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ORIGINAL ARTICLE

Evaluation of Wound Healing, Antioxidant and Antimicrobial Effects of *Cinnamomum zeylanicum* Extracts: *In Vivo* and *In Vitro* Approaches

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ABSTRAK

Cinnamomum zeylanicum mempunyai pelbagai sifat biologi, termasuk kesan antioksidan, antibakteria dan anti-radang, yang menunjukkan potensinya dalam merangsang penyembuhan luka. Kajian komprehensif telah dilakukan menggunakan model luka eksisi pada tikus Wister untuk menilai keupayaan penyembuhan luka bagi ekstrak tumbuhan ini. Sebanyak 48 tikus Wister dibahagikan kepada empat kumpulan iaitu kawalan normal, kawalan kenderaan, povidone-iodine sebagai rawatan piawai dan rawatan dengan ekstrak tumbuhan. Kontraksi luka diukur berdasarkan masa penyembuhan luka sepenuhnya dan pemerhatian makroskopik. Selain itu, pewarnaan hematoksilin dan eosin digunakan untuk menilai mikrostruktur kulit. Di samping itu, ujian aktiviti penyingkiran radikal bebas DPPH pula dilakukan untuk mengukur kesan antioksidan ekstrak tumbuhan. Keputusan menunjukkan bahawa luka yang dirawat dengan ekstrak tumbuhan sembuh dengan lebih cepat berbanding dengan kumpulan kawalan ($p<0.05$). Analisis histopatologi bagi kumpulan yang dirawat dengan ekstrak tumbuhan menunjukkan peningkatan re-epitelialisasi, neovaskularisasi dan aktiviti fibroblas yang lebih aktif, bersama dengan pengurangan pengumpulan sel radang. Selain itu, ekstrak etanol menunjukkan aktiviti antimikrob yang kuat, terutamanya terhadap bakteria Gram-positif, dengan merosakkan selaput sel dan menyebabkan kebocoran kandungan intrasel. Penemuan ini menyorot potensi ekstrak Cinnamomum zeylanicum dalam mempercepatkan penyembuhan luka, yang kemungkinannya disebabkan oleh sifat antioksidan dan antimikrob yang berkesan.

Kata kunci: *Antimikrob; antioksidan; Cinnamomum zeylanicum; histopatologi; kulit; penyembuhan luka*

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ABSTRACT

Cinnamomum zeylanicum possesses various biological properties, including antioxidant, antibacterial, and anti-inflammatory effects, which suggest it might promote wound healing. A comprehensive study was done using an excision wound model in Wister rat to determine the wound healing abilities of this plant extracts. A total of 48 Wister rats were distributed equally into four groups such as normal control, vehicle control, povidone-iodine as standard treatment and treatment with plant extract. Wound contraction was determined by the complete healed day of wounds and their macroscopic observation. Besides, hematoxylin and eosin staining was done to evaluate the skin microstructure. In addition, the 1,1-Diphenyl-2Picrylhydrazyl (DPPH) free radical scavenging activity assay was used to measure the antioxidant effect of plant extracts. The results indicated that wounds treated with the plant extract healed significantly faster compared to the control group ($p < 0.05$). Histopathology of the plant-treated group showed enhanced re-epithelialisation, neovascularisation, and accelerated fibroblast activity, along with reduced inflammatory cell accumulation. Additionally, the ethanolic extract displayed strong antimicrobial activity, particularly against Gram-positive bacteria, by disrupting cell envelopes and causing leakage of intracellular contents. These findings highlight the potential of *Cinnamomum zeylanicum* extract in accelerating wound healing, likely due to its potent antioxidant and antimicrobial properties.

Keywords: Antimicrobial; antioxidant; *Cinnamomum zeylanicum*; histopathology; skin; wound healing

INTRODUCTION

Cinnamomum zeylanicum (*C. zeylanicum*), also referred to as cinnamon, belongs to the Lauraceae family and is predominantly found in Asia and various tropical areas (Ranasinghe et al. 2013). It is a perennial tree with 10-15 meters in height which has a wide range of medicinal applications across the world (Kowalska et al. 2021). The plant consists with various parts including leaves, barks, seeds and fruits used as the medicinal and edible materials (Rao & Gan 2014). Traditionally it is used as tranquilisers, aphrodisiac and breath or mouth sweeteners (Saleem et al. 2015). It is believed that boiling *C. zeylanicum* plants consume to cure flu, headache, joint pain, gastric, intestinal, respiratory and cardiac diseases (Yan et al. 2010). It helps in digestion and provide relief from gas in the alimentary tract (Kawatra & Rajagopalan 2015). *C. zeylanicum* demonstrates a diverse spectrum of biological processes, including anti-inflammatory effects (Lee et al. 2005), antioxidant properties (Lee et al. 2003; Singh et al. 2007), antimicrobial activities (Matan et al. 2006), anti-diabetic functions (Khan et al. 2003), as well as tumor inhibitory effects (Kwon et al. 2009; Schoene et al. 2005).

