In vivo wound healing activity of ethanolic extract of *Terminalia catappa* L. leaves in mice (*Mus musculus*)

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Abstract. The leaves of Indian almond (*Terminalia catappa* L.) is known as a folk medicine. However, the leaves has not been explored scientifically for its wound healing activity. Therefore, current research was designed to evaluate the wound healing activity of ethanolic extract of both green (GL) and brown (BL) Indian almond leaves in mice (*Mus musculus*). Incision wounds was inflicted on mice under chloroform anesthesia. Group C served as control without treatment, while group V treated with vaseline. Group Pi was positive control and treated with povidone iodine, group GL20, GL40, and GL 60 treated with simple ointment containing 20, 40 and 60% (w/w) ethanol extract of the GL, whereas group BL20, BL 40, and BL60 used BL with the same concentration. All the mouse were treated topically once a day. Wound healing potential was assessed with the percentage of the wound healing until day 16. The results found that, all groups of mice treated with ethanol extract of either GL or BL higher than 20% showed significantly wound healing activity compared to group of mice treated with simple ointment group, Pi, or control. The present study provides a scientific rationale for the use of the leaves extracts of *T. catappa* in the treatment of wound.

1. Introduction

Wound healing is a repairing process of the tissue from the damage. This process consist of four step: blood coagulation, inflammation, cell proliferation, lesion contraction and remodeling [1]. Successful of wound healing process involves particular factors such as soluble mediators, blood cells, extracellular matrix, biochemical mediators, extra-cellular matrix molecules, and parenchymal cells in a time frame [2].

Previous research revealed that some extract of the plant has capability as wound healing such as *Argyreia nervosa* [3], *Napoleona vogelii* [4], and *Pereskia aculeate* [5]. Another potential plant that can be used as wound healing is *Terminalia catappa* L. The *T. catappa* (Combretaceae), commonly known as Indian almond, Ketapang (Indonesian, Javanese), is a large growing deciduous tree up to 20 meters tall that is widely distributed in both tropical and sub-tropical regions, including Indonesia [6-8].

Previous research stated that the water extract of *T. catappa* leaves can be applied as a traditional medicine to cure antipyretic, hemostatic, hepatitis, and liver-related diseases [9, 10]. Some important phytochemicals compounds such as alkaloids demethoxy-kanugin, gamatay, glabrin, glabrosaponin,
kaempferol, kanjone, kanugin, karangin, neoglubrin, pinnatin, pongamol, pongapin, quercitin, saponin, b-sitosterol, and tannin are found in *T. catappa* leaves [8, 11]. Allyn et al [6] revealed that *T. catappa* brown and green leaves showed an antibacterial activity which might be useful as part of wound healing. Though the research regarding wound healing activity of *T. catappa* leaves has been done [12], However, the comparison of the wound healing activity between green and brown leaves of *T. catappa* has never been done. Thus, present research aimed to evaluate the wound healing activity of ethanolic extract of green (GL) and (BL) of *T. catappa* leaves.

2. Materials and methods

2.1. Plant material

The leaves of *T. catappa* were collected from around Mulawarman University campus, Gunung Kelua, Samarinda, East Kalimantan, Indonesia.

2.2. Animal preparation

A group of 54 male mice (weight ± 30g) was used and obtained from the local animal market around Mulawarman University. Mice were fed on a standard pellet diet and water ad libitum and maintained at 24–28°C, relative humidity (30%-70%), with a 12 h light/12 h dark cycle. All the experiments were conducted in accordance with the internationally accepted laboratory, following NC3Rs Animal research reporting of in vivo experiments (ARRIVE) animal ethical guidelines.

2.3. Extraction

The freshly collected both green and brown leaves were shade-dried, cut, and pulverized using a mechanical grinder. The powdered leaves were macerated with 90% ethanol for 3 days, with occasional shaking. The extract was subjected to preliminary phytochemical tests after drying by using standard methods [13].

