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Research Article

Efficcacy of Tagetes erecta Linn. leaf extract cream on rat dermal wound healing

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ABSTRACT
Dermal wound of the human body are a problem that must be cured immediately. Therefore it is necessary to look for new herbal medicine options. The purpose of this study was to determine the effectiveness of the dose of Tagetes erecta Linn. leaf extract cream on rat dermal wound healing. This study is an animal treatment in Wistar rats. Rats were divided into 3 groups, namely groups I, II and III. All rats in the treatment groups were excision on the back skin. Group I (control) after excision was given the drug nitrofurazone cream, group II was given T. erecta Linn. leaf extract cream 3% and group III was given T. erecta Linn. leaf extract cream 4%. T. erecta Linn. leaf extract cream 3% and 4% play a role in increasing the strength of wound contraction, the percentage of wound contraction and accelerating the epithelialization period compared with the positive control group (nitrofurazone). T. erecta Linn. leaf extract cream 3% and 4% better as antibacterial than positive control (nitrofurazone). T. erecta Linn. leaf extract cream 3% and 4% increase protein and DNA content in the wound area compared with the positive control group (nitrofurazone). T. erecta Linn. leaf extract cream 3% and 4% can be followed by clinical trials in humans so that it can be an option for treating on dermal wounds healing.

Keywords: Tagetes erecta Linn. leaf extract cream, wound healing, epithelialization period.

INTRODUCTION

Dermal wound on the human body are a problem that must be cured immediately. Therefore it is necessary to look for new natural treatment options, both sourced from animals and plants. For this purpose we have tried to examine the contents of natural materials derived from animals and plants. We have tried to show 8 proteins fraction of earth worms (Parwanto et al, 2016). In the future, we hope that earthworm extract can be used for dermal wound healing. Beside that it has been demonstrated a formulation of Lantana camara Linn. leaf extract ointment (Parwanto et al, 2013) and look for a doses effectiveness for wound healing in rats (Parwanto, 2017). After the injury, there will be growth of dermal tissue to cover the wound. To accelerate wound healing, new drug preparations should be sought. The search for new drug preparations can be done by involving various extract of natural ingredients, including Tagetes erecta Linn. leaf extract.

The results of previous studies showed that isolation of steroids and phenolic acids from T erecta Linn. leaf extract was carried out using column chromatography (Jayavant et al, 2020). The other study showed that 19 phytochemicals were contained by leaf extract of L. camara Linn. The main bioactive compounds are tetra decanoic acid, 2,6,10- trimethyl 14-ethylene-14-pemtadecme, N-hexadecanmic acid, 15-hydroxy penta decanoic acid and stigmasterol (Devika and Justin, 2014). In addition, has been reported that qualitative phytochemical screening of T. erecta Linn. oil. The screening results showed that T. erecta Linn. contained terpenoids, flavonoids, alkaloids, quinones, phenols, coumanins, carbohydrates and tannins.The screning shows that T. erecta Linn. contains terpenoids, flavonoids, alkaloids, quinones, phenols, coumanins, carbohydrates and tannins (Rajvanshi and Dwivedi, 2017). Furthermore, it has been proven quantitatively that lipophilic extract from T. erecta plena Linn. var. Hawaii contains carotenoids (up to 80.51±1.15 mg% equivalent to β-carotene) and flavonoids (up to 0.7120±0.0060% equivalent to patuletin) (Maliuhina et al, 2020). Beside that, T. erecta Linn. also contains such as quercetin and its glycoside derivatives, ellagic acid, chlorogenic acid, kaempferol, myricitrin and routine (Marrelli et al, 2013). Another study has also been carried out to evaluate the chemical content of T.erecta Linn. leaf extract were cultivated at Bandungan,

Central Java, Indonesia. Gas chromatography-mass spectrometry (GC-MS) was used to perform 17 active compounds with a retention time of 31,284 to 50,614. The three compounds with the highest content are neophytadine 43.88%, 9,12,15-acid-methyl ester Octadecadienoic 13.45%, and hexadecanoic acid-methyl ester 13.24% (Edy et al, 2017). There have been pharmacological studies of T. erecta Linn. leaf extract.

T. erecta Linn. extract has various pharmacological effects, including anti-bacterial, wound healing, antioxidant, antidiabetic, antihyperlipidemic, hepatoprotective, original and repelent, nematosidal, insecticidal, analgesic and cytotoxic (Karwani & Sisoidia, 2015]. Research on wound healing in white rats has been carried out with cream extract of T. erecta Linn. 5% and 10%. The results showed that T. erecta Linn. extract cream was more beneficial to wound healing compared with C. asiatica extract (Chartterjee, 2011). Other studies have shown the effect of T. erecta Linn. 2.5% extract gel on wound healing in white rats (Kiranmai et al, 2011). In addition, the use of T. erecta Linn.for traditional treatment has been carried out.