Several studies have investigated that the bioactive phytochemicals present in *C. zeylanicum*, including cinnamaldehyde, eugenol, eucalyptol, linalool, α-terpineol, β-caryophyllene, camphor, cinnamyl acetate, α-humulene, α-pinene, δ-cadinene, p-cymene and limonene (Rao & Gan 2014; Unlu et al. 2010). Additionally, various phenolic and flavonoid compounds such as catechin, epicatechin and procyanidin B2 have been isolated from *C. zeylanicum*. These phytochemicals hold promise as potential sources for novel wound healing medicine (Ahmad at al. 2018). Moreover,

phytochemicals of the plant have been found to accelerate the wound healing process due to their anti-inflammatory, antioxidant, and antimicrobial properties (Ahmad et al. 2021). Several studies have demonstrated the wound-healing properties of *C. zeylanicum*. For instance, recent findings have reported its efficacy in promoting cellular repair and combating microbial infections in wound models (Kamath et al. 2003; Yuan et al. 2018). By conducting a comprehensive analysis, this study aimed to determine the efficacy of *C. zeylanicum* in promoting wound healing, assess its ability to neutralise free radicals, and investigate its effectiveness against various pathogenic bacteria. The findings of this research contributed to the understanding of the therapeutic potential of *C. zeylanicum* and supported its use in developing natural remedies for wound management.

MATERIALS AND METHODS

Plant Collection and Extraction

Bark materials of *C. zeylanicum* were collected from local market and authenticated by a botanist from the Bangladesh National Herbarium (Verification no. DACB 91376). The ethanolic extract was prepared using the maceration method with some modification (Huda et al. 2019). About 4 kg of dried *C. zeylanicum* barks were cut into small pieces and blended into powder. Then it was soaked into 20 liters of ethanol for 15 days. Then cotton filtration technique was used to filter the extract and a light dark colour solution was collected. Rotary evaporator was used to evaporate the ethanolic portion from extracts of *C. zeylanicum*. Extracts were stored at 80°C temperature to prepare for freeze drying process. After freeze drying, extract was obtained into fine particles using mortal pestle.

Topical Ointment Preparation

The topical medicament of *C. zeylanicum* extract was prepared using paraffin which is chemically inert and does not affect the skin. A pilot study was experimented to optimise the dose of plant extract, where, five different doses of extract were used: 0.1%, 0.5%, 1.0%, 2.0% and 4.0%. 2.0% concentration of *C. zeylanicum* extracts was optimised as the experimental dose base on the lowest amount and best efficacy compared to other groups in the excision wound model. Based on the pilot study results, the topical ointments were prepared by mixing the 2.0% concentration of *C. zeylanicum* extract with the paraffin ointment. In brief, firstly, fine particles were obtained from extracts using mortar and pestle. Then, the weighed extract and paraffin ointment were thoroughly mixed in a clean glass using two stainless steel spatulas. To make uniformity of medicament, this process was done three times for each preparation. Finally, the ointment was transferred into a jars with tight seal and label (Ahmad et al. 2022).

Experimental Animals and Treatment

The study was approved by the University of Asia Pacific Animal Ethics Committee (ethical approval number: UAP/REC/2022/111). A total of 69 Wister rats were sourced from the Jahangirnagar University Animal Research Center, Savar. All rats were 100 - 130 gm weight and 2-3 months old. They were acclimatised to the laboratory environment for one week, where the temperature was maintained at 22 ± 2°C, humidity at 60 ± 10%, and a day/night cycle of 12 hours each. The rats had access to food pellets and water *ad libitum*. Ketamine hydrochloride (Gonoshasthaya pharmaceuticals ltd, Bangladesh) was injected intraperitoneally to anesthetise at 0.2 mL/100

g body weight of rat. For this study, 69 Wister rats were utilised where 21 rats were used for pilot study and 48 rats were used for the final study. For the pilot study, 21 rats were equally subdivided into seven groups: vehicle, standard treatment and five different concentrations of extracts: 0.1%, 0.5%, 1.0%, 2.0% and 4.0%. On the other hand, 48 rats were equally subdivided into four groups: normal group without treatment (NC), vehicle dressing (VC), povidone-iodine ointment dressing as standard treatment (ST) and 2.0% of *C. zeylanicum* extract dressing (CE). Excision wound model had set for determining the wound healing effect in this experiment (Ahmad et al. 2021). After anesthetising the rats, hair on the dorsal surface was removed and four wounds were created on each rat using 6 mm biopsy punch. The day of introducing the wounds was considered as day zero. In order to ensure full healing, the wounds were dressed starting on day zero and they stayed that way until they had fully healed. All treatments were administered topically once daily. Wound dressing began on day zero and continued until complete healing. On day 3 and day 9, wound tissues from three rats in each group were collected for histological analysis.

Macroscopic Observation and Measurement of Wound Contraction

Two parameters including macroscopic appearance and wound contraction were determined for evaluating of excision wound healing. A digital camera and Vernier caliper were used to capture images and measured of wound diameter respectively. The diameter of wounds was measured at four directions on day 3, 6, 9, 10, and continued until complete healing (Fernandes et al. 2015). Wound area pictures were taken of each rat for macroscopic examination on same days as well as the measurement of wound diameter.

