Research Article
Sciences of Phytochemistry

A comparative study of in-vivo wound healing properties of Tithonia Diversifolia. A gray crude extracts to Silver Sulphadiazine in Albino Wistar rats.

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Abstract: One species of flowering plant in the Asteraceae family is *Tithonia diversifolia* A. Gray (*T. diversifolia*), which grows as a shrub or weed. Significant anti-infective therapeutic characteristics, such as anti-mycobacterial, antifungal, antibacterial, anti-inflammatory, anti-malaria, and anthelmintic effects, have been discovered in the plant's extracts. Ethnic communities have been using the plant extract to cure wounds. They appear to prefer it above conventional treatments in many circumstances, to the point where their aqueous solution may be smuggled into medical institutions in order to augment the care provided. The purpose of this study was to compare *T. diversifolia* with silver sulphadiazine's capacity for wound healing. For 72 hours, a 70% ethanol alone and water alone was used to extract the plant. After the extracts dried out, the powder was measured and 10 mL of reconstituted volume was assessed at various concentrations for the purpose of treating wounds. Silver sulphadiazine was used as the positive control and distilled water as the negative control. According to the findings, the aqueous extract had a 48.0% healing rate after 14 days of treatment, ethanol had a 20.0% healing rate, and silver sulphadiazine had a 22.0% healing rate. It is possible to draw the conclusion that the aqueous extract concentration of 0.6 mg/10 mL demonstrated a higher healing percentage than silver sulphadiazine and the ethanol extract.

Introduction

Natural products have been used by people of diverse ethnic backgrounds for various human problems since the beginning of time. This can be partly attributed to the therapeutic properties of the biologically active substances found in different plants (1-7). Mangoyi et al. (2014) demonstrated that plant extracts possess significant anti-infective medicinal properties, including anti-proliferative, anti-mycobacterial, anti-fungal, anti-bacterial, and anti-inflammatory activities. Despite the effectiveness of traditional medicine, which relies on indigenous health ideas, beliefs, and practices to diagnose, treat, and prevent illnesses (7), modern chemical science has revealed the structural diversity and complex properties of plant-based natural compounds. These properties are often correlated with their effectiveness, as evidenced by the successful use of morphine and its analogues for pain management.

The focus of this investigation is on *Tithonia diversifolia* (*T. diversifolia*) A. Gray (see Figure 1), a weedy flowering plant from the Asteraceae family. This plant is indigenous to southern African countries like Zambia and other parts of the world, including Mexico. It is known for its phytochemical compounds that aid in wound healing and its ability to thrive in severe environments. *T. diversifolia*, commonly known as Kalula-lula or Dundu in Zambia and the Mexican sunflower in Mexico, has been traditionally used by ethnic groups to treat various illnesses due to its

widespread availability and resilience (2, 4, 8-10).

Figure 1. Tithonia diversifolia plant's (A) leaves (B) stem and (C) flower.

Despite the availability of over-the-counter wound creams, *T. diversifolia* is still widely used, particularly for wound treatment. It is favored for being inexpensive and, in some cases, more effective than conventional wound creams (2, 4, 9, 12). Research has shown that the plant's phytochemical properties are effective against several illnesses, such as malaria, cancer, hepatotoxicity, and liver-related issues (12-18). In 2019, Komakech et al. investigated the potency of *T. diversifolia* methanol leaf extract formulated as gels on rats with experimentally induced wounds. By the 17th day of treatment, 100% wound closure was observed in both gel formulations of the plant methanolic extract and the standard gel, indicating the plant's effectiveness in wound healing (21). In other studies, *T. diversifolia* was taken orally or to bathe the affected area to cure microbial infections in the sexual organs, according to a study conducted in Uganda. According to Owoyele et al. (2004), there is scientific evidence to support the use of *T. diversifolia* extracts in the treatment of wounds, as they have been shown to effectively reduce inflammation in both the acute and chronic phases (24). Omokhua et al., 2018, results for the phytochemical quantification showed that both *T. diversifolia* and *T. rotundifolia* are rich in phenolics, flavonoids and hydrolysable tannins. The major phytochemicals displayed by both species are known to be responsible for biological activities such as anti-inflammatory, antiviral, antioxidant, antimicrobial and wound healing properties (25).

The purpose of this study is to compare the healing properties of silver sulphadiazine, a common wound cream, with the crude extract of *T. diversifolia*. This plant is often preferred by ethnic groups for wound treatment over conventional creams. This study aims to investigate why this preference exists and how the herbal remedy's effectiveness compares to that of silver sulphadiazine. The primary questions are: Why is there so much faith in the herbal remedy? Does it have any healing power for wounds? If so, how does it stack up against the often-criticised conventional cream, silver sulphadiazine?