Utilization of T. erecta Linn. has been carried out among others as an antiseptic ingredient and to overcome kidney problems, muscle aches and ulcers. Part of T. erecta Linn.flower is used to cure fever, epilepsy, stomach ache, scabies, liver disease, eye pain, rheumatism and bronchitis (Karwani & Sisoidia, 2015). It has been reproted that methanol extract of T. erecta Linn. could protect human skin against photo-aging by attenuating oxidative damage, suppressing matrix metalloproteinase expression and/or activity as well as by stimulating collagen synthesis (Kang et al, 2018). Previous studies have reported that aqueous and alcoholic extracts of T. erecta Linn. has anxiolytic effect (Manisha et al, 2013). In addition, that each part of the plant of T. erecta Linn. has a certain phytochemical content. The main constituents of each part of the plant of T. erecta Linn. have different pharmacological effects such as anti-nociceptive, anti-inflammatory, anti-fungal effects, insecticides, larvicidal, hepatoprotective, antipyretic, wound healing, antibacterial, antimicrobial, antiepileptic, and antifungal properties (Singh et al, 2020). Another studies have shown that T. erecta Linn. flower extract is proven to have the ability of central nervous system stimulant, antidepressant, and antipyretic activity (Shetty et al., 2009). Based on the description of T. erecta Linn. in health, we would like focus on the use of this plants for dermal wound healing.

Wound healing is a process that occurs if there is physical interference with cells and tissues. So that wound healing is more effective and does not cause hypersensitive effects, it can be done by providing potential plant extract. Data shows that many plants have the potential for wound healing. Therefore, ethnobotany experts collect data about plants that have the potential to wound healing (Arun et al, 2013). T. erecta Linn. also has wide ethno medicinal importance attributed to the bioactive compounds and essential oil found in leaves and having commercial application as medicine (Mir et al, 2019). Furthermore was demonstrated the healing activity of the lipophilic extract of T. erecta Linn. (Maliuhina et al, 2020). Related to this, it turns out DNA and protein play a role in wound healing.

Increased DNA content in wound healing indicates cellular hyperplasia. Along with this, there is an increase in total protein that shows active synthesis and deposition of matrix proteins in granulation tissue. The wound healing process depends on the regulation of biosynthesis, deposition and maturation of collagen. Increased content and deposition of collagen accelerate wound healing. Increasing the amount of collagen can increase the total cell count as a result of increased cell division (Sumitra et al, 2005). A previous study has reported that various extract of T. erecta Linn. contains carotenoid lutein. Carotenoid lutein in T. erecta Linn. acts as an antioxidant thereby reducing the level of cell damage. In addition, caroteoid lutein also has the effect of reducing inflammation and immunosuppressants (Hajare et al, 2013). Decreasing the level of cell damage can be done in the process of dermal wound healing.

In addition of dermal wound healing, the level of cell damage is also influenced by the infecting bacteria. Previous study has revealed the role of T. erecta Linn. extract as an anti-bacterial. T. erecta Linn. extract also has antibacterial effects against Escherichia coli, Proteus vulgaris, Proteus mirabilis, Aeromonas sobria, Aeromonas hydrophila, Plesiomonas shigelloides and Salmonella enterica. In addition, extract of T. erecta Linn. also have antibacterial effects against Bacillus subtilis, Bacillus cereus, Bacillus circulans and Staphylococcus aureus (Jain et al, 2012). T. erecta Linn. 2.5% extract gel has also been shown to be antibacterial. The inhibition zone diameter of the extract against S. aureus was 17.87 mm, whereas for E. coli it was 15.33 mm (Edy et al, 2019). Gel preparations have been proven stable after being tested using a climate chamber (Memmert) with a temperature of 45 °C±2 °C and a relative humidity of 75%±5% for 90 days

(Edy et al, 2017). As a consequence, increasing the number of bacteria inhibits the wound healing process. Therefore it is necessary to use T. erecta Linn. extract in various preparations for wound healing.

The formula of T. erecta Linn. leaf extract cream needs to be carefully optimized. This is based on our experience in making L. camara Linn. leaf extract ointment 20% and 24%. The L. camara Linn. leaf extract ointment conforms to the parameters of organoleptic, homogeneity and pH, but does not meet the spread power ointment requirements (Parwanto et al, 2013). In addition, T. erecta Linn. extract has also been successfully formulated into various pharmaceutical forms such as gel, cream, anti-mosquito lotion and hair dye (Edy & Parwanto, 2019). A previous study has proven the effect of T. erecta Linn.2.5% leaf extract cream (Gopi et al, 2012) or T. erecta Linn. 5% extract gel on wound healing (Kiranmai et al, 2011). The purpose of this study was to determine the effect of T. erecta Linn. leaf extract cream 3% and 4% on dermal wound healing in Wistar rats.