2.4. Formulation of the extract

Two types of formulations, green and brown leaves extract with different concentration: 20, 40, 60% were prepared. Simple ointment was prepared using the vaselin as a base for the various ethanolic extract of *T. catappa* leaves.

2.5. Wound healing trial

In total 54 male mice (weight ± 30g) were distributed randomly into 9 groups of six in each. Group C: Control (without any treatment); Group V: Treated with vaseline ointment; Group Pi: Treated with Povide iodine ointment; Group GL20-60%: treated with various concentration (20, 40, 60%) ointment of green leaves extract of *T. catappa*, BL20-60%: treated with various concentration (20, 40, 60%) ointment of brown leaves extract of *T. catappa*. All mice in each group were anaesthetized by the open mask method with anaesthetic chloroform. To develop wounds, a single full thickness 1.5 cm in length superficial excision was made on the mid-dorsum, after back hair removal and alcohol disinfection. All the mice were treated topically once a day with various extract. The wound healing potential was assessed and monitored with the percentage of the wound healing until day 16.

2.6. Statistical analysis

The percentage results obtained from wound models have been expressed as mean ± SEM. The data was evaluated by one way ANOVA followed by Duncan post hoc test, *P<0.05* was considered as significant

3. Results
Qualitative phytochemical analysis of green and brown ethanolic extract leaves of *T. catappa* revealed the presence of alkaloids, saponin, triterpenoid, quinon, phenolic, tannin, and flavonoid both in green and brown leaves of *T. catappa* extract (Table 1). However, no steroid has been found in both of them.

**Table 1.** Phytochemicals content of green and brown leaves ethanolic extract of *Terminalia catappa*

| Phytochemicals | Green leaves | Brown leaves |
|----------------|--------------|--------------|
| Alkaloid       | +            | +            |
| Saponin        | +            | +            |
| Steroid        | -            | -            |
| Triterpenoid   | +            | +            |
| Quinon         | +            | +            |
| Phenolic       | +            | +            |
| Tannin         | +            | +            |
| Flavonoid      | +            | +            |

The measurement of the progress of the wound healing induced by the vaseline (Control +), Povide iodine, GL and BL ointments, in the excision wound model were shown in Table 2. It was observed that all groups of mice treated with ethanol extract of either GL or BL *T. catappa* showed significantly better wound healing activity than group of mice treated with vaseline ointment group, Pi, or control. The mice in the group GL20 and BL 40 showed completely close wound at the day 12, while BL20 at the day 13. However, the GL60 ointment found to be slower for wound healing than other groups.

**Table 2.** The percentage of wound healing induced by green and brown leaves ethanolic extract *Terminalia catappa* simple ointment in mice (*Mus musculus*)

| Day* | C       | V       | Pi       | GL20    | GL40    | GL60    | BL20    | BL40    | BL60    |
|------|---------|---------|----------|---------|---------|---------|---------|---------|---------|
|      | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 1    | 11.82±0.74 | 3.68±0.79 | 10.65±1.11 | 20.50±1.80 | 11.68±0.71 | 13.68±2.43 | 6.50±0.87 | 5.33±0.47 | 13.33±2.93 |
| 2    | 15.50±0.73 | 10.98±1.29 | 18.00±0.97 | 26.33±2.22 | 25.15±0.83 | 21.15±3.59 | 9.68±0.88 | 12.33±2.14 | 19.50±1.92 |
| 3    | 23.17±1.09 | 15.65±0.69 | 25.33±0.73 | 30.35±1.67 | 31.33±1.66 | 25.33±4.02 | 15.83±0.88 | 20.15±1.41 | 25.18±0.57 |
| 4    | 30.82±1.16 | 29.83±1.28 | 30.33±0.88 | 35.85±3.24 | 38.00±1.78 | 29.00±4.07 | 22.83±1.44 | 24.68±1.36 | 36.50±0.31 |
| 5    | 37.50±1.00 | 36.68±1.06 | 38.35±0.84 | 41.68±2.58 | 46.33±0.88 | 32.00±4.11 | 30.33±1.13 | 37.68±0.88 | 43.50±0.58 |
| 6    | 43.15±0.83 | 43.83±1.12 | 44.15±1.18 | 45.18±2.76 | 54.15±1.84 | 36.65±3.99 | 36.50±1.29 | 43.33±0.99 | 50.15±1.47 |
4. Discussion