Histological Analysis

Three rats from each experimental group were sacrificed on both day 3 and day 9. Skin tissues from the wound site were collected and fixed in 10% formaldehyde for histopathological investigation. Then, paraffin wax was used to embed them and followed by slicing the tissue using a microtome. Hematoxylin and eosin (H&E) dye was used to stain these slices. Next, the slides were examined using a light microscope at 10x magnifications, and pictures were taken for histomorphological analysis. A blinded method utilising a modified 0 to 3 numerical scale was employed to score the stained sections based on the histological characteristics of the skin. Histologic scoring of skin was described in Table 1 (Abramov et al. 2007).

Phytochemicals Screening of *C. zeylanicum* Extracts

The crude ethanol extracts of *C. zeylanicum* were tested to detect secondary metabolites such as alkaloids, flavonoids, polyphenols, tannins, saponins, steroids and glycosides in accordance with standard methods (Rani et al. 2021).

Total Phenolic Contents

The presence of total phenolic contents (TPCs) in the bark extract was determined using the Folin-Ciocalteu Reagent (FCR) (Sigma-aldrich, Missouri, United States) with gallic acid (Dalian Chem, India) as the standard reference according to a previous method with some modification (Abeysekera et al. 2013). In summary, 1 mL of bark extract was dissolved with 5 mL FCR solution into a

TABLE 1: Scoring for histomorphological study of skin tissue

Variable	Scoring			
	0	1	2	3
Re-epithelialisation	None	Partial	Complete but immature or thin	Complete and mature
Inflammation cell infiltration	None	Scant	Moderate	Abundant
Fibroblast proliferation	None	Scant	Moderate	Abundant
Angiogenesis	None	Up to five vessels per HPF (High-Power Field)	6–10 vessels per HPF	More than 10 vessels per HPF
Granulation tissue formation	Immature	Mild maturation	Moderate maturation	Fully matured

test tube. Prior to incubation, 4 mL sodium carbonate solution was added to the test tube. Incubation was for 30 minutes at 20°C temperature for initiating the reaction with extracts. Using a UV spectrophotometer, the final solutions' absorbance was measured at 765 nm. Gallic acid was used as a standard reference to prepare the standard curve. TPC of the *C. zeylanicum* extracts was calculated as the gallic acid equivalent.

Total Flavonoid Contents

Total flavonoid contents (TFCs) was determined using aluminium chloride colourimetric method according to previous method described by Bao et al. (2005) with some modifications. In brief, 1 mL of *C. zeylanicum* extracts solution was dissolved with 3 ml ethanol, followed by adding of 0.2 mL of 10% $AlCl_3$, 0.3 mL $NaNO_2$, 0.2 mL of IM NaOH and 5.6 mL of distilled H_2O. Solution was incubated for 30 minutes at 37°C temperature. After developing a yellowish color of solution, absorbance was recorded at 510 nm using UV spectrophotometer. Catechin (Dalian Chem, India) was used to prepare the standard curve as a standard reference. TFC of the *C. zeylanicum* extracts was calculated as the catechin equivalent.

1,1-Diphenyl-2Picrylhydrazyl (DPPH) Scavenging Capacity Assay

The antioxidant activity of *C. zeylanicum* extract was assessed based on its capacity to scavenge 1,1-Diphenyl-2Picrylhydrazyl (DPPH) (Smeriglio et al. 2017). Four different concentrations (25, 50, 75 and 100 g/ml) of extract were prepared with 1 mL of 0.1 mM DPPH solution. Solution was incubated for 30 minutes at room temperature in the dark condition. Absorbance of the final solutions was recorded at 517 nm using ultraviolet (UV) spectrophotometer. The standard curve was prepared using ascorbic acid (Dalian Chem, India) as a standard reference. DPPH radical scavenging activity of *C. zeylanicum* can be calculated following equation; DPPH radical scavenging activity (% inhibition) = [1- (Absorbance of sample/ Absorbance of control)] x 100. Then, IC_{50} was determined using plot graph of the percentage of inhibitions against log concentration.

Antimicrobial Activity

- Collection and identification of microorganism from wound area on rat's skin

A sample of microorganisms was obtained from the wound fluid and tissue debris of an infected rat from the infected wound on rat skin using a cotton-tipped swab. The swab was then streaked onto a blood agar plate with a sterile loop and incubated at 37˚C for 18-24 hours. After incubation, the plate was inspected for bacterial growth. The bacterial colonies that developed on the blood agar were subsequently Gram-stained to assess the characteristics of the bacterial cell wall. Additionally, a catalase test was conducted to further confirm the bacterial identity.

- Collection and identification of microorganism

To assess the antimicrobial activity of *C. zeylanicum* extract, five microorganisms were collected from Ayesha Memorial Hospital in Bangladesh. The strains included *Escherichia coli* ATCC-O157, *Streptococcus spp.* ATCC-15913, *Salmonella enterica* serotype Typhi ATCC-6539, *Salmonella enterica* serotype Paratyphi A ATCC 9150, and *Staphylococcus aureus* ATCC 25923. Additionally, a sample was collected from an infected diabetic rat and identified as *Staphylococcus aureus* using the blood agar method. In total, six microorganisms, including the rat-derived sample, were collected and cultured at 37˚C on Mueller-Hinton agar media (HiMedia, India) for the study.