MATERIALS AND METHODS

Study area

The research project was divided into 3 stages, namely the first stage is plant cultivation of T. erceta Linn., the second stage is making T. erecta Linn. leaf extract cream, while the third stage is treatment of T. erecta Linn. leaf extract cream in Wistar rats. Cultivation of T. erceta Linn. carried out on agricultural land in the Bandungan District, Semarang Regency, Central Java Province, Indonesia. The cultivation time is from July to November 2017. The making of T. erecta Linn. leaf extract cream was carried out at the Biomedical Laboratory, Faculty of Medicine, Universitas Trisakti, Indonesia from December 2017 to March 2018. The treatment of T. erecta Linn. leaf extract cream on the Wistar rat was carried out from April 2018 to August 2018 in Animal Laboratory, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia.

Ethical clearance approval

The study was only done after the recommendation approval by "Komisi Etik Riset, Fakultas Kedokteran, Universitas Trisakti, Indonesia" No. 90/KER/FK/V/2016.

Formulation of T. erecta Linn. leaf extract cream

T. erecta Linn. identified beforehand to determine the species of plants. Leaves of T. erecta Linn. obtained by cultivated at Bandungan, Semarang, Central Java, Indonesia. Leaves of T. erecta Linn. covered with a black cloth and then dried in the sun to dry. The dried leaves are then weighed and made powder. Extraction of T. erecta Linn. powder using ethanol 70% and then filtered to yield a viscous supernatant as crude extract. Aliquot of the extract lyophilized and the results are weighed. T. erecta Linn. leaf extract is used as an active ingredient of cream. Composition of the cream base ingredient has been optimized. Cream base ingredient consist of 14.2 grams stearic acid, 10 grams glycerol, 0.25 grams borax, 1 grams triethanolamine, 0.1 grams nipagin, 0.05 grams nipasol, 9.5 mL mono sodium phosphate (NaH2PO4) 2.55%, 0.5 mL NaH2PO4 1.85% and aquabidestilata add to100 mL. T. erecta Linn. leaf extract cream 3% and 4% are made by adding 3 grams and 4 grams of T. erecta Linn. leaf extract into each the cream base ingredients.

Animal maintenance

In this study involve 24 of Wistar male rats (Rattus novergicus, Wistar starin), aged between 3-4 months with average weight of 150-200 gram were used. The animals were made aclimatization in the cage for 1 week before treatment. Rats were fed and drunk of libitum according to standards. Treatment rooms are equipped with air conditioners with temperatures of 22±3 °C, relative humidity of 55±5%, and artificial fluorescent lamps (12:12 hours, light and dark cycles).

Experimental design

Randomly, Wistar rats are grouped into 3 groups. Group I: Wistar rats were dermal back excision and treated with nitrofurazone (positive control). Group II: Wistar rats were dermal back excision and treated with T. erecta Linn. leaf extract cream 3%. Group III: Wistar rats were dermal back excision and treated with T. erecta Linn. leaf extract cream 4%. Combination of ketamine (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally were used for rats anesthetized before dermal back excision. Administration of nitrofurazone and T. erecta Linn. leaf extract cream daily, for 16 days after back skin excision. Granulation tissue was taken on days 4, 8, 12 and 16 for analysis.

Measurement of dermal wound healing

Graphical planimetry methods was used to measurement of rat wound on days 0, 4, 8, 12 and 16. The size of the wound is marked on all edges of the wound using paper with ink markers. The paper is then attached to graph paper, then measured manually in units of mm^2. The strength of wound contraction (SWC) is the difference of wound on the measurement at day 0 compared with the specified day. The percentage of wound contractions (PWC) is calculated using the

formula as follows PWC={(area on day 0-area on final day)/area on day 0)×100} (Dwivedi and Chaudhary, 2012). Epithelialization period is determined by the number of days needed for epithelialization at the site of the wound (Sumitra et al, 2005).

Calculation of bacteria colonies number

In this study we used 2 media, phosphate-buffered saline (PBS) and Azide blood agar (ABA). The composition of PBS consisted of 8 grams sodium chloride, 0.2 grams kalium chloride, 1.42 grams disodium phosphate and 0.42 grams potassium phosphate monobasic. Final pH of PBS is 7.4, stored at 25 °C. The composition of ABA media consists of 3 grams meat extract, 10 grams tryptose, 5 grams sodium chloride, 0.2 grams sodium azide, 15 grams agar powder, deionized water is added to 1000 mL. Final pH of ABA is 7.3, stored at 25 °C. The number of bacteria in the granulation tissue was calculated on the 6th day of treatment (Hirsch et al, 2008). One hundred µL homogenate granulation tissue was taken to count bacterial colonies. The solution series was inoculated in the PBS for isolation, while the slective culture medium was ABA. Petri was incubated aerobically at 37 °C for 24 hours. Bacteria colonies number expressed as log of 10 colony-forming units (CFU) per gram of tissue. Number of bacteria colonies $>1\times10^5$ declared granulation tissue infected with bacteria (O'Meara et al, 2006).