Experimental Section

Materials

Plants collected from Kabwe Great North Road campus of Mulungushi University, Kabwe, Zambia. Silver sulphadiazine obtained from reputable licenced pharmacists from Kabwe, Zambia. Lidocaine (local anaesthetic), obtained from the Veterinary Extension office in Kapiri Mposhi District, Zambia.

The following reagents were gotten from Merck South Africa, and they were all analytical grade. The following are reagent: ferrous chloride ($FeCl_3$), hydrochloric acid (HCl), magnesium ribbons, acetic hydride, Dragendorff's reagent, sulphuric acid (H_2SO_4), and ethanol (75%).

Preparation of Crude Extracts

The standard process for the extraction method has been established by John-Dewole and Oni (2013) and Hildebrand et al. (1970) (27-28). Washing the plant leaves after collection and two weeks of room temperature drying were the steps in the process. After that, the leaves were ground into a powder and stored as stock in a cool, dry location.

Water Maceration

A 1000 mL beaker containing 50 g of stock powder was filled with 500 mL of distilled water to dilute the powder with the solvent to concentration of 1.0 g of powder to 10 mL of solvent. After that, the solution was given a ferocious 24 h shake using an electronic shaker before being left to soak and dissolve for a full 72 hours. The solution was filtered and the filtrate evaporated to dry at 34 °C in an oven. The residue was weighed and stored in a cool dry place as stock for water extracts for treatment of wounds.

Ethanol Maceration

From the dry stock powder, 50 g was weighed and put in a clean dry 1000 mL beaker. Then 75% ethanol was added to the dry powder in a beaker and placed in an electric shaker for 24 h. After which it was kept without shaking with merely mild stirring for 2 more days. The solution was filtered, and the filtrate evaporated to dry at 34 °C in an oven. The residue was weighed and stored in a cool, dry place as stock for ethanol extracts for treatment of wounds.

Phytochemical Screening

The phytochemical tests for the crude extracts of the plant were done according to the following methods.

Terpenes

The Lieberman-Burchard was prepared according to Daka and Chansa 2023, Amadi, et al, 2013, Adu et al, 2019, 50 mL of acetic anhydride was pipetted into an amber glass vial and put in an ice bath. Then, allowing 30 min to stay, 5 mL of concentrated sulphuric acid was measured and carefully added to the acetic anhydride in the vial. Then, from the stock filtrate, 1.0 mL was mixed and observations were made (1, 28-29).

Tannins

In Nigussie et al. 2021 report, 2.0 mL of filtrate stock solution of the crude extract was used, a few drops of 10% Iron (III) chloride ($FeCl_3$) in the pale yellow solution. The observation to look out for are: Blackish-blue colour means Gallic tannins are present, while greenblackish shows the presence of catechol tannins (1, 30).

Flavonoids

The dried crude extract 5.0 g was weighed then, soaked in 20.0 mL of 75% ethanol and soaked for 24 h in a cool dry place. The solution was filtered, then 10.0 mL was used where 10 drops of hydrochloric acid (HCl) and 4 stripes of magnesium ribbons (4.0 mm). The observations were noted (1, 31).

Alkaloids

According to Daka and Chansa 2023, an orange-red precipitate formation with 1.0 mL of Dragendorff's reagent to 2.0 mL of the extract, suggests the presence of alkaloids. This was also in agreement with Chaudhary et al. 2010, (1, 31-32).

Saponins

From the dried stock, a 5.0 g was weighed and soaked in 5.0 mL of distilled water. After which it was warmed in the water bath, the persistent froth after 3 drops of olive oil, this was done according to the protocol of Tilaoui et al. 2021 (33).

Animal Model Setup

The population for the experiment was set at 15, and the animal model setup experiment was carried out in accordance with Abdullahi et al., 2014, which was also verified by Charan and Kantharia, 2013. The E value, which is based on an ANOVA, must be measured for the procedure to work. According to the approach, E should fall between 10 and 20. If it is less than 10, then adding more animals suggests that significant results could be produced; however, if it is greater than 20, adding more animals won't enhance the likelihood of significant results (23, 26). The Equation 1 was used for the animal number determination.

$$E = Total\ number\ of\ animals - Number\ of\ groups$$

Equation 1 (23, 26).

In our case, we had 4 groups of three animals, based on the E evaluation formula in equation 1, which gives the E value within the range of requirements of equation 1. Meanwhile, to ascertain the correct concentrations required to treat with, for final evaluation of a comparative effect of a crude extract, two other sets of four rats were used for treatment for 14 days with ethanol extract and aqueous extract. The results from the trials gave results for the best concentration of *T. diversifolia* crude extract to be used in the final comparative studies.