Zone of Inhibition Measurement

The disk-diffusion method was utilised to assess the antibacterial efficacy of ethanol extract from *C. zeylanicum* by measuring the zones of inhibition. Six petri dishes containing cultures of various microorganisms were prepared, and three wells (each with a diameter of 12 mm) were created in each dish using a cork borer. Among these, one well was filled with a low concentration (5%) of the *C. zeylanicum* extract, another with a high concentration (10%), and the third served as a control. Each petri dish was subdivided into four quadrants, with three designated for the treatment wells and the remaining quadrant reserved for a standard. For the purpose of establishing a comparison, an amoxicillin (30 µg) disk was incorporated as the standard, based on concentrations determined from a preliminary study. The plates were then incubated at 37˚C for a period of 24 hours. Following incubation, the diameters of the zones of inhibition (mm) surrounding each concentration of *C. zeylanicum* extract and the amoxicillin were recorded, thereby facilitating the evaluation of antibacterial activity. This entire process was replicated thrice to ensure the reliability of the results.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Minimum inhibitory concentration (MIC) was determined using the micro-dilution method. Varying concentrations of ethanolic *C. zeylanicum* extracts (2%, 5% and 10%) were prepared and added to 2 ml of Mueller Hinton Broth medium in separate tubes. Each tube was then inoculated with 20 µl of bacterial suspension, standardised to a turbidity equivalent to 0.5 McFarland. The tubes were incubated at 37˚C for 24 hours. The MIC was determined by observing the lowest concentration of cinnamon extract that inhibited visible bacterial growth. This

process was performed in triplicate to ensure accuracy. In addition, minimum bactericidal concentration (MBC) was determined by re-culturing the samples from the tubes that were used in the MIC determination. After incubation, the contents of the tubes showing no visible growth were streaked onto fresh agar plates and incubated again at 37°C for 24 hours. The MBC was identified as the lowest concentration of *C. zeylanicum* extract that resulted in no bacterial growth on the agar plates.

Statistical Analysis

Quantitative data were expressed as means ± standard errors (SE). One-way analysis of variance (ANOVA), associated with Tukey's HSD post-hoc test was used to compare the statistical data using SPSS statistics 23.0 software package, where, $p \leq 0.05$ was considered significant.

RESULTS

Macroscopic Assessment of Wound Healing

Macroscopic appearance of wounds in the four experimental groups on days 0, 3, 6, 9, 10, 11, 12 and 13 were depicted in the Figure 1. According to this macroscopic view, wounds

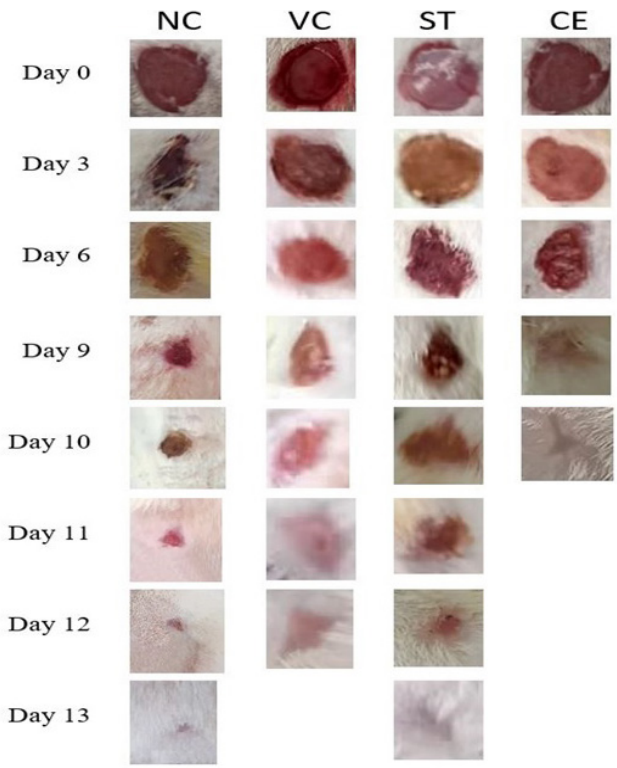

FIGURE 1: Macroscopic view of wounds of rat experimental groups: NC, VC, ST and CE against treatment day 0, 3, 6, 9, 10, 11, 12 and 13. Normal group without treatment (NC), vehicle dressing (VC), povidone-iodine ointment dressing as standard treatment (ST) and 2.0% of C. zeylanicum extract dressing (CE). Scale bar was 20 u.

treated with 2% *C. zeylanicum* extract started to heal around day 10 whereas, the VC group started healing from day 12, the ST group and the NC started to heal around day 13. The end of inflammation stage and the start of proliferation stage were seen in wounds treated with CE on day 3 and on day 6, a scab began to emerge. The pinkish pigment gradually gave way to the whitish fibrous tissue that was visible on day 10 after coming off the scab. In contrast, granular and fibrous tissues in other groups become visible later on days 9-11 and days 12-13, respectively.