Biochemical measurements

DNA and protein content in the granulation tissue were extracted in 5% trichloroacetic acid (trichloroacetic acid=TCA). Ten mL of TCA (5%) added to the tissue (100 mg dry weight of the tissue) was heated at 90 ^{0}C for 30 minutes in a water bath to extract DNA and proteins. The solution is centrifuged and the supernatant is used to measure DNA and protein levels. Measurement of DNA content using the Burton method, whereas protein measurement using the Lowry method, (Sumitra et al, 2005).

Histology analysis

At the end of the treatment phase, rats were anesthetized using a combination of xylazine (10 mg/kg) and ketamine (100 mg/kg), and skin tissue (0.8×0.5 cm) were collected into a 10% formalin solution for histological analysis. After the skin tissue is fixed, then wash with running water. The next phase is dehydrated with multilevel alcohol, and then washed with xylene. After the dehydration and washing phase, skin tissue is wrapped in paraffin (melting point 56 °C). Hematoxylin and Eosin (H&E) are used to paint the tissue. Tissue histology observations were carried out using a microscope and documented with photomicrographs.

Statistical analysis

Data of all groups were estimated by one-way analysis of variance (ANOVA) and declared significance if $p<0.05$. If there are differences between groups, followed by the least significant difference (LSD) test.

RESULTS

T. erecta Linn. were used in this study as a result of plant cultivation on agricultural land in Bandungan District, Semarang Regency, Central Java Province, Indonesia (Fig. 1). The geographical location of Bandungan District which is on the southern slopes of Mount Ungaran. Based on Urchin Tracking Module (UTM) coordinates, Bandungan District is located between 425275 - 435093 mE and 9197640 - 9205676 mN.

There are differences in the effect of T. erecta Linn. leaf extract cream compared with nitrofurazone on wound healing (Fig. 2). T. erecta Linn. leaf extract cream 4% is faster healing process, followed by the T. erecta Linn. leaf extract cream 3% and the nitrofurazone group (positive control). The speed of wound healing is characterized by a decrease in wound size (Table 1). Wound size on 4th day showed that group I>II (P=0,022), I>III (P=0.000), II>III (P=0.024). Wound size on 8th day showed that group I>II, III(0.000), while group II=III (0.059). Wound size on 12th day showed that group I>II (0.006), group I>III (0.000) and group II>III (0.022). Wound size on 16th day showed that group I>II>III (0.000).

Strength of wound contraction is a healing response to reduce of wound size, then the damaged tissue is completely repaired. Strength of wound contraction between groups I, II and III at day 0 of tretament as initial tretament day, as well as a base line data. Strength of wound contraction between groups I, II and III at 4th, 8th and 16th days were significantly different (P=0.000). The results of this study showed that T. erecta Linn. leaf extract cream 3% and 4% at 4th, 8th and 16th days had better of strength wound contraction compared with positive controls (nitrofurazone) (P=0.000) (Table 2). Strength of wound contraction on 4th day showed that group I<II (P=0,022), I<III (P=0.000), II<III (P=0.024). Strength of wound contraction on 8th day showed that group I<II, III(P=0.000), while group II=III (P=0.059). Strength of wound contraction on 12th day showed that group I<II (P=0.006), group I>III (P=0.000) and group II<III (P=0.022).

Strength of wound contraction on 16th day showed that group I<II<III (P=0.000).

Percentage of wound contractions between groups I, II and III at day 0 of tretament is initial tretament day, as well as a base line data. Percentage of wound contractions between groups I, II and III at 4th, 8th, 12th and 16th days were significantly different (P=0.000). The percentage of wound contraction in group II and III are stronger than group I (Table 3). T. erecta Linn. leaf extract cream increase the percentage of rat dermal wound healing compared with nitrofurazone. T. erecta Linn. leaf extract cream 3% and 4% at 4th, 8th and 16th days had better on percentage of wound contraction compared with positive controls (nitrofurazone).

We used microscopic observation for determine of epithelialization. Base on the result of epithelialization period in this study, we are divided into 3 stages of the wound healing process, namely early, middle and advanced stages (Fig. 3). In the early stages, only 1 cutaneous muscle is seen in the field of view on a microscope with a magnification of 40X (Fig. 3. A). In the middle stage, two cutenous muscles appear in the field of view on a microscope. The distance between the two cutaneous muscles is getting closer which shows progress in wound healing (Fig. 3. B, C and D). At an advanced stage, two cutenous muscles appear close together (Fig. 3. E). In the advanced stages of wound healing, epithelialization appears on the surface of the wound healing area (Fig. 3. F).

Number of bacterial colonies (NBC) at 6th day in this study showed that in group I the highest, then group III, while group II was lowest (P=0.000) (Fig. 5). DNA levels at 8th, 12th and 16th showed that group I was lower than group II and III (P<0.05). The all groups showed that the DNA content at the 8th day was highest and decreased at the 12th day and the lowest at the 16th day. At the 16th day, groups II and III did not differ in their DNA content (P=0.457), both groups had higher DNA content compared with group I (P=0.000)(Table 4). Proteins levels at 8th, 12th and 16th showed that group I was lowest compared with group II and III (P<0.05). The all groups showed that the proteins levels at the 8th day was lowest and increased at the 12th day and than decreased at the 16th day (P<0.05). The proteins levels at 8th day and 12th day observation showed that group I was lowest compared with group II, while group III was higher (P=0.000). The proteins levels at 16th day observation showed that group I was lowest compared with group II and III (P=0.000), likewise group II was lower compared with group III (P=0.013) (Table 5).

