cultivated overnight, had their cell concentrations adjusted to an optical density (OD600) of 0.1. A quantity of 100 mL from each pathogenic bacterial culture was uniformly distributed onto nutrient agar (NA). Subsequently, a sterile paper disk (0.6 mm) was positioned onto the NA. Ten

microliters of each extract were applied to the center of the paper disk, with DMSO serving as negative controls. The extracts were granted a diffusion period of 10 min before the NA plates were incubated at 37 °C overnight. The emergence of an inhibition zone around the paper disks was subsequently quantified [20,21].

The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) values were determined by the use of the broth microdilution assay. Extracts from the leaves and inflorescences of Hang Kra Rog Phu Phan ST1 were serially diluted twofold in a 96-well plate containing NB to achieve a range of concentrations. The bacterial inoculum, which had been cultivated overnight, was adjusted to an OD600 of 0.1 before 100 µL was introduced to each well. DMSO was used as negative controls. The 96-well plates were subsequently incubated at 37 °C overnight. To denote MIC and MBC values, 50 µL of iodonitrotetrazolium chloride (INT) was added to each well of the 96-well plate and incubated at 37 °C for 30 min. Wells that displayed bacterial growth turned pink, while those without bacterial growth remained yellow [22].

Data analysis

The determination of *in vitro* antibacterial activity was conducted using an experimental design, which was subsequently followed by a descriptive analysis. For cell viability and cell migration, the data was represented as the mean ± standard deviation (SD). A 1-way analysis of variance (ANOVA) was utilized for data comparison, which was then followed by Duncan's multiple range test (DMRT) for multiple comparisons. Statistical significance was determined at *p*-values less than 0.05.

Results and discussion

Effect of *C. sativa* (Hang Kra Rog Phu Phan ST1) extract enhances cell proliferation of BJ cells in complete and serum-deprived medium

The influence of *C. sativa* extracts on BJ fibroblast cell viability and proliferation was investigated in both serum-supplemented and serum-depleted media. In serum conditions, the growth stimulatory effects of leaf extract decreased with concentration. As a result, it can promote cell proliferation at low concentrations and exhibit cytotoxic effects at high concentrations (**Figure 2(A)**). In contrast, no concentration-dependent effect was observed in serum-deprived conditions. Specifically, concentrations ranging from 0.391 to 25 µg/mL of the extract enhanced proliferation at 24 h, followed by a decrease at 48 h. Higher concentrations (50 - 200 µg/mL) also increased proliferation, albeit less pronouncedly and exhibited no significant difference between 24 and 48 h incubation periods (**Figure 2(B)**). Furthermore, the inflorescence extracts also promoted cell proliferation at low concentrations. Notably, proliferation increased by approximately 36 % at 50 µg/mL in serum-deprived conditions. However, cells cultured only in serum-depleted media displayed heightened sensitivity to the extract's effects at higher concentrations (100 - 200 µg/mL) (**Figures 2(C) - 2(D)**). The observed phenomenon could potentially be attributed to the presence of proteins, hormones, nutrients or growth factors in Fetal Bovine Serum (FBS) that mitigate cell death or interact with the effects of the extracts. However, it is important to note that conditions of serum deprivation can instigate cellular stress, which may subsequently influence cell viability and proliferation. Consequently, careful consideration must be given to the duration of serum deprivation and the concentrations of plant extracts utilized. This is to ensure that the effects observed are specifically attributable to the extracts and are not merely a manifestation of cellular stress or cytotoxicity.

The results indicate that the ethanolic extract derived from both the leaf and inflorescence of *C. sativa* can enhance cellular proliferation upon treatment. Additionally, the leaf extract demonstrates efficacy in

promoting short-term cellular proliferation, whereas the inflorescence extract exhibits effectiveness over longer durations of treatment.