Wound Contraction Measurement

In the Figure 2, the bar chart displayed the mean value of required days for complete wound healing in various experimental animal groups: NC, VC, ST, and CE. Here, CE-treated group achieved complete wound healing much faster than the other experimental groups. Treated group with CE restored the wound far more quickly than the other groups. These findings were supported by statistical analysis, where $p<0.05$ indicated a significant difference compared to the control groups (NC, VC, ST), while no significant differences were observed among the control groups themselves.

Histopathological Examinations

Figure 3 showed the histological appearance of skin wound. Table 2 depicted the scores obtained from the histopathological examination of the skins of four experimental groups by H&E staining. Histological results in all groups were equivalent in terms of re-epithelialisation, fibroblast cell proliferation, neovascularisation, and granulation tissue formation; however, inflammatory cell infiltration was antipodal. Except for inflammatory cell infiltration, other characteristics of skin histology determined by H&E stain were immature at this time, and the score ranged from 0.11 ± 0.07 to 0.17 ± 0.08. On day 3, the inflammatory cells scored 1.67 ± 0.12 to 2.17 ± 0.08, suggesting mild to moderate infiltration. Inflammatory cell infiltration was more prominent in CE group than NC and VC

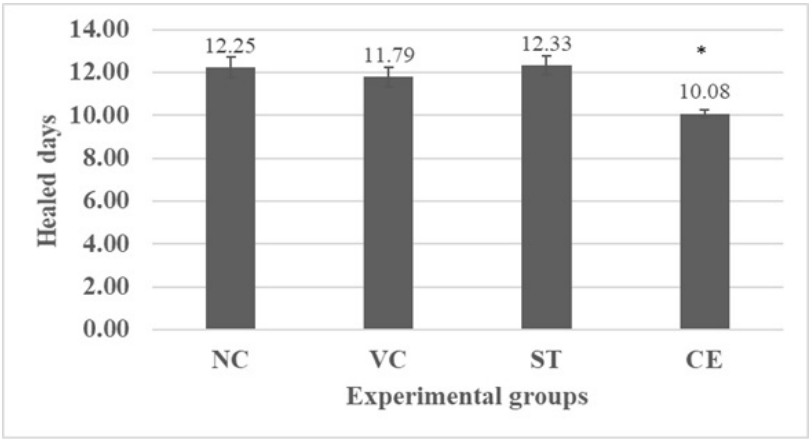

FIGURE 2: Bar chart represented the mean value of wounds healed day of rat experimental groups: NC, VC, ST and CE. Statistically significant results indicated as (*) $p < 0.05$ versus control groups (NC, VC and ST). Normal group without treatment (NC), vehicle dressing (VC), povidone-iodine ointment dressing as standard treatment (ST) and 2.0% of *C. zeylanicum* extract dressing (CE)

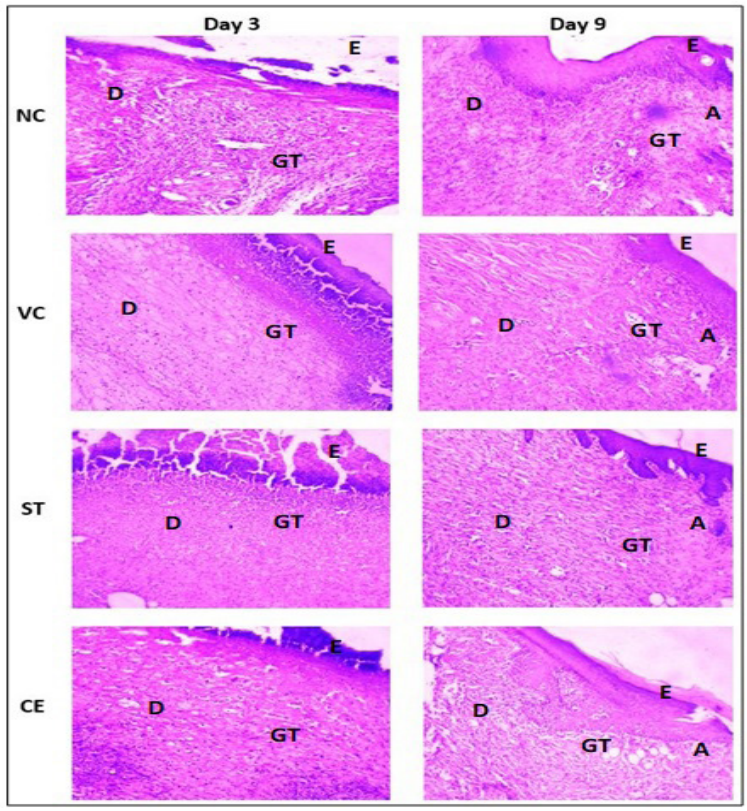

FIGURE 3: Histopathological view of skin wound by H & E staining of four experimental groups: NC, VC, ST and CE at day 3 and day 9 of post-wounding. Pictures of stain were at x10 magnification. Alphabets indicated the events during wound healing; E = Epidermis, D = Dermis, A = Angiogenesis, GT = Granulation tissue. Normal group without treatment (NC), vehicle dressing (VC), povidone-iodine ointment dressing as standard treatment (ST) and 2.0% of C. zeylanicum extract dressing (CE). Scale bar was 100 u