Animal Wounds and Treatment

Animal groups were divided into Positive control (PC), negative control (NC), and *T. diversifolia* subdivided as ethanol (E) and water (W) extract treated. The treatment was also varied in terms of the concentration of the crude extract. They were labelled and weighed using a beam balance, then, the area of interest was shaved, and cleaned with 75% ethanol (aseptic technique) and each rat was anaesthetized through intraperitoneal injections with 0.25 mL of lidocaine (local anaesthetic) four times in a clockwise manner (Abdullahi et al., 2014) (23). The process of injecting the rats was done by a technician from the Veterinary extension office.

Hot Plate Method

Using the hot plate method, the square metal plate of with 2.5 x 2.5 x 0.2 cm measurements was heated to some temperature, then the shaved part of the rat was placed for seconds and the same procedure was repeated until the wound with the required size was produced on all the animals. They were observed frequently for signs of pain or discomfort, and appropriate treatment was given after some seconds.

Administration of *Tithonia Diversifolia* Extract and Silver Sulphadiazine

Each set of tests was grouped: Positive control samples were administered with the approved burn cream silver sulphadiazine. The negative controls were only administered with distilled water. Then for each a crude extract from distilled water and another crude extracted by ethanol was set. The width and length

were measured by using a ruler, and the colour change (pink, red), dryness, shape or raised features of the wound were carefully observed. The observations and measurements continued until when any of the groups showed signs of complete recovery.

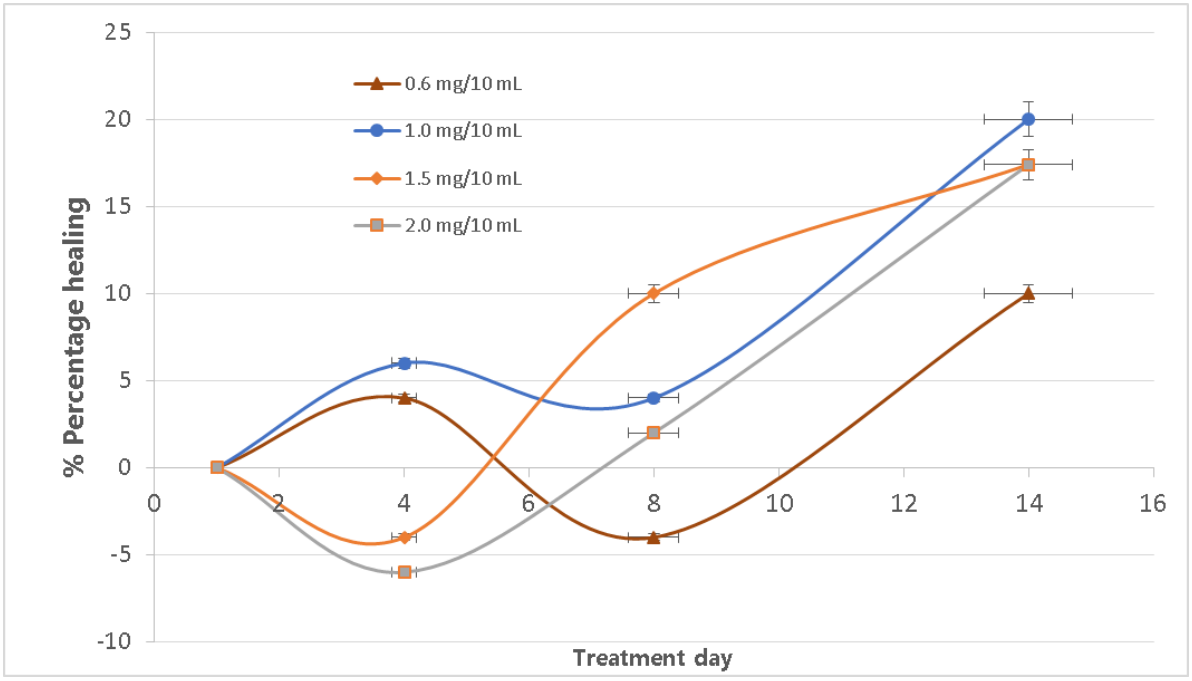

Figure 2. Wound healing percentage of the ethanol extract groups.