DISCUSSION

Identification of T. erecta Linn. was carried out according to instructions in the book Flora of Java (specifically for Spermatophyta) (Backer CA and van den Brink B, 1963-1968). The result of T. erecta Linn. extraction are used as active ingredients of the cream. Furthermore, the cream containing T. erecta Linn. leaf extract was tested for its effect on wound healing in Wistar rats. We have experience extracting another specimen namely L. camara Linn. and has been tested as an ointment for rat dermal wound healing (Parwanto et al, 2013, 2017). Next, we will to examine effects of T. erecta Linn. extract cream compared with L. camara Linn. extract cream in rat dermal wound healing.

The base cream were used in this study has been simulated. T. erecta Linn. leaf extract cream contains active ingredients namely T. erecta Linn. leaf extract. T. erecta Linn. 3% and 4% leaf extract cream in this study were semi-solid, with the distinctive aroma of T. erecta Linn. extract, slightly black green, pH 5, homogeneous and not thick, spread power of 5.2 cm. The characteristics of the cream fulfill as topical preparations that are suitable for use. In addition, bacterial media is has been prepared and easy to use for isolation and culture.

Base on wound size, T. erecta Linn. leaf extract cream 3% and 4% are faster healing process in Wistar rats compared to nitrofurazone. In addition to the reduction of wound size, strength of wound contraction and percentage of wound contraction can also be used as an indicator of the speed of wound healing. Base on strength of wound contraction, T. erecta Linn. leaf extract cream 3% and 4% are stronger on healing process in Wistar rats compared with nitrofurazone. Based on these results, we expect that T. erecta Linn. 3% and 4% leaf extract cream can be used as a healing agent for dermal wounds. The results of this study according to the results of previous studies which showed the effectiveness of T. erecta Linn. on rat dermal wound healing (Kiranmai et al, 2011). Furthermore demonstrated that strength of wound contraction in control rats 21.6% to 68.3% from 4th day to 12th day and 80.6% to 98.1% from 14th day to 20th day (Murthy et al, 2013). In addition, recent research was conducted to examine the effect of anti-ulcer activity of T. erecta Linn. leaf extract gel 2.5% and 5% compared with a standard drug, triamcinolone. The results showed that T. erecta Linn. leaf extract gel showed significant oral ulcer protective activity (83.6%) compared with triamcinolone. This fact demonstrated that the T. erecta Linn. leaf extract

gel has better potential against oral ulcers (Lakshana et al, 2020).

T. erecta Linn. leaf extract cream 3% and 4% at 4th, 8th and 16th days had better on percentage of wound contraction compared with positive controls (nitrofurazone). This fact is consistent with studies of T. erecta Linn. extract in rat dermal wound healing (Kiranmai et al, 2011). The results of this study are consistent with other studies, which show that ethanol extract of T. erecta Linn. have different percentage of wound contraction between 4th, 8th and 16th days (Chartterjee et al, 2011). If we pay attention, the data about strength of wound contraction is related to the percentage of wound contraction. Base on data in this study, T. erecta Linn. leaf extract cream 3 % and 4% showed strength of wound contraction and percentage of wound contraction are better than nitrofurazone, then clinical trials need to be done on human wound healing.

The result of epithelialization period analysis between groups I, II and III were significant different ($P<0.05$). Effect of T. erecta Linn. leaf extract cream on epithelialization period of dermal wound healing in Wistar rats showed that group I>II, III ($P=0.000$), while group II=III ($P=0.232$) (Fig. 4). In the rat dermal wound healing, the effect of T. erecta Linn. leaf extract cream 3% on epithelialization period was not significantly different compare with T. erecta Linn. leaf extract cream 4%. The effect on epithelialization period of the two creams is shorter than the nitrofurazone cream. Because T. erecta Linn. cream is faster in wound healing than nitrofurazone cream, subsequently T. erecta Linn. leaf extract cream 3% and 4% have the potential to be developed as a wound healing drug. Previous studies have shown that epithelialization period of T. erecta Linn. leaf extract gel 2.5% was faster than T. erecta Linn. leaf extract cream 3% and 4% in this study, however T. erecta Linn. leaf extract cream 3% and 4% can bee developved as an alternative to wound healing. The results of other studies showed that epithelialization period of ethanol extract of T. erecta Linn. 5% (w/w) was significantly different compared with T. erecta Linn.10% (w/w). Epitelialization period of ethanol extract of T. erecta Linn. 5% is 18.00±0.51 days, while ethanol extract of T. erecta Linn. 10% is 14.16±0.75 days (Chartterjee et al, 2011). Beside that, complete epithelization and healing in control rats were observed on 24th day (Murthy et al, 2013). Apparently the epithelialization period of dermal wound healing in Wistar rats in this study is different from mice. The results of studies on the injured mice, then treated with nitrofurazone showed that epithelialization period was around 14.67 days (Belachev et al, 2020). The evidence shows that different epithelialization periods are caused by different types of experimental animals.