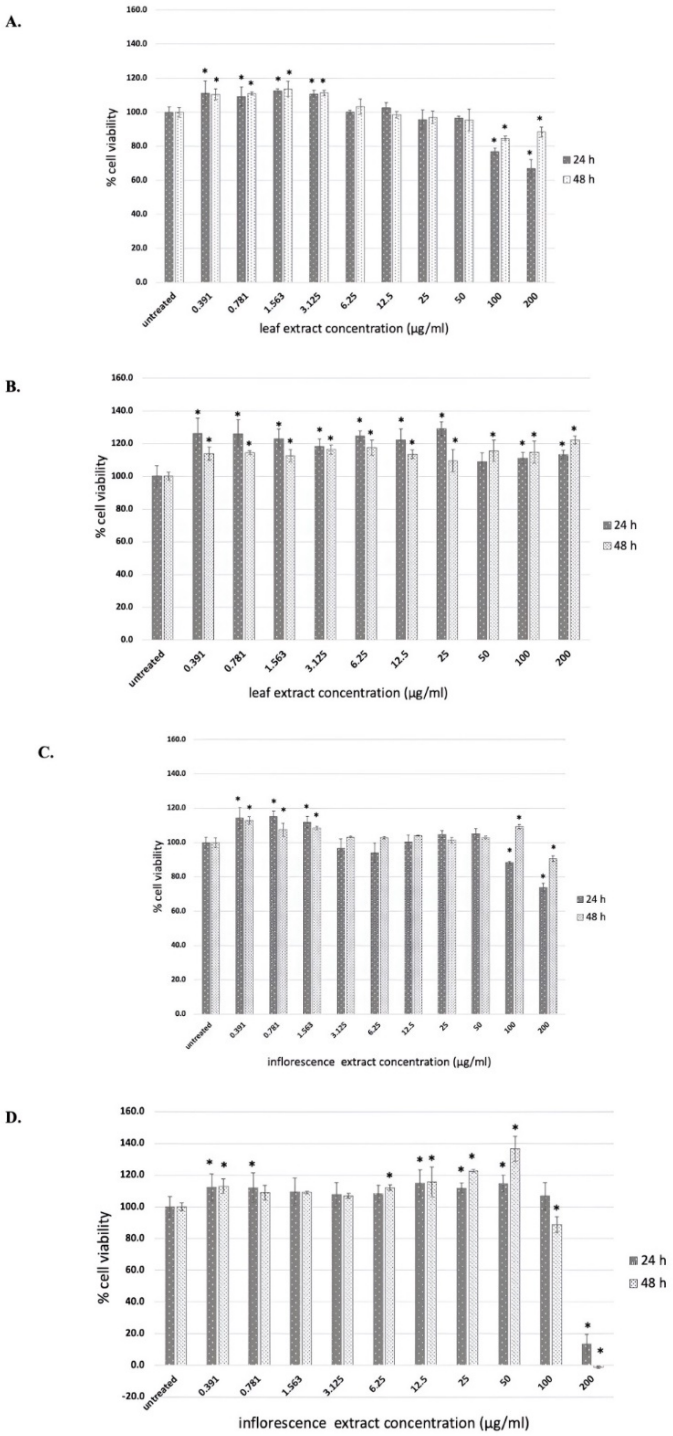

Figure 2 Effects of different concentrations of leaf and inflorescence extracts of *Cannabis sativa* on the proliferation of BJ fibroblasts in serum-supplemented and serum-depleted media. (A) Leaf extract in serum-supplemented media. (B) Leaf extract in serum-depleted media. (C) Inflorescence extract in serum-supplemented media. (D) Inflorescence extract in serum-depleted media. Data are represented as mean ± SD (n = 3; triplicates). $^{*}p < 0.05$ compared to the control group (untreated cells).

Evaluation of *C. sativa* (Hang Kra Rog Phu Phan ST1) extracts for wound-healing activity using an *in vitro* scratch assay