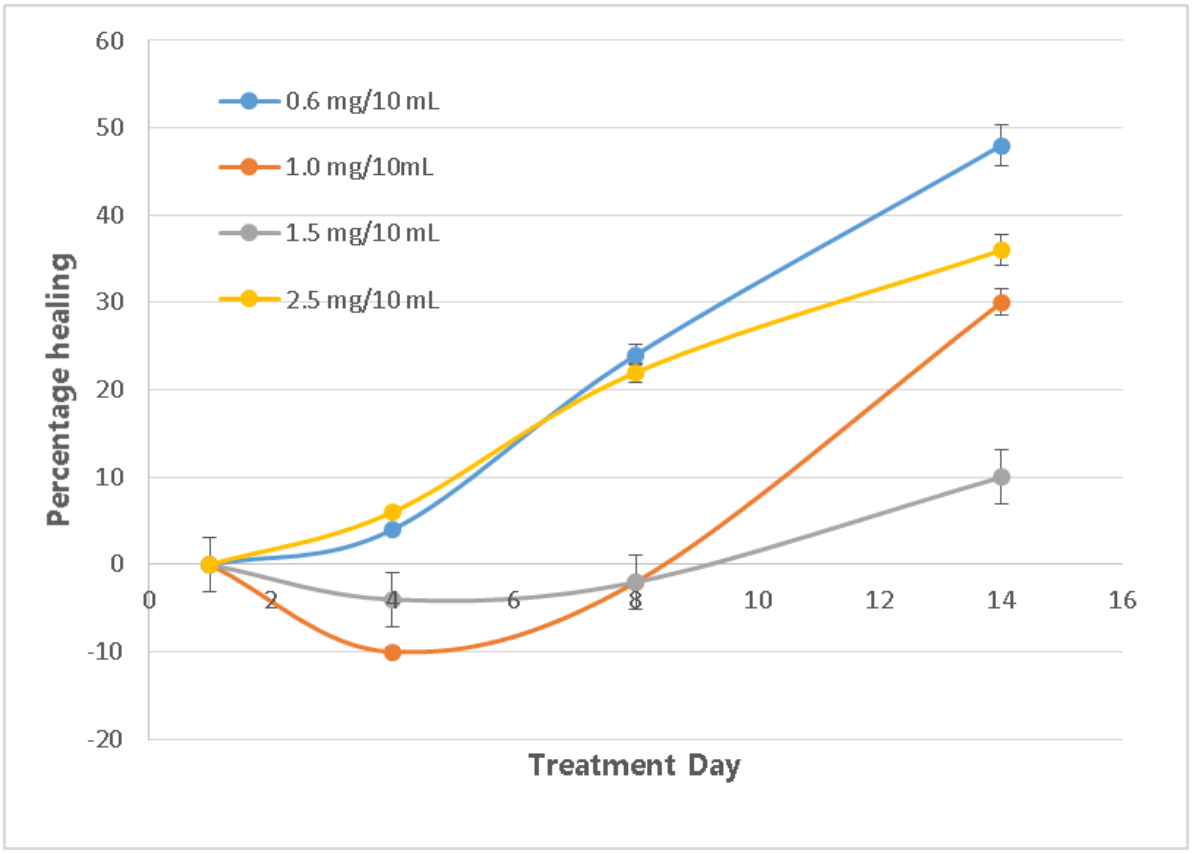

Figure 3. Wound healing percentage of the water extract groups.