Base on data in this study indicated that the T. erecta Linn. leaf extract cream 3% and 4% has a better role as an antibacterial than a positive control (nitrofurazone). The results of this study are consistent with studies showing that the ethyl acetate fraction of T. erecta Linn. has activity against S. aureus (Trinh et al, 2020). Beside that, it has been demonstrated that T. erecta Linn. had antimicrobial activity inhibiting the growth of S. agalactiae and suggesting that they may be useful in the treatment of S. agalactiae infections in humans and animals (Mekvimol et al, 2020).

Data in this study indicated that the process of wound healing involves the role of DNA. Indications of wound healing involving DNA in this studies in accordance with the statement that wound healing is a complex process with stages that are not linear, but there is an overlapping process involving 1,000 genes to achieve closure of dermal wounds (Cooper et al, 2005). The dynamics of changes in DNA levels on wound healing in this study are similar to the results of our previous study of the efficacy of L. camara Linn. leaf extracts ointment on dermal wound healing (Parwanto, 2017). We have not found references to the effects of T. erecta Linn. on DNA levels in wound healing. Nevertheless, a previous studies in rats showed the effect of Potentilla fulgens on increasing DNA levels up to 60.0% on the 16th day (Kundu et al, 2016). Furthermore, the ointment consists of Cordia obliqua Willd. and Vigna radiata Linn. extract proven to increase DNA synthesis, thereby increasing tissue regeneration and accelerating wound contraction (Kadhiravan et al, 2017).

CONCLUSION

T. erecta Linn. leaf extract cream 3% and 4% better act as antibacterial than positive control (nitrofurazone). T. erecta Linn. leaf extract cream 3% and 4% play a role in increasing the strength of wound contraction, the percentage of wound contraction and accelerating the time of epithelialization compared with the positive control group (nitrofurazone). T. erecta Linn. leaf extract cream 3% and 4% increased the DNA and protein content in the injured area compared with the positive control group (nitrofurazone). We recommend that T. erecta Linn. leaf extract cream 3% and 4% can be continued to clinical trials on human dermal wound healing.

SIGNIFICANCE STATEMENT

This study discovered that T. erecta Linn. leaf extract cream 3% and 4% were faster than

nitrofurazone cream on the wound healing proces in Wistar rat. Scientific evidence from this study in the future can be followed by clinical trials in humans so that it can be an option for treating on dermal wounds healing.

AUTHOR'S CONTRIBUTIONS

MLEP and DT: Schemed and designed experiment. MLEP and HJE: Collecting and interpretation of the results. All authors analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of interest: No

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Table 1: Effect of Tagetes erecta Linn. leaf extract cream on wound size in Wistar rats.

		Days of observation				
Variables	Group	0 day (Means ± SD)	4th day Means ± SD	8 th day Means ± SD	12 th day Means ± SD	16 th day Means ± SD
WS (mm^2)	I	400.00 ± 0.00	344.59 ± 6.33	276.56 ± 10.29	85.20 ± 1.73	29.48 ± 2.71
	II	400.00 ± 0.00	334.77 ± 10.04	244.50 ± 6.86	80.10 ± 2.55	22.26 ± 1.27
	III	400.00 ± 0.00	325.07 ± 6.98	234.02 ± 13.35	75.93 ± 4.93	16.76 ± 2.65
	P	-	I>II (0.022) I>III (0.000) II>III(0.024)	I>II, III(0.000) II=III(0.059)	I>II (0.006) I>III (0.000) II>III (0.022)	I>II>III (0.000)

Abbreviations: WS=wound size, group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%, mm^2=millimeter square, SD=standard of deviation, P=significance value.

Table 2: Effect of Tagetes erecta Linn. leaf extract cream on strength of wound contraction in Wistar rats.

		Days of observation				
Variables	Group	Δ WS 0 day – 0 day (Means ± SD)	Δ WS 0 day – 4th day Means ± SD	Δ WS 0 day – 8th day Means ± SD	Δ WS 0 day – 12th day Means ± SD	Δ WS 0 day – 16th day Means ± SD
SWC (mm^2)	I	0.00 ± 0.00	55.41 ± 6.33	123.44 ± 10.29	314.80 ± 1.73	370.52 ± 2.71
	II	0.00 ± 0.00	65.23 ± 10.04	155.50 ± 6.86	319.90 ± 2.55	377.74 ± 1.27
	III	0.00 ± 0.00	74.93 ± 6.98	165.98 ± 13.35	324.07 ± 4.93	383.24 ± 2.65
P		-	I<II (0.022) I<III (0.000) II<III (0.024)	I<II, III (0.000) II=III (0.059)	I<II (0.006) I<III (0.000) II<III (0.022)	I<II<III (0.000)

Abbreviations: SWC=strength of wound contraction, Δ WS= difference of wound size on the measurement at 0 day observation compared with the specified day, group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%, mm^2=millimeter square, SD=standard of deviation, P=significance value.