The wound-healing potential of *Cannabis sativa* extract was evaluated using the scratch assay, a widely employed method to quantify cell migration capacity, which is a critical aspect of the wound-healing process [23]. In this study, BJ cells were exposed to non-toxic concentrations (0.39 - 50 µg/mL) of leaf or inflorescence extracts in serum-free conditions. Cell migration was assessed at 6 and 12 h post-treatment and the extent of wound closure was quantified using Image J software. Our findings demonstrate that *C. sativa* extracts significantly promote the migration of human fibroblasts. As early as 6 h post-treatment, the average wound closure of extract-treated cells was significantly higher compared to the untreated group. Specifically, cells treated with leaf extract concentrations of 3.12, 6.25 and 12.5 µg/mL achieved wound closure of 93.40 ± 0.84, 87.74 ± 3.81 and 87.05 ± 3.2 5%, respectively (**Figures 3(A)**, **4** and **5**). Although leaf extracts exhibited greater activity compared to inflorescence extract at 6 h, by the 12-hour time point, cells treated with extracts from both sources within the concentration range of 0.391 - 6.25 µg/mL demonstrated wound closure exceeding 90 %. Notably, the most effective closure was observed with 3.12 µg/mL of leaf extract, achieving 96.4 ± 2.1 % wound closure **(Figures 3(B)**, **4** and **5**). Wound healing progresses through a well-defined sequence encompassing hemostasis, inflammation, cell proliferation and remodeling. Fibroblasts serve as contributors during the proliferative phase. These cells actively synthesize and secrete various extracellular matrix (ECM) components, including collagen, glycosaminoglycans and proteoglycans. This deposited ECM establishes a temporary scaffold that facilitates cellular migration, adhesion and differentiation. Additionally, fibroblasts promote capillary formation (angiogenesis), a crucial process for delivering oxygen and nutrients to the developing tissue, fostering a microenvironment that supports successful wound repair [24].

Our research showed that *C. sativa* Hang Kra Rog Phu Phan ST1 leaf and inflorescence extracts can stimulate wound healing activity utilizing antibacterial activity, cell migration and proliferation. The *C. sativa* plant's inflorescences contain significant concentrations of THC and CBD, the 2 most researched and well-known cannabinoids. Though in smaller amounts than in flowers, cannabinoids are also present in leaves. Furthermore, terpenoids can be detected in both leaves and inflorescences, with larger amounts seen in the inflorescence [25]. Gerasymchuk [10] *et al.* found that 2 µM THC increased cell survival while 2 µM CBD decreased it. THC had a stronger and faster effect on human dermal fibroblasts than CBD, even though both substances increased cell migration. However, CBD decreases cell viability dose-dependently, with effects observed at concentrations exceeding 2 µM and apoptosis induction was observed at high doses of 10 µM and above [26]. The study of CBD on HaCat keratinocytes showed CBD can induce cell proliferation when activated at a low concentration (0.6 µg/mL) in long-term culture by inducing vascular endothelial growth factor (VEGF) gene expression [27]. While high dose of THC significantly inhibits cell proliferation and cell migration on both amniotic epithelial cell line and primary cells through the regulation of matrix metalloproteinases (MMP2 and MMP9) [28]. *C. sativa* has been thoroughly investigated and 750 secondary metabolite compounds have been identified - including polyphenols, terpenoids, alkaloids and cannabinoids [29]. Further research is needed to determine how the extracts affect the healing of wounds. Sangiovanni *et al.* [30] reported that the production of inflammatory mediators involved in wound healing and inflammatory processes occurring in human dermal fibroblasts and HaCaT cells can be inhibited by *C. sativa* ethanolic extract. Specifically, 84 genes are related to either wound healing or the inflammatory response. The expression of 16 genes involved in wound healing and 26 genes involved in inflammation is elevated following TNFα treatment. As opposed to pure CBD, which was only able to downregulate a small number of genes, the extract was able to lower all the upregulated genes, and its effect was greater [30].