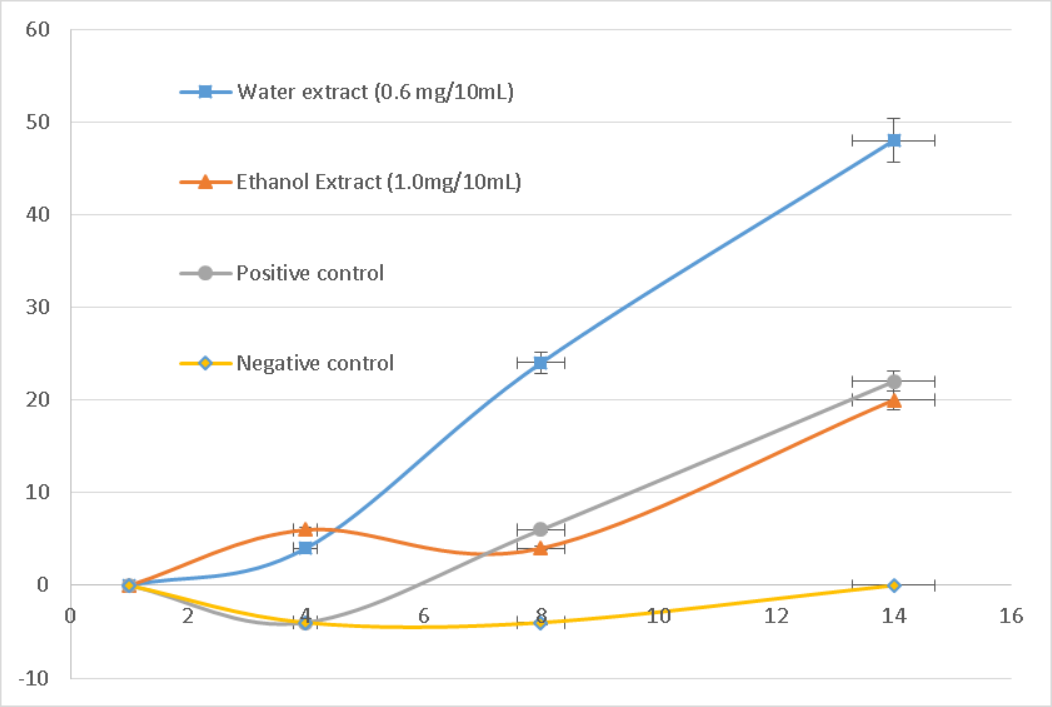

Figure 4. Wound healing percentage of the crude extract groups versus silver sulphadiazine.

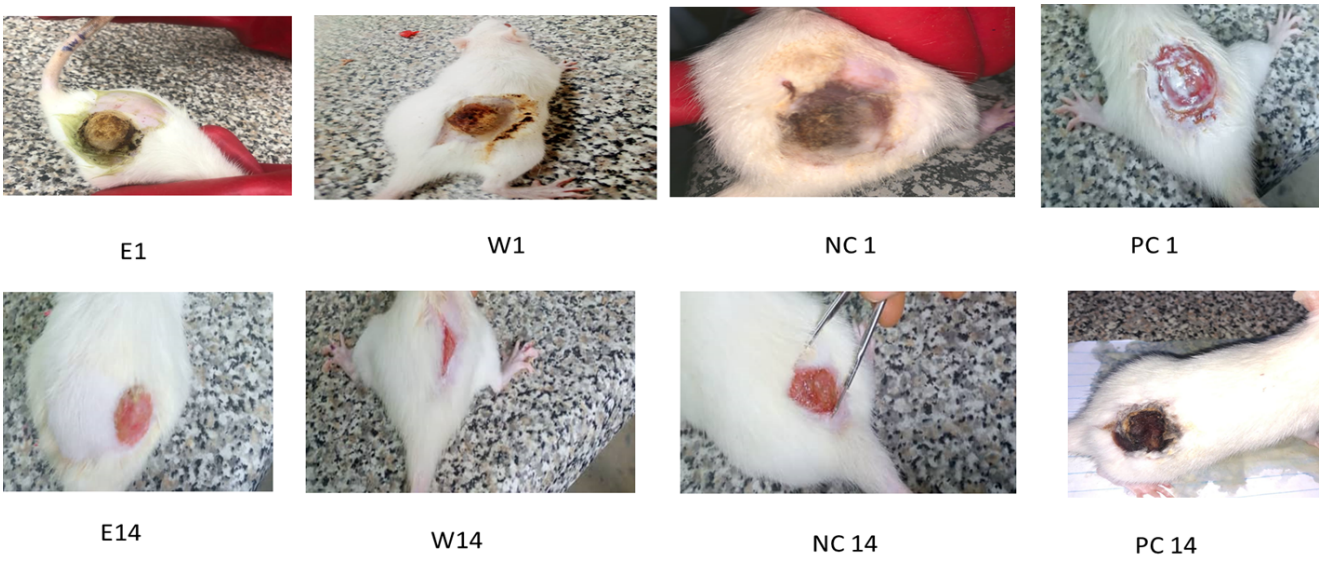

Figure 5. Wound healing properties and stages of each treatment. Note: (E) is ethanol extract, (W) is water extract, (NC) is negative control, and (PC1) is positive control. The numbers 1 and 14 refer to the days of observation.

Statistical Data Evaluation

IBM SPSS 2.0, 2024 was used to analyse the data and conduct statistical analyses, which included the following: Paired T-test, mean treatment duration, and treatment continuity. The effectiveness of treatment was assessed over the period compared to the untreated group using the Paired T-test. In order to determine whether the observed percentage of healing was statistically significant, this was done. In order to track the overall tread over time and determine which treatment had the least amount mean size of the wounds to show effectiveness, throughout the course of the trial.

Results

Determination of Optimal Crude Extract

The various concentrations of the ethanol and aqueous crude extract were dissolved in water and applied to the trial set of sample rats. The length and width of the wounds were measured on days 1, 4, 8 and 14. The trend in the size of the wounds was evaluated as a percentage of initial size calculated using Equation 2.

$$\%\ wound\ healing = \frac{Size_{Initial} - Size_{Final}}{Size_{Initial}} \times 100\%$$

Equation 2 (22)

When the healing percentage trend was analyzed in relation to each concentration, the ethanol extract data could be understood as shown in Figure 2, while the water extract is shown in Figure 3.