Table 3: Effect of Tagetes erecta Linn. leaf extract cream on percentage of wound contraction in Wistar rats.

		Days of observation				
Variables	Group	0 day (Means ± SD)	4th day Means ± SD	8 th day Means ± SD	12 th day Means ± SD	16 th day Means ± SD
PWC (%)	I	0.00 ± 0.00	13.85 ± 1.58	30.86 ± 2.57	78.70 ± 0.43	92.63 ± 0.68
	II	0.00 ± 0.00	16.31 ± 2.51	38.88 ± 1.72	79.98 ± 0.64	94.44 ± 0.32
	III	0.00 ± 0.00	18.73 ± 1.74	41.49 ± 3.34	81.02 ± 1.23	95.81 ± 0.66
	P	-	I<II (0.022) I<III (0.000) II<III (0.023)	I<II, III (0.000) II=III (0.059)	I<II (0.006) I<III (0.000) II<III (0.021)	I<II<III (0.000)

Abbreviations: PWC=percentage of wound contraction, group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%, mm^2=millimeter square, SD=standard of deviation, P=significance value.

Table 4: Effect of Tagetes erecta Linn. leaf extract cream on DNA levels of wound healing in Wistar rats.

		Days of observation		
Variables	Groups	8 Means ± SD	12 Means ± SD	16 Means ± SD
DNA Levels (mg/mL)	I	38.37 ± 0.44	29.33 ± 0.84	23.74 ± 0.71
	II	54.35 ± 0.47	42.15 ± 1.21	32.59 ± 1.00
	III	55.85 ± 0.85	45.94 ± 0.75	32.29 ± 0.76
	P	I<II<III (0.000)	I<II<III (0.000)	I<II, III (0.000) II=III (0.473)

Abbreviations: DNA=deoxyribo nucleic acid, Group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%, SD=standard of deviation, mg/dL=milligram per deciliter, P=significance value.

Table 5: Effect of Tagetes erecta Linn. leaf extract cream on protein levels of wound healing in Wistar rats.

		Days of observation		
Variables	Groups	8 Means ± SD	12 Means ± SD	16 Means ± SD
Protein	I	10.58 ± 0.42	14.39 ± 0.41	13.29 ± 0.43

levels (mg/mL)	II	16.66 ± 0.52	26.37 ± 0.58	21.53 ± 0.95
	III	18.19 ± 0.36	28.64 ± 0.41	22.34 ± 0.51
	P	I<II<III (0.000)	I<II<III (0.000)	I<II, III (0.000); II<III (0.013)

Abbreviations: Group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%, SD=standard of deviation, mg/dL=milligram per deciliter, P=significance value.

Fig.1: Cultivation of Tagetes erecta Linn. in Bandungan District, Semarang Regency, Central Java province, Indonesia. A. T. erecta Linn. seedlings in a poly bag. B. T. erecta Linn. at a young age (flowers are still fresh). C. T. erecta Linn. with fresh flowers. D. T. erecta Linn. in old age (flowers have dried). Photographer by Hosea Jaya Edy on October 3, 2017.

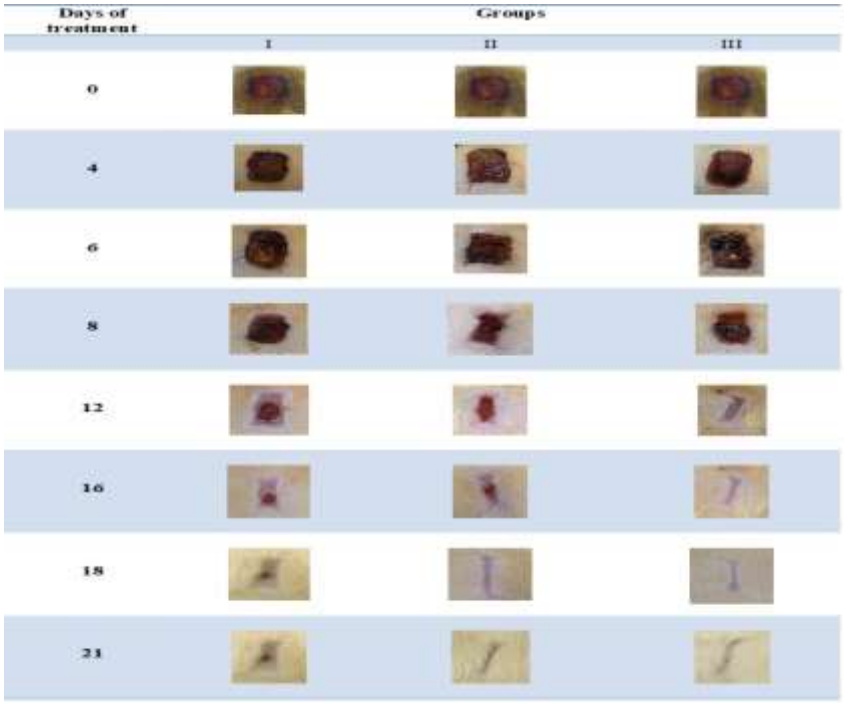

Fig.2: Effect of T. erecta Linn.leaf extract cream on wound healing proces in Wistar rats. Group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%.