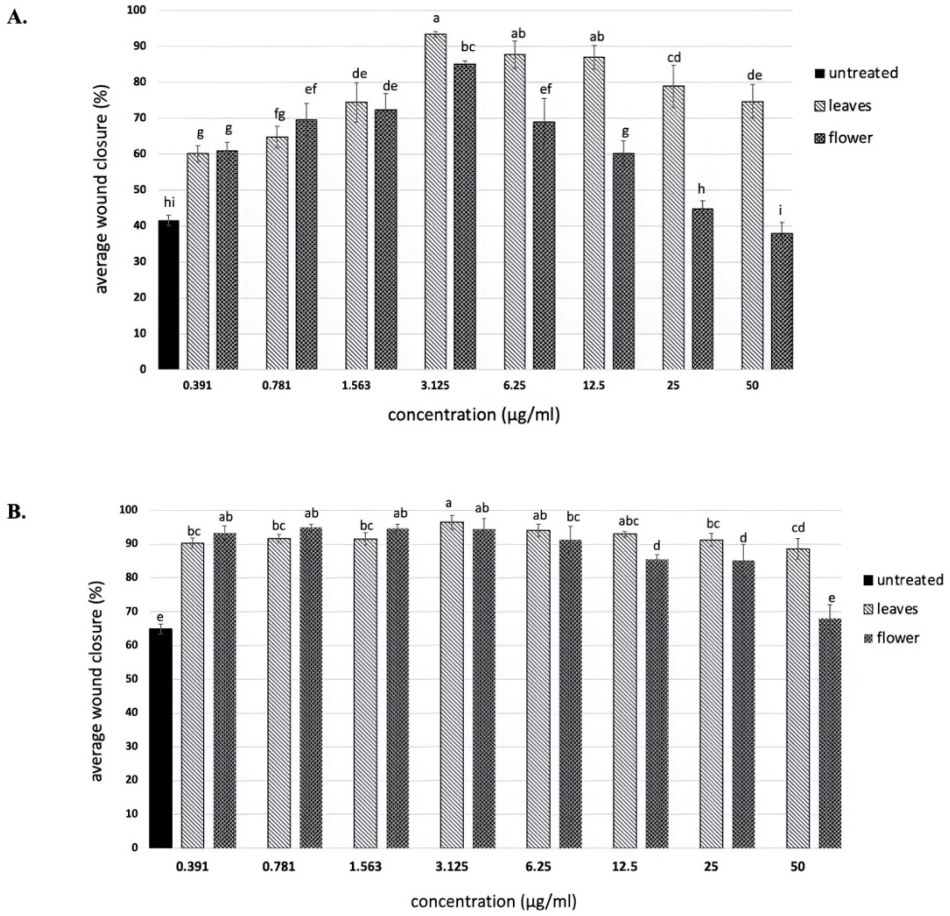

Figure 3 Effects of different concentrations of leaf and inflorescence extracts of *Cannabis sativa* on the cell migration of BJ fibroblasts in serum-depleted media at 6 (A) and 12 h (B) of treatments. Data are represented as mean ± SD (n = 3; triplicates). The superscript letters denote statistically significant differences ($p < 0.05$) between the treatment group receiving leaf or inflorescence extract compared to the untreated cells (control group).

Antibacterial activity

The antibacterial properties of inflorescence and leaf extracts from Hang Kra Rog Phu Phan ST1 were assessed against 5 pathogenic bacteria: *Staphylococcus epidermidis* TISTR 518, *Klebsiella pneumoniae* TISTR 1383, *Pseudomonas aeruginosa* TISTR 2370, *Staphylococcus aureus* TISTR 1466 and *Bacillus cereus* TISTR 2373. The most significant inhibition zone, measuring 13 mm, was observed with the inflorescence extract against *S. aureus* TISTR 1466 (**Figure 6**). This was followed by a 9 mm inhibition zone against *S. aureus* TISTR 1466 and *S. epidermidis* TISTR 518, achieved by both the inflorescence and leaf extracts (**Table 1**). In a related study by Ali *et al.* [31] the seed oil of *C. sativa* showed substantial antibacterial activity, with inhibition zones ranging from 21 to 28 mm against *B. subtilis* and *S. aureus*. Similarly, the petroleum ether extract from the whole plant exhibited significant antibacterial activity, with inhibition zones of 23 to 28 mm against both *B. subtilis* and *S. aureus*. The methanol extract from the entire plant also demonstrated notable antibacterial activity, with a 12 mm inhibition zone specifically against *Staphylococcus aureus* [32]. Rattanasuk and Phiwthong [33] reported that the extracts from *Spathiphyllum wallisii* have antimicrobial properties against human pathogenic bacteria. Their findings suggested that *S.*

wallisii extracts can prohibit the growth of *S. aureus* TISTR 1466, presenting a zone of inhibition ranging from 0.6 to 2.0 cm.