The concentration of ethanol extracts seemed to have the better profile of healing based on tread was 1.0 mg/10 mL, it produced generally a higher percentage recovery of the wound area, and in its process time did not show a negative percentage, meaning the wound increased in size. Similarly, for water, 0.6 mg/10 mL showed no negative percentage and generally, showed a higher percentage wound healing, hence for comparative studies 1.0 mg/10 mL and 0.6 mg/10 mL were used for ethanol and water respectively.

The graphical representation for the comparative studies can be seen in Figure 4 . The data shows that the 0.6 mg/10 mL had a higher percentage of 48% healing by day 14 compared to the others which showed half the healing percentage. The ethanol extract seemed to almost resemble the positive control. Meanwhile, distilled water which was a negative control seemed the wound was just generating.

From the graph, it is also clear that the aqueous extract of T.Diversifolia showed a higher percentage of healing by day 14 of treatment, this too is evident in the images of sizes of the wounds as shown in Figure 5 below.

Phytochemical Screening

The phytochemical tests results are shown in the Table 1. The '+' signifies a positive test, while '-' signifies a negative test for the specific phytochemical compounds of interest.

Table 1. Phytochemical constituents of the aqueous (water) and ethanol extract of T. Diversifolia.

Metabolite group	Ethanol extract	Aqueous extract
Flavonoids	+	+
Saponins	+	+
Tannins	+++	+++
Terpenoids	++	+++
Alkaloids	++	++

Note: + Present in mild concentration, ++ definitely present, +++ abundantly present

Discussion

Optimal Concentration

As demonstrated in Figures 2 and 3 above, the water and ethanol extracts showed that by day 14 of the wound treatment process, solutions containing 0.6 mg/10 mL and 1.0 mg/10 mL, respectively, had higher healing percentages than the other solutions. This may be explained by the possibility that the larger dosage was harmful to the animal cells in the open wounds where it was applied, which would have caused the wounds' observed increasing size, which is indicated by a negative healing percentage. This is in line with the findings of Abdalla et al. (2018), who discovered that the plant had cytotoxic effects though their extracts were orally administered. According to their findings, cytotoxicity was detected at as little as 0.05 mg/mL (25, 37). Nonetheless, the underlying pathophysiological approach should recognise the wound healing mechanism as complex (19, 33-34). The in vivo trials add to the intricacy because individual animals respond to therapy differently than groups do. Therefore, the toxicity of plant extracts may have contributed to a reaction in healing that increased the average size of the wound when higher concentrated doses were used to treat wounds. According to reports, the plant extracts have anthelmintic characteristics, which may eradicate the parasites by either killing them or interfering with their normal cycle of reproduction. Consequently, at the cellular level at the application site of the wound, the results can appear as a larger wound, potentially reducing the effectiveness of the plant extracts as demonstrated by the favorable outcomes of anthelmintic evaluations. Meanwhile, Figure 4 shows that the ethanol extract exhibited a decrease from day 4 to day 8, which could be attributed to a phenomenon called tolerance, where organisms require a higher dose of treatment before they respond (41).

Thus, for experimental purposes, the optimal working concentrations for the water and ethanol extracts were determined to be 0.6 mg/10 mL and 1.0 mg/10 mL, respectively, for comparative studies. In the meantime, the ethanol extract at 0.6 mg/10 mL showed a positive start, but the increase in wound size by day 8, with later reduction, may be related to the potent component for wound healing being less soluble in the ethanol extract. It may also be related to some side reactions when ethanol was used as a solvent, affecting some active metabolites for wound treatment and thereby minimizing their potency by transesterifying the carboxylic and aldehyde components. Therefore, 0.6 mg/10 mL of ethanol could not be used as the optimal concentration for comparative studies.