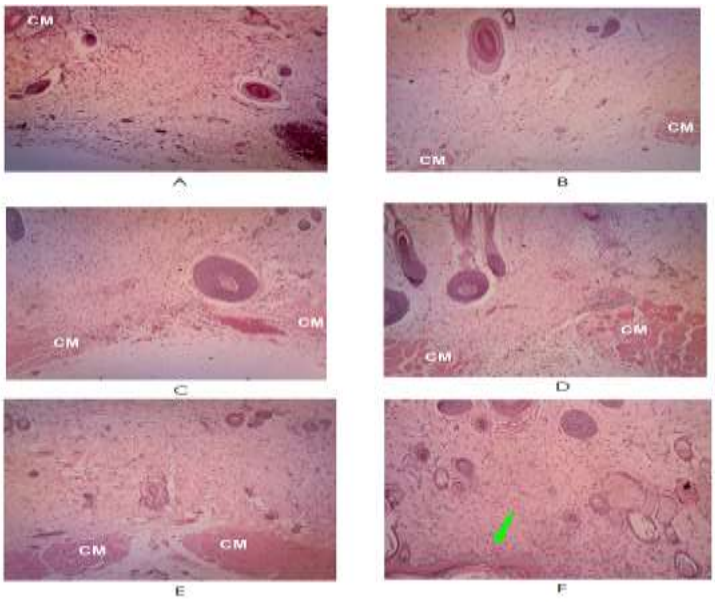

Fig.3: Histological observation of dermal wound healing process in Wistar rats (hematoxylin and eosin painting, magnification 40X). A. Wound healing in the early stage. B, C, and D. Wound healing in the middle stage. E. Wound healing in the advanced stage. F. Wound healing area (lime arrow).

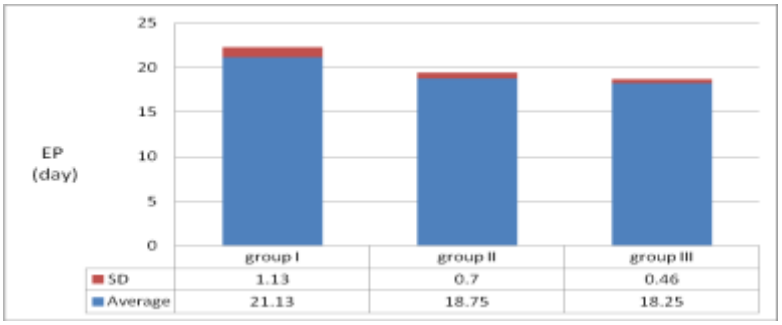

Fig.4: Effect of T. erecta Linn. leaf extract cream on epithelialization period (EP) of dermal wound healing in Wistar rats. Group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%, SD=standard of deviation, p=significance value, group I>II, III (P=0.000), while group II=III (P=0.232).

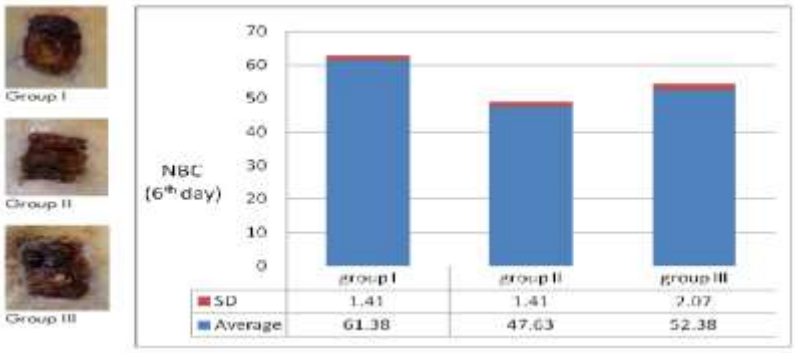

Fig.5: Effect of T. erecta Linn. leaf extract cream on the number of bacterial colonies (NBC) on the 6th day of wound healing in Wistar rats. Group I=Wistar rats were back skin incision and treated with nitrofurazone (positive control), group II=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 3%, group III=Wistar rats were back skin incision and treated with T. erecta Linn.leaf extract cream 4%. NBC at the 6th day on wound healing in Wistar rats showed that group I>III>II (P=0.000).