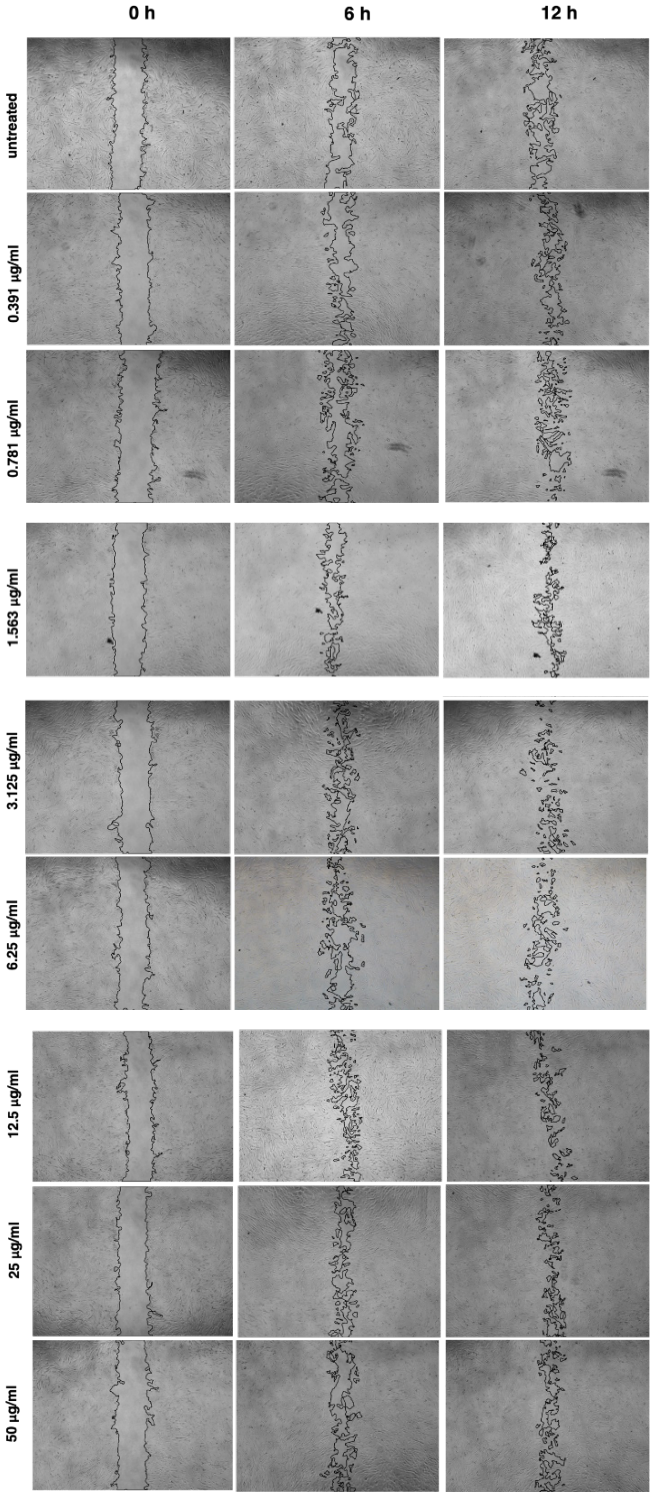

Figure 4 The effects of different concentrations of leaf extracts of *Cannabis sativa* on the cell migration of BJ fibroblasts in serum-depleted media were monitored at 6 and 12 h after treatment by an inverted light microscope (magnification ×100). The images were analyzed using the ImageJ program. The black lines in the images mark the boundaries of the scratched gaps.