The wound healing and tissue repair which is caused by incision or excision are the complex processes, involving some biochemical process and cellular reactions. The process of wound healing starts with inflammation, repairing, and remodeling of the injured tissue. The wound itself is a rupture of epithelial integrity of the skin that might be caused by violence or trauma. The rupture of epithelial integrity usually follows by disruption of the structure and function of underlying normal tissue [14].

The results of the present study indicate that ethanolic leaves extract ointment both green and brown leaves of *T. catappa* exhibited significant wound healing activity. This wound healing was demonstrated by a significant enhance in the percentage of wound repair post tissue damage. This may be due to the effect of *T. catappa* extract which contained phytochemicals that increased collagen synthesis. Similar results has been found by Das [15], stating that phytochemical constituent in the aqueous crude extract of *Stevia rebaudiana* improved the wound healing process in mice.

The use of plant extract as a traditional medicine has been known many years with high degree of success [4, 16, 17]. Plant extract that contains phytochemicals help increase wound healing process. Present results found that both green and brown *T. catappa* leaves contained some important phytochemical contents, such as saponin, triterpenoid, quinon, phenolic, tannin, and flavonoid which increased wound healing activity. This finding in line with past research stated that phytochemical constituents such as triterpenoids [18] and flavonoids [19, 20] are known to increase the wound healing process mainly due to their astringent, antimicrobial and free radical scavenging activity. Another study of plant extract related to wound healing also found that the roots of *Mimosa pudica* which contained phenol constituents played an important role in wound healing process [21-23]. In addition, alkaloid constituent also showed antibacterial activity which useful for wound healing activity due to infections complicate the post-operative wound healing process [24].

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5. Conclusion
This research highlighted the wound healing activity of the ethanolic extract of *Terminalia catappa* green and brown leaves. The results indicated that both green and brown leaves ethanolic extract facilitated wound healing to repair the damage tissue in mice. The ointment of 20% green leaves ethanolic extract is recommended to increase wound healing activity compare to brown leaves. This finding suggested that ethanolic extract of *T. catappa* green leaves benefit to be implemented as wound healing biological agent and uphold the traditional use of the plant for the treatment of wounds.

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References
[1] Hermes D, Dudek D N, Maria M D, Horta L P, Lima E N, de Fatima A, Sanches A C Modolo L V 2013 In vivo wound healing and antiulcer properties of white sweet potato (*Ipomoea batatas*) *Journal Of Advanced Research* 4(4) pp 411-5 PubMed PMID: 25685447 Pubmed Central PMCID: 4293869
[2] Lodhi S, Singhai A K 2013 Wound healing effect of flavonoid rich fraction and luteolin isolated from *Martynia annua* Linn. on streptozotocin induced diabetic rats *Asian Pacific Journal of Tropical Medicine* 6(4) pp 253-9
[3] Gupta H, Bhati V S, Singhal A K 2011 Wound healing activity of *Argyreia nervosa* leaves extract *International Journal of Applied and Basic Medical Research* 1(1) p 36
[4] Adiele L C, Adiele R C, Enye J C 2014 Wound healing effect of methanolic leaf extract of *Napoelonea vogelii* (Family: Lecythidaceae) in rats *Asian Pacific Journal of Tropical Medicine* 7(8) pp 620-4
[5] Carvalho E G, Soares C P, Blau L, Menegon R F, Joaquim W M 2014 Wound healing properties and mucilage content of *Pereskia aculeata* from different substrates *Revista Brasileira de Farmacognosia* 24(6) pp 677-82
[6] Allyn O Q, Kusumawati E, Nugroho R A 2018 Antimicrobial activity of *Terminalia catappa* brown leaf extract against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 *F1000Research* 7
[7] Nugroho R A, Manurung H, Nur F M, Prahastika W 2017 *Terminalia catappa* L. extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in Betta sp archives of Polish Fisheries 25(2) pp 103-15
[8] Nugroho R A, Manurung H, Saraswati D, Ladyescha D, Nur F M 2016 The effects of *Terminalia catappa* leaf extract on the haematological profile of ornamental fish *Betta splendens* *Bioscientifica: Journal of Biology and Biology Education* 8(2) pp 241-8
[9] Arjariya S, Nema N 2014 Estimation of antipyretic, palliative and anti-inflammatory potential of methanolic extract of *Terminalia catappa* Bulletin of Pharmaceutical Research 4(1) pp 37-42
[10] Chyau C C, Ko P T, Mau J L 2006 Antioxidant properties of aqueous extracts from *Terminalia catappa* leaves *LWT-Food Science and Technology* 39(10) pp 1099-108
[11] Caruso G 2015 Use of Plant Products as Candidate Fish Meal Substitutes: An emerging issue in aquaculture *Productions Fisheries and Aquaculture Journal* 6(3) pp 1-3

