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Research Article

Evaluation Of Wound Healing Activity Of Methanolic Leaves And Stem Bark Extract Of *Piliostigma Thoninngii* In Albino Rats

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ABSTRACT

Piliostigma thonningii is used in many parts of West Africa including Kebbi State, Nigeria for management of wounds, chronic ulcer, gastric heart pain and headache. The present study was carried out to evaluate the wound healing effect of methanolic leaves and stem bark extract of *P. thonningii* in animal model. The wound healing effect was evaluated using excision wound model and ointment formulation of 10 and 50% w/w of both extracts were used respectively. Phytochemical screening was carried out using standard laboratory methods. The results revealed a dose and time dependent significant ($P<0.05$) increase in percentage wound contraction in the standard drug (penicillin), leaves and stem bark ointment extracts of *P. thonningii* treated groups from day 3 to day 15. Maximum wound contraction were observed at day 12 – day 15 in all the groups. Significant ($P<0.05$) decrease in epithelization period were observed in penicillin ointment (13.25±0.63 days), 50% leaves ointment extract (10.75±0.75 days) and 50% stem bark ointment extract (9.75±0.7500 days) of *P. thonningii* treated groups respectively when compared with control (16.00±1.16 days) and normal base (15.50±0.65 days) treated groups. Phytochemical constituents detected includes alkaloids, steroids, flavonoids, saponins, tannins and anthraquinones. These findings justify the ethnomedicinal uses of *P. thonningii* for management of wound.

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INTRODUCTION

In most of the developing world like Nigeria, plants products play an important role in the treatment of wound [1]. Crude extracts from different plants have been used to treat skin infections such as sores, bites, burns and lacerations. Nearly 80% of people living in the developing countries especially in Africa depend on herbal medicine for their health needs [2]. Plants have the immense potential for the management and treatment of wounds. A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. These phytomedicine are not only cheap, effective and affordable but are also safe. The presence of various life-sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential wound healing properties.

Medicinal plants are coming into prominence because of the over-use of conventional medicines such as antibiotics which has resulted in the development of resistance in many infectious organisms. It has been estimated that nearly 6 million people suffer from chronic wounds worldwide [3]. The chronicity could probably be attributed to the inefficacy of conventional drugs or resistance of microorganisms to such drugs. Unhealed wounds constantly produce inflammatory mediators that produce pain and swelling at the wound site. Chronic wounds may even lead to multiple organ failure or death of the patients [3]. Though several conventional drugs are known to increase healing in different type of wounds, these drugs are complicated, expensive and their availability is still limited [4].

Piliostigma thonningii has been used and reported for different and varied medicinal purposes. For example, it has been reported from many Africa countries that various parts of the plant are used to treat wounds, ulcers, gastric and heart pain, gingivitis, and as an antipyretic [5, 6]. Many herbal drugs have been used in management and treatment of wounds over the years. Plants or chemical entities derived from plants need to be identified and formulated for the treatment and management of wounds. The aim of wound care is to promote wound healing in the shortest time possible with minimal pain, discomfort, and scarring to the patient and must occur in a physiological environment, conducive to tissue repair and regeneration [7]. Therefore, the present research was designed to evaluate the wound healing potential of *Piliostigma thonningii* leaves and stem bark.

MATERIALS AND METHODS

Collection and Identification of plant material

Fresh leaves and stem bark of *Piliostigma thonningii* were collected within the premises of Kebbi State University of Science and Technology, Aleiro campus, which were then identified and authenticated by Dr. D. Singh of Department of Biological Science, Kebbi State University of Science and Technology, Aleiro. A sample with voucher number 109 has been deposited for future reference at the department's Herbarium.

Piliostigma thonningii Leaves and Stem Bark Methanolic extraction

The leaves and stem bark of *Piliostigma thonningii* were separately air dried under room temperature until constant weight was obtained. The dried leaves and stem bark were then pulverized respectively using a wooden mortar and pestles into coarse powders. One hundred grams (100g) each of the pulverized plant material was dissolved in 1000mL of methanol for 72 hours with constant shaking and filtered using muslin cloth. The filtrate was concentrated in an oven at 40°C and stored in a refrigerator at 4°C prior to the commencement of this study.

