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, neoglabrin, 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 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 vaseline 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*	Groups								
	C	V	Pi	GL20	GL40	GL60	BL20	BL40	BL60
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00[a]	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

7	57.50±1.45	49.65±0.89	57.18±1.35	52.65±4.38	63.85±1.18	40.35±2.91	44.00±0.99	50.65±1.25	55.83±0.56
8	63.35±1.31	56.85±1.04	63.68±1.23	65.65±2.67	69.50±1.00	47.83±2.56	54.33±1.15	64.00±0.99	64.85±1.04
9	71.00±1.02	65.15±1.88	70.83±1.09	66.50±2.12	79.00±1.94	58.00±3.20	58.50±1.56	71.33±1.77	74.68±1.41
10	76.50±1.09	74.00±1.85	77.50±1.09	80.33±2.05	86.50±2.12	66.00±2.69	70.83±1.38	83.18±1.21	83.03±0.88
11	82.17±0.56	81.50±2.18	84.18±1.28	91.00±1.56	91.85±1.34	81.50±1.80	81.50±1.74	90.33±1.23	88.35±1.34
12	84.85±1.04	89.18±0.89	90.50±0.92	100.00±0.00	100.00±0.00	89.00±0.43	89.65±0.89	100.00±0.00	100.00±0.00
13	90.00±0.87	92.00±1.30	100.00±0.00	100.00±0.00	100.00±0.00	93.33±1.51	100.00±0.00	100.00±0.00	100.00±0.00
14	95.17±0.57	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
15	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
16	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

*) = post wounding day. C = Control without treatment, V = vaseline, Pi = positive control and treated with povidone iodine, GL20, GL40, and GL 60 = Simple ointment containing 20, 40 and 60% (w/w) ethanol extract of the Green Leaves (GL), whereas group BL20, BL 40, and BL60 = Simple ointment containing 20, 40 and 60% (w/w) ethanol extract of the Brown Leaves (BL). Values are expressed as mean ± SEM (N=6), $P<0.05$ when compared to control.

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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