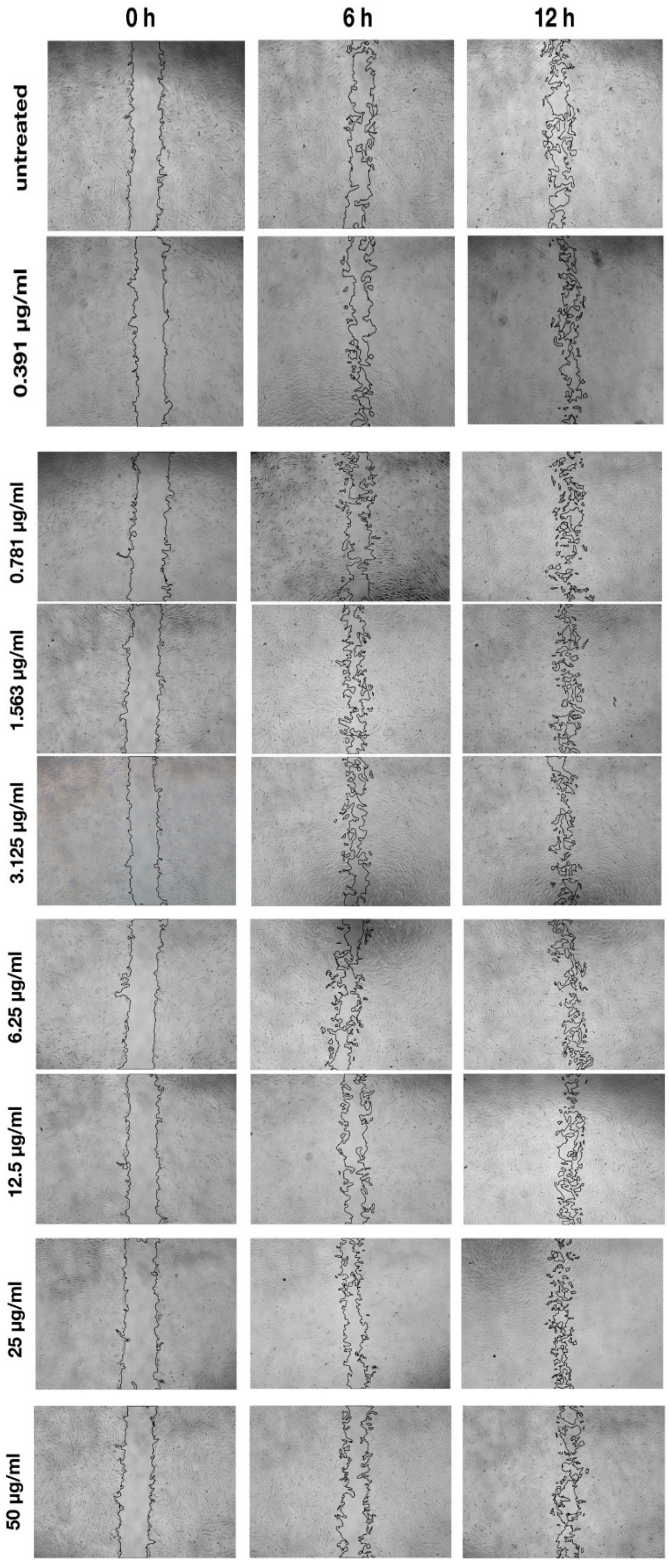

Figure 5 The effects of different concentrations of inflorescence extracts of *Cannabis sativa* on the cell migration of BJ fibroblasts in serum-depleted media were monitored at 6 and 12 h after treatment by an inverted light microscope (magnification ×100). The images were analyzed using the ImageJ program. The black lines in the images mark the boundaries of the scratched gaps.