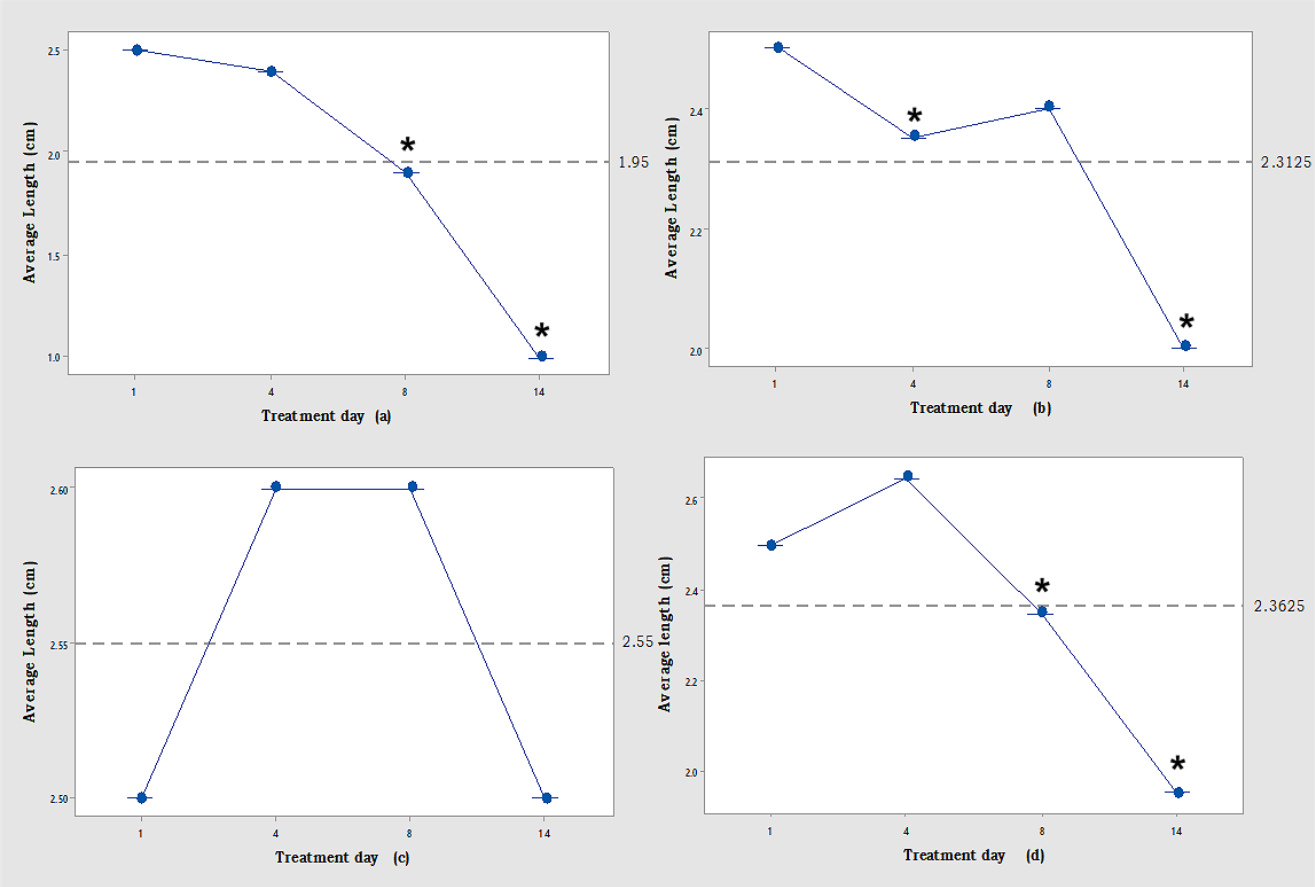

Figure 6. Tread in average lengths in cm and the group mean for the treatment period. Note: (a) water extract (0.6mg/10mL) , (b) ethanol extract (1.0mg/10mL), (c) negative control, and (d) positive control.

Phytochemical Screening

According to the results of the phytochemical testing, the *T. diversifolia* crude extract is high in concentration of tannins, terpenoids, and alkaloids, while, flavonoids and saponins in moderate concentrations. The results, particularly in leaves and stems, are in line with those of other reports (14, 25, 28, 34-36). The plant extracts are essential for wound healing because it has been reported that they contain all the aforementioned components. According to reports, the plant's aqueous extracts contain terpenoids (sesquiterpenes), flavonoids (Hispidulin), and tannins. Moreover, flavonoids affect angiogenesis, re-epithelialization, oxidative stress, and inflammation. They also activate macrophages, fibroblasts, and endothelial cells by promoting the production of TGF-β1, VEGF, Ang, Tie, Smad 2 and 3, and IL-10. (42). These compounds are known to have antifungal, anti-inflammatory, and immunosuppressive qualities that make them beneficial for wound healing (35-36). Therefore, it is possible to draw the conclusion that the different components of the *T. Diversifolia* aqueous extracts collaborated to cure the wound. In the meantime, as shown in Figures 2 and 3 above, if a high concentration of extracts is applied, the phytochemical compounds of some component metabolites may start to act against the body on the wound and create an impression of the increased size of the wound, which manifests as a negative percentage healing.