Qualitative Phytochemical Screening

Piliostigma thonningii leaves and stem bark extracts were analyzed for glycosides, alkaloids,

saponins, tannins, flavonoids, steroids, carbonhydrates Antraquinones and quinones using standard laboratory procedures [8, 9, 10].

Experimental Animals

Forty (40) albino rats of both sexes weighing 150-250g were purchased from the Animal House, department of biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria and were transported to the Department of Biochemistry Laboratory, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria and used for this study. They were fed with growers mash (Vital Feeds, Nigeria) and tap water *ad libitum*. The albino rats were housed under standard laboratory environment and allowed to acclimatize to the laboratory conditions for 10 days before the commencement of the study. All animals in this research were treated in accordance with "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985).

Excision wound

The animals were anesthetized by using ketamine (100 mg/kg, im). An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved to a circular diameter of 40 mm by means of razor blades one day prior to the experiment and the shaved area was cleaned with 70% v/v ethanol before excision wounds were created. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2 [11].

Animal Groupings and Treatments

The rats were divided into five (7) groups of four (4) rats each for evaluation of wound healing activity of methanolic leaves and stem bark extract of *Piliostigma thonningii* extract. Wound treatment commenced on the second day of wound excision. The normal base, extract and reference drugs ointments were respectively applied topically to the wounds 24 hourly for 15 days as follows;

Group one (1) served as untreated control,

Group two (2) was treated with normal base only,

Group three (3) served as standard group, treated with Penicillin ointment.

Group four (4) was treated with 10% w/w of methanolic leaves extract of *P. thonningii.*

Group five (5) was treated with 50% w/w of methanolic leaves extract of *P. thonningii*

Group six (6) was treated with 10% w/w of methanolic stem bark extract of *P. thonningii.*

Group seven (7) was treated with 50% w/w of methanolic stem bark extract of *P. thonningii*

Wound size / contraction

Wound size measurement can be used to monitor the progress of healing through changes in the area of the wound with time. The size of the wound was measured at a regular interval of 72 hours. An excision wound area was measured by vernier calipers and millimeter ruler and expressed in percentage of healed wound area [12]. The evaluated surface area was then engaged to determine the percentage of wound contraction, taking initial size of wound, $500mm^2$, as 100%, by using the following formula as:

$$\text{Wound contraction (\%)} = \frac{\text{initial wound size} - \text{specific day wound size} \times 100}{\text{Initial wound size}}$$

Epithelialization period

It was evaluated by noting the number of days requisite for the escher to fall off from the wound surface exclusive of leaving a raw wound behind [12].

Statistical Analysis

The values were presented as mean ± S.E.M. The significance of the difference of the mean value with respect to control group was analyzed by one way ANOVA followed by Dunnet's t-test. *$P<0.05$ was as considered to be significantly different.

RESULTS AND DISCUSSION

The percentage yield of methanolic extract of *P. thonningii* leaves and stem bark was found to be 29% and 12% respectively. Phytoconstituents present in *P. thonningii* leaves and stem bark are present in table 1.

Phytochemicals	*P.thonningii Leaves*	*P.thonningii Stem Bark*
Saponin	+	+
Tannin	+	ND
Cardiac Glycosides	+	ND
Flavonoids	ND	+
Anthraquinones	+	+
Carbohydrates	+	+
Alkaloids	+	+
Steroids	+	+
Quinines	+	+

+ = detected; ND = Not Detected

The effect of methanolic extract of *P. thonningii* leaves and stem bark on wound size and percentage contraction is presented in table 2. The result showed significant (P<0.05) reduction in wound size and mean percentage wound contraction in all extract and penicillin treated groups at day 3 and day 6 respectively compared to control and normal base treated groups. The wound showed dose dependent complete epithelization in the 50% and 10% leaves ointment (10.75±0.75 days; 12.75±0.85 days), 50% and 10% stem bark ointment (9.75±0.75 days; 10.5±0.86 days) respectively which was not significantly (P>0.05) different from standard drug penicillin (13.25±0.63 days) but significantly (P<0.05) different from control (16.00±1.16 days) and normal base (15.50±0.65 days) treated groups (table 3).