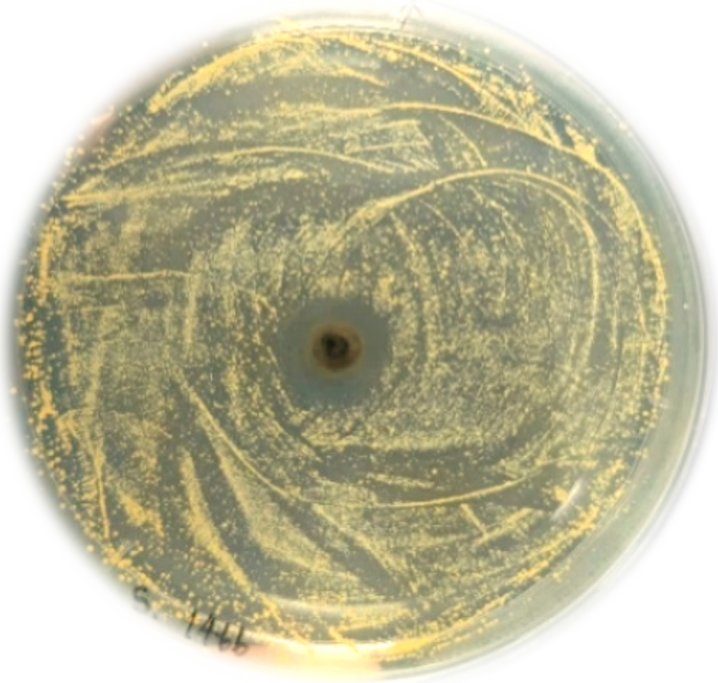

Figure 6 The agar disc diffusion results of the inflorescence extract against *S. aureus* TISTR 1466.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the inflorescences and leaf extracts from Hang Kra Rog Phu Phan ST1 were ascertained using the microbroth dilution and colorimetric assay. The results indicated that the lowest MIC and MBC values, less than 0.048 and 0.048 mg/mL, respectively, were derived from the inflorescences extract against *S. aureus* TISTR 1466. This was succeeded by the leaf extract, which displayed MIC and MBC values of 0.09 and 0.19 mg/mL, respectively against *S. aureus* TISTR 1466 (**Table 1**). These findings are consistent with a previous report by Serventi *et al.* [34] who demonstrated the antibacterial effectiveness of the hydroalcoholic extract of *C. sativa* cv. *strawberry* inflorescences. Their study exhibited MIC values ranging from 15.74 to 62.99 µg/mL against *S. aureus*. In a study conducted by Csakvari *et al.* [35] the antibacterial potential of silver nanoparticles synthesized using various *C. sativa* leaf extracts was investigated. The results suggested that the combination of silver nanoparticles and *C. sativa* leaf extracts exhibited potent antibacterial activity against *K. pneumoniae*, *P. fluorescens* and *S. aureus*. Similarly, Iseppi *et al.* [36] investigated the antibacterial effectiveness of essential oils derived from fiber-type *C. sativa* against bacterial pathogens. Their findings revealed MIC values ranging from 2 - 32 µg/mL against *S. aureus*, 1-16 µg/mL against *S. epidermidis* and 1 - 16 µg/mL against *B. cereus* [36]. Moreover, Frassinetti *et al.* [37] reported the antibacterial activity of hemp seed extract. The results suggested that the hemp seed extract concentration at 0.5 - 2.5 mg/mL can inhibit the growth of *S. aureus* ATCC 35556 and *S. aureus* ATCC 25923.

Table 1 The antibacterial activity of Hang Kra Rog Phu Phan ST1 inflorescence and leaf extracts against tested bacteria.

Bacteria	Inflorescence extract			Leaf extract			DMSO
	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)	Inhibition zone (cm)	MIC (mg/mL)	MBC (mg/mL)	
Staphylococcus epidermidis TISTR 518	9	1.56	12.5	0.9	3.12	25	-
Klebsiella pneumoniae TISTR 1383	7	6.25	12.5	0.7	12.5	25	-
Pseudomonas aeruginosa TISTR 2370	7	1.56	25	0.8	3.12	25	-
Staphylococcus aureus TISTR 1466	13	< 0.048	0.048	0.9	0.09	0.19	-
Bacillus cereus TISTR 2373	7	-	-	0.8	6.25	12.5	-

Conclusions

Our study provides compelling evidence for the potential wound-healing properties of Cannabis sativa extracts, particularly those derived from the leaves of the Hang Kra Rog Phu Phan ST1 cultivar. The ethanolic leaf extract exhibited potent antibacterial activity against several pathogenic bacterial strains, including *S. aureus* and *S. epidermidis*, which are common causes of wound infections. Notably, the leaf extract demonstrated superior performance in promoting fibroblast cell proliferation and migration compared to the inflorescence extract, especially at earlier time points. The ability of the leaf extract to stimulate rapid cell migration and wound closure is a crucial attribute for effective wound healing, as it contributes to the early stages of the healing process, including inflammation and tissue remodeling. While the precise mechanisms underlying the wound-healing effects of the *C. sativa* leaf extract require further investigation, the unique phytochemical composition, including cannabinoids, terpenes and phenolic compounds, is likely responsible for its bioactivities. The synergistic interactions between these compounds may contribute to the observed antibacterial, proliferative and pro-migratory effects on fibroblasts, which play a pivotal role in wound repair processes. These findings pave the way for the development of novel wound-healing therapies based on *C. sativa* leaf extracts.

Acknowledgements

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