Therapy Effectiveness

When the two extracts of T. diversifolia were tested against silver sulphadiazine, it was shown that the water extract at 0.6 mg/10 mL had a better healing percentage than the other tests, which included the negative and positive controls, and the ethanol extract at a 1.0 mg/10 mL concentration (Figures 4 and 5). It is important to acknowledge that even if the wounds were produced symmetrically, the healing process deviates from the standardized wound shape due to the asymmetrical nature of wound healing. The complexity of wound healing systems causes this variation (19-20, 23, 33). Based on Figure 4, it was determined that on day 14, the healing percentages for the positive control and the 1.0 mg/10 mL ethanol extract were similar, at 22% and 20%, respectively. When the average healing length and overall mean lengths for wounds were evaluated, the results were as shown in Figure 6.

Except for the negative control group, where the average length of the wounds remained nearly the same as at the start of the treatment process, the wounds in other animal groups showed a gradual

healing pattern. Meanwhile, in the negative control group, even though there seemed to be a reduction, it only reduced to the initial wound size throughout the treatment. This was a significant finding, indicating that the immune systems of the animals, over the treatment period and conditions, did not substantially reduce the wound size. Therefore, this provides a better basis for comparative analysis of results. This was also evident when specific individual lengths for each group treatment were subjected to a paired t-test in IBM SPSS 2.0, with the negative control data as pretreatment. The test showed no statistical significance between the conventional medicine and the negative control.

With the degree of freedom of 3, and 95% confidence level, the critical values is 3.182. It was found that statistically, there was no significant difference between day 1 and 4 in almost all treatment groups. This could be attributed to all animals responding to different treatments. But for day 4 to day 8 in the water extract a statistically significant chance was observed. When the initial day and final day were compared it was found that in all groups except the negative control had shown statistical difference in response to treatment.

When each treatment group was compared to a negative control group, at 95% confidence level, the *t*-value of 1.965 which was lower than critical value of 2.365, both the water and ethanol extracted crude of *T. diversifolia* shown to be statistically significant difference. The *p*-value for water was 0.029 less than 0.05 at 95% confidence level. The *t*-value of 2.741 greater than the critical value of 2.365 for 7 degrees of freedom. The ethanol extract had the *p*-value of 0.029, less than 0.05 at 95% confidence level, the *t*-value of 2.747 greater than the critical value of 2.365 for 7 degrees of freedom.

The results of the Tukey test seems correlates with the ethnic group belief that the sulphadiazine does not provide the much needed help compared to the solution of *T. diversifolia* extracts. When the overall lengths and treatment processes for all groups and cases were evaluated for homogeneity, the results showed there were significant differences in length distributions on wound sizes as they healed, but there was no significant difference in treatment processes themselves in all groups p-values of 0.018 for lengths across groups and group treatment p-value of 0.772 respectively. The results are consistent with Mohamad et al. 2022, which showed (39).

Hence, the differences in the healing of wounds' sizes now could be associated with the content of the phytochemical compounds and active ingredients of silver sulphadiazine and not the process. Hence, it could be concluded by saying aqueous and ethanol extracts of *T. diversifolia* statistically possess wound healing potency better than the positive control. The results are similar to other works, which show that the same treatment of groups of animals shows significant differences (38-40).

Conclusion

The aqueous and ethanol extracts of *T. diversifolia* contain relatively high levels of terpenes, tannins, and alkaloids, while saponins and flavonoids are present in moderate amounts. These phytochemical compounds work synergistically, providing antibacterial, antifungal, anti-inflammatory, and analgesic properties that aid in wound healing. In comparative tests, the 0.6 mg/10 mL aqueous extract performed relatively well compared to the 1.0 mg/10 mL ethanol extract of *T. diversifolia* and silver sulphadiazine. The aqueous extract showed a healing percentage of 48.0%, which was twice that of silver sulphadiazine and the ethanol extract for the same period and under similar treatment conditions for Wister rats. The ethanol crude extract of *T. diversifolia* showed a 20.0% healing percentage over a period of 14 days, which compared well with the 22.0% of silver sulphadiazine. The statistical analysis also showed that there is no significant difference between the negative control (untreated) and the positive control in terms of wound length reduction over the test period, with a *p*-value of 0.09, which is greater than 0.05 at a 95% confidence level. Meanwhile, both the water and ethanol extracts showed statistically significant wound size reduction over the time. Hence, it can be concluded that the *T. diversifolia* extract showed relatively significant potency in wound healing compared to the positive control, silver sulphadiazine.

Declarations

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Conflict of Interest
The authors declare no conflicting interest.

Data Availability
The unpublished data is available upon request to the corresponding author.

Ethics Statement
The Ethic clearance was sorted and the acceptance number is SMHS-MU4-2023-036. The protocols were followed and we engaged the Vet extension officer to help with the management

Funding Information
Not applicable.

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