[12] Khan A A, Kumar V, Singh B K, Singh R 2014 Evaluation of wound healing property of *Terminalia catappa* on excision wound models in Wistar rats *Drug Research* 64(5) pp 225-8 PubMed PMID: 24132703

[13] Trease G, Evans W 1989 A text book of pharmacognosy. ELSB Bailiere Tindall. 11 ed. London: Bailiere Tindall pp 45-50

[14] Mekonnen A, Sidamo T, Asres K, Engidawork E 2013 In vivo wound healing activity and phytochemical screening of the crude extract and various fractions of *Kalanchoe petiﬁtiana* A. Rich (Crassulaceae) leaves in mice *Journal of Ethnopharmacology* 145(2) pp 638-46 PubMed PMID: 23228912

[15] Das K 2013 Wound healing potential of aqueous crude extract of *Stevia rebaudiana* in mice *Revista Brasileira de Farmacognosia* 23(2) pp 351-7

[16] Esimone C, Nworu C, Jackson C 2008 Cutaneous wound healing activity of a herbal ointment containing the leaf extract of *Jatropha curcas* L.(Euphorbiaceae) *International Journal of Applied Research in Natural Products* 1(4) pp 1-4

[17] Mesfin F, Demissew S, Teklehaymanot T 2009 An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, *Ethiopia Journal of Ethnobiology and Ethnomedicine* 5(1) p 28

[18] Scortichini M, Rossi M P 1991 Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burriill) Winslow et al *Journal of Applied Bacteriology* 71(2) pp 109-12

[19] Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M 1996 Comparative study on the antibacterial activity of phytochemical ﬂavanones against methicillin-resistant *Staphylococcus aureus* *Journal of Ethnopharmacology* 50(1) pp 27-34

[20] Devi P S, Shyamala D C 1999 Protective effect of quercetin in cisplatin-induced cell injury in the rat kidney *Indian Journal of Pharmacology* 31(6) p 422

[21] Kokane D D, More R Y, Kale M B, Nehete M N, Mehendale P C, Gadgoli C H 2009 Evaluation of wound healing activity of root of *Mimosa pudica* *Journal of Ethnopharmacology* 124(2) pp 311-5

[22] Kumarasamyraja D, Jeganathan N, Manavalan R 2012 A review on medicinal plants with potential wound healing activity *International Journal of Pharmacy and Pharmaceutical Sciences* 2 pp 105-11

[23] Silambujanaki P, Bala T C H, Anil Kumar K, Chitra V 2011 Wound healing activity of *Glycosmis arborea* leaf extract in rats *Journal of Ethnopharmacology* 134(1) pp 198-201

[24] Bhattacharyya P, Chakrabarty P, Chowdhury B 1985 Glycozolidol, an antibacterial carbazole alkaloid from *Glycosmis pentaphylla* *Phytochemistry* 24(4) pp 882-3