TABLE 2: Scores obtained from four experimental groups: NC, VC, ST and CE. All data were given as mean ± S.E. for six animals in each group. Statistically significant results were indicated as (a) $p < 0.05$ vs control groups (NC and ST), (b) $p < 0.05$ vs control groups (NC and VC), (c) $p < 0.05$ vs control groups (NC, VC and ST)

Groups	Re-Epithelialisation		Inflammatory cell infiltration		Fibroblast cell proliferation		Neo-Vascularization		Granulation tissue formation	
	Day 3	Day 9	Day 3	Day 9	Day 3	Day 9	Day 3	Day 9	Day 3	Day 9
NC	0.11 ± 0.07	2.17 ± 0.08	1.67 ± 0.12	1.17 ± 0.12	1.11 ± 0.07	2.06 ± 0.06	0.11 ± 0.07	2.11 ± 0.07	0.11 ± 0.07	2.11 ± 0.07
VC	0.17 ± 0.08	2.33 ± 0.12	1.78 ± 0.09	1.11 ± 0.07	1.17 ± 0.08	2.11 ± 0.07	0.11 ± 0.07	2.11 ± 0.07	0.17 ± 0.08	2.22 ± 0.12
ST	0.11 ± 0.07	2.22 ± 0.09	2.06 ± 0.10	1.11 ± 0.07	1.17 ± 0.08	2.22 ± 0.09	0.06 ± 0.06	2.22 ± 0.09	0.11 ± 0.07	2.11 ± 0.07
CE	0.17 ± 0.08	2.72 ± 0.09[a]	2.17 ± 0.08[b]	0.83 ± 0.12	1.17 ± 0.08	2.67 ± 0.08[c]	0.11 ± 0.07	2.50 ± 0.12	0.22 ± 0.12	2.56 ± 0.13

groups. Later on, the ratings ranged from 0.83 ± 0.12 to 1.17 ± 0.12, with the group treated with CE having the least infiltration. On day 9, re-epithelialisation of CE group was more noticeable than NC and ST group, similarly fibroblast cell was more significant in CE group than the other three control groups (NC, VC & ST).

Identification of Phytochemicals

The phytochemical screening of crude ethanolic extracts of *C. zeylanicum* revealed the existence of various secondary compounds, including flavonoids, polyphenols, alkaloids, saponins, tannins, steroids and glycosides, as indicated in Table 3.

Determination of TPCs and TFCs

Constituting major group of phytochemical compounds, phenol components are extremely important for their antioxidant activity. The *C. zeylanicum* extract had TPCs of 31.01 ± 1.54 mg/g in gallic acid equivalent (GAE). In the ethanolic extract of *C. zeylanicum* also found flavonoid compounds. TFCs in *C. zeylanicum* is 59.28 ± 1.64 mg/g in rutin equivalent (RE) which was quite rich in range (Table 4).

TABLE 3: Quantitative phytochemical evaluation of *C. zeylanicum* extract

Name of phytochemicals	Intensity of *C. zeylanicum* ethanolic extract's phytochemicals
Alkaloids	+++
Polyphenols	++
Flavonoids	+++
Tannins	+
Saponins	+
Steroids	+
Glycosides	+

High concentration (+++); moderate concentration (++); and low concentration (+)

Free-Radical Scavenging Activity of *C. zeylanicum*

Antioxidant effect of ethanolic *C. zeylanicum* bark extract were done by DPPH radical scavenging capacity. Strong antioxidants react with DPPH stable free radicals, resulting in the transformation of a strong violet color into a colourless molecule. The degree of discoloration can use to measure as an indicator of the antioxidant activity. In the experiment, The *C. zeylanicum* ethanolic extract was found to have a high DPPH activity of 79.20%, which was a strong indicator of having antioxidant action as shown in the Figure 4.

Identification of the Microorganism Collected from Wound of Rat's Skin

The gram-stain confirmed the presence of gram-positive cocci and the colonies showed typical morphological characteristics of *Staphylococcus aureus*, including round, convex, smooth and opaque appearance.

Antimicrobial Activity

The result of zone of inhibition demonstrated in Table 5 had shown a significant inhibitory effect of ethanolic extract of cinnamon on different bacterial strain. High concentration

TABLE 4: Quantitative phytochemical evaluation of *C. zeylanicum* extract

Phytochemicals (per gram of *C. zeylanicum* extract)	Concentration (mg/g)
Polyphenols	31.01 ± 1.54
Flavonoids	59.28 ± 1.64

