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Wound healing effect of methanolic leaf extract of *Napoleona vogelii* (Family: Lecythidaceae) in rats

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ABSTRACT

Objective: To investigate the wound healing property of *Napoleona vogelii* leaf extract in folkloric medicine. **Methods:** Both sexes of adult albino rats (n=25) were used in this study and another group (n=30) were subjected to acute toxicity test (LD_{50}) of the plant extract. For the LD_{50}, three randomized groups of 5 rats were first treated with 10, 100, 1 000 mg/kg body weight (bw), orally. This was followed by a second treatment of 1500, 3000, and 5 000 mg/kg bw of the leaf extract with continual monitoring of the animals for mortality or non-mortality. Incision wounds (1.5 cm) were created on the skin of five groups of 5 rats using surgical blade under anesthesia. The first group was topically treated with petroleum jelly alone, group 2 was topically applied 400 mg/mL w/v of the reference drug, Neobacin, while group 3-5 were topically treated with 5-50 mg/mL w/v of the