Table 2: Effect of methanolic leaves extract of Piliostigma thonningii on wound size and contraction

Wound Area (mm^2) SEM and % Wound Contraction In Parenthesis							
Day	**Control**	**Normal Base (Vehicle)**	**Penicillin ointment**	**10% leaves ointment**	**50% leaves ointment**	**10% Stem Bark ointment**	**50% Stem Bark ointment**
Day 0	500.00±0.00 (0)	500.00±0.00 (0)	500.00±0.00 (0)	500.00±0.00 (0)	500.00±0.00 (0)	500.00±0.00 (0)	500.00±0.00 (0)
Day 3	396.50±13.25 (20.7)	382.23±9.41 (23.53)	368.31±13.00 (26.34)	339.36±4.76* (32.12)	313.86±16.25* (37.22)	272.25±15.06* (45.6)	229.25±16.14* (54.15)
Day 6	315.00±5.20 (37)	286.36±9.05 (42.72)	232.50±4.33* (53.5)	228.75±7.18* (54.25)	203.25±9.23* (59.35)	165.5±28.814* (66.9)	159.5±18.355* (68.1)
Day 9	186.33±26.91 (62.73)	158.25±19.26 (68.35)	105±32.04* (79)	90.5±6.19 ** (81.9)	71.75±17.60* (85.65)	46.5±9.912* (90.7)	21.5±7.089 * (95.7)
Day 12	114.33±38.46 (77.13)	96.25±28.92 (80.75)	32.75±18.41* (93.45)	22.25±5.11 * (95.55)	12.25±7.88* (97.55)	9.75±5.921* (98.05)	1.5±1.500* (99.7)
Day 15	40.00±23.09 (92)	29.00±20 (94.2)	5.25±3.09* (99)	5.75±3.60* (98.85)	0.00±0.00* (100)	2.25±1.436* (99.55)	0.00±0.000* (100)

*Each value is the mean ± S.E.M. of four rats; *P <0.05 is considered significantly different*

Table 3: Effect of Methanol Leaves and Stem Bark Extract of Piliostigma thonningii on Epithelisation Period

Treatments (dose)	Epithelization Period (days)
Control (untreated)	16.00±1.16
Normal base (Vehicle) (100% w/w)	15.50±0.65
Penicillin ointment (10% w/w)	13.25±0.63
10% leaves ointment (10%w/w)	12.75±0.85*
50% leaves ointment (50% w/w)	10.75±0.75**
10% stem bark ointment (10%w/w)	10.50±0.86**
50% stem bark ointment (50%w/w)	09.75±0.75**

*P <0.05 and **P <0.05 are considered significantly different compared to control

A wound occurs when the integrity of any tissue is compromised (eg skin breaks, muscle tears, burns or a bone fracture [13]. Irrespective of the type of wound, the response to injury is immediate via mechanism s such as barrier protection, inflammatory phase, fibroplastic phase and the remodelling phase. Protection of the exposed wound surface is vital as bacterial infection may manifest. Bacterial contamination delays the process of healing due to release of bacterial toxins that provoke necrosis, suppuration and thrombosis. The pharmacological activities of medicinal plants are due to the presence of bioactive chemical constituents present as secondary metabolites. Tannins and saponins are known to exert numerous pharmacological effects including antimicrobial and anti-inflammatory activity [14, 15]. Flanonoids and Tannins act as free radical scavengers known to enhance wound healing since high levels of reactive oxygen species in wound sites promotes collage breakdown, destruction of the extracellular matrix, reduced angiogenesis and re-epithelization while alkaloids have been shown to have analgesic effects [16]. Steroids and polyphenols have also been reported for its wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation, thereby reduce cell necrosis and improving vascularity [17]. In the present study, the remarkable acceleration of healing within the leaves and stem bark ointment treated groups may be attributed the presence of these phytochemicals. Wound healing effect of *Piliostigma thonningii* leaves and stem bark ointment could be a function of either the individual or the synergistic effects of the phytochemical constituents. These phytochemical constituents may promote the process of wound healing by increasing the viability of collagen fibrils, increasing the strength of collagen fibers either by increasing the circulation or by preventing the cell damage or by promoting the DNA synthesis [18].

CONCLUSION

The methanolic leaves and stem bark ointment of *Piliostigma thonningii* exhibited accelerated wound healing effect via rapid wound contraction and re-epithelization period respectively which was comparable to the standard drug penicillin. These findings justify the medicinal uses of *P. thonningii* for management of wound. Further studies need to be undertaken to isolate, purify, characterize and elucidate the underlying wound healing mechanism of bioactive component(s) of this plant.

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