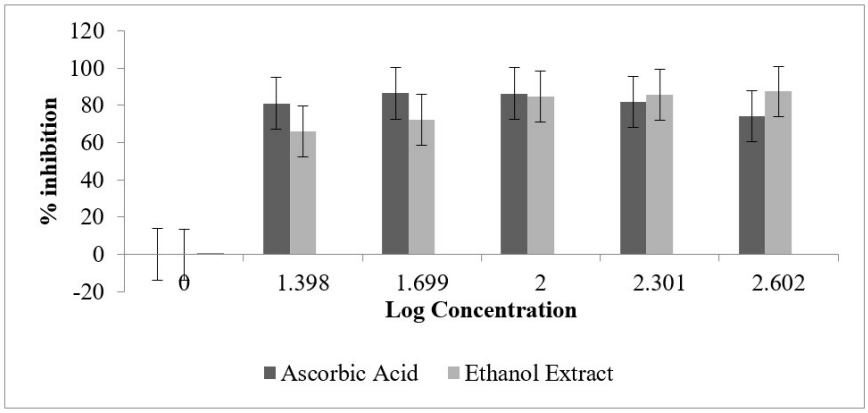

FIGURE 4: Percentage inhibition by ascorbic acid and *C. zeylanicum* extract

of extract (10%) had proved to be satisfactorily effective against *Escherichia coli*, *Salmonella enterica* serotype Paratyphi and the sample that was collected from infected rat compared to the amoxicillin disk. The high concentration was also effective against *Salmonella enterica* serotypes Typhi, *Staphylococcus aureus, Streptococcus spp.* Low concentration (2%) of *C. zeylanicum* was also effective against these bacteria but the efficacy was not significant.

Turbidity was observed at 2% (++) and 5% (+), indicating some bacterial growth at these concentrations (Table 6). At 10%, the sample was clear (-), indicating inhibition of growth. Thus, the MIC for *Escherichia coli* was 10%. Both *Salmonella enterica* serotype Paratyphi and *Salmonella enterica* serotype Typhi showed clear samples across all tested concentrations (2%, 5%, 10%). This suggested that even the lowest concentration (2%) was sufficient to inhibit the growth of these

TABLE 5: Zone of inhibition data

Groups	Zone of Inhibition (mm)		
	***C. zeylanicum* at low concentration (5%)**	***C. zeylanicum* at high Concentration (10%)**	**Amoxicillin**
Escherichia coli	7.69 ± 0.68	21.46 ± 0.64	22.74 ± 0.69
Salmonella enterica serotype Paratyphi	10.32 ± 0.33	21.98 ± 1.26	22.58 ± 2.05
Salmonella enterica serotype typhi	7.38 ± 0.36	15.76 ± 0.89	25.43 ± 1.83
Staphylococcus aureus	4.94 ± 0.25	15.65 ± 0.31	25.21 ± 0.57
Streptococcus spp	6.49 ± 0.25	11.92 ± 1.36	19.21 ± 0.10
Sample infected skin (*Staphylococcus aureus*)	7.83 ± 0.30	21.17 ± 0.94	37.75 ± 1.01
Control	0	0	0

TABLE 6: Determination of MIC of *C. zeylanicum* extract against microorganisms

Groups	Concentration (%)	Turbidity
Escherichia coli	2	++
	5	+
	10	-
Salmonella enterica serotype Paratyphi	2	+
	5	-
	10	-
Salmonella enterica serotype typhi	2	+
	5	-
	10	-
Staphylococcus aureus	2	++
	5	-
	10	-
Streptococcus spp	2	++
	5	++
	10	-
Sample *(Staphylococcus aureus)*	2	++
	5	-
	10	-

Description: ++: Turbid, +: Clear Enough, -: Clear

TABLE 7: Determination of MBC of various concentration *C. zeylanicum* extracts against microorganisms

Groups	Concentration (%)	Microbial Growth
Escherichia coli	2	+
	5	+
	10	-
Salmonella enterica serotype Paratyphi	2	+
	5	-
	10	-
Salmonella enterica serotype typhi	2	+
	5	-
	10	-
Staphylococcus aureus	2	+
	5	+
	10	-
Streptococcus spp	2	+
	5	+
	10	-
Sample *(Staphylococcus aureus)*	2	+
	5	+
	10	-

Description: +: Grown, -: Ungrown

bacteria, indicating an MIC of 2% for both strains. Turbidity was observed at 2% and 5% (++), with a clear sample (-) at 10%. The MIC for *Streptococcus spp.* was therefore 10%. Similarly, turbidity was observed at 2% (++) and 5% (+), but at 10%, the sample was clear (-) for both ATCC strain and sample of *Staphylococcus aureus*. Therefore, the MIC for *Staphylococcus aureus* was 10%. In a word, the MIC for all bacteria except *Salmonella enterica* serotypes was 10%, while the MIC for the *Salmonella enterica* serotypes was 2%. No microbial growth was observed at 5% and 10% (-), but there was growth at 2% (+).

In the Table 7 showed that MBC for *Escherichia coli* was 5%. Both *Salmonella enterica* serotype Paratyphi and *Salmonella enterica* serotype Typhi showed that no microbial growth (-) at all tested concentrations (2%, 5%, 10%). This indicated that the MBC for both strains was 2%. Microbial growth of *Staphylococcus aureus* and *Streptococcus spp* was observed at 2% (+) but not at 5% and 10% (-). This indicated that the MBC for both microbes were 5%. The MBC for *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus spp.* was 5%, while the MBC for *Salmonella enterica* serotypes was 2%.

DISCUSSION

A critical stage in the healing process that results in wound closure is wound contraction.

The ability of the tissue to heal itself, the angiogenesis process, and the type and extent of tissue damage all play a role in how quickly the tissue recovers (Priya et al. 2004). As a result, assessments of wound contraction and visual presentations serve as reliable criteria in the evaluation of macroscopic wound healing (Gal et al. 2008). The current study has reported similar outcomes from the macroscopic observations. *C. zeylanicum* extract stimulates the wound's surrounding cells to increase their multiplication throughout the healing process. The results from macroscopic view showed that *C. zeylanicum* promotes wound healing activities by stimulating the surrounding tissues to increase their proliferation during wound healing. After the inflammation stage on day 3, wounds treated with *C. zeylanicum* showed better healing progress than the other three groups. The findings are highly correlated with anti-inflammatory capabilities of *C. zeylanicum*. During the early stage of inflammatory phase (day 3 to day 6), the wound healing showed slow progress due to the presence of inflammation. The reduction of inflammation is up to the mark as it reduced redness, swelling and also reduced the overall healing time. Inflammatory cells invade the damaged location in tissues swiftly following the development of a clot that is stable (Henry & Garner 2003). Inflammatory cells particularly neutrophil infiltrate into wound area and remove the necrotic tissue, debris and microbial sources (Westman et al. 2019). Inflammatory cells, mediators and growth factors play potential role in wound healing process (Landén et al. 2016). Proliferative effects of *C. zeylanicum* begin to operate on day 6, and wound closure speeds up. *C. zeylanicum* may reduce the swelling, redness, and discomfort associated with wounds which accelerates the healing process.

C. zeylanicum treated group exhibited expeditious healing than the other three groups. Antiseptics like povidone-iodine inhibited fibroblast migration and proliferation in a way dependent on dosage. As a result, cell migration into the wound area, matrix development collagen synthesis, fibroblast proliferation, and wound contraction were delayed in the standard treatment (Thomas et al. 2009). The paraffin had not any significant wound healing activity, so it is safe to use as a base for the ointment preparation (Mazliadiyana et al. 2017; Nur et al. 2018).

The ethanolic extract of *C. zeylanicum* showed the presence of alkaloids and flavonoids. Alkaloids are bioactive chemicals found in medicinal plants that have a wide range of biological functions. Alkaloids exhibit a wide range of bioactivities and pharmacological activities, including antimalarial, anticancer, antibacterial and antihyperglycemic (Cushnie et al. 2014; Kittakoop et al. 2014; Qiu et al. 2014; Wink 2015). Flavonoids are widely known for their antioxidant capabilities, which help to inhibit tumor growth and development. Previous research has also found that flavonoids can help preventing coronary heart disease, as well as guard against platelet buildup, microorganisms, liver toxins, viruses, cancers, free radicals and allergies (Stephen & Ejikeme 2016). The TPC of *C. zeylanicum* ethanolic extract was determined. The results of this investigation demonstrated that polyphenols are abundant in the ethenolic extract of *C. zeylanicum*. Previous research found that phenolic compounds have redox characteristics since they are key plant components with antioxidant action (Soobrattee et al. 2005).

Tissue injury can cause oxidative stress due to overproduction of free radical, particularly reactive oxygen species (ROS) (Pizzino et al. 2017). ROS can delay wound healing. Therefore, wound healing process can be

accelerated by reducing the ROS levels (Dissemond et al. 2002). Antioxidants can enhance the wound contraction by scavenging of ROS. In this study, antioxidant property of the extract has been evaluated by DPPH free radical scavenging assay. Amongst methods available for determining plant antioxidant capacity, the DPPH test is the most appropriate (Mensor et al. 2001). This study determined that *C. zeylanicum* extract has significant free radicals scavenging activity, which would help to prevent oxidative damage and promote wound healing process.

The antibacterial activity of *C. zeylanicum* extract was assessed against both Gram-positive and Gram-negative bacteria using various methods, including disc diffusion, well diffusion, MIC and MBC. The extract showed strong antibacterial effects, with its effectiveness differing based on the bacterial type. The largest inhibition zones were observed for *Staphylococcus aureus*, especially in well diffusion assays, which exhibited greater antibacterial activity than disc diffusion due to direct contact between the extract and bacteria (Behbahani & Fooladi 2018; Behbahani et al. 2017). The MIC and MBC results indicated that Gram-positive bacteria were inhibited at lower concentrations of the extract compared to Gram-negative bacteria, likely because Gram-positive bacteria have a simpler cell membrane structure, making them more vulnerable to antimicrobial agents. In contrast, the complex cell membranes of Gram-negative bacteria, which contain lipopolysaccharides and phospholipids, may reduce the diffusion of lipophilic antimicrobial compounds, making them less susceptible to the extract (Singh et al. 2016; Veras et al. 2017).

CONCLUSION

In conclusion, the present study revealed that the *C. zeylanicum* treated group recovered much more quickly than the other three groups. The *C. zeylanicum* treated group had superior re-epithelialisation, angiogenesis and a decrease in inflammatory cells in the granulation tissue, according to histologic examination. Further, phytochemical, antioxidant and antimicrobial studies also revealed that *C. zeylanicum* can accelerate the wound-healing process. Therefore, the study concluded that the ethanolic extract from *C. zeylanicum* holds significant promise as a remedy for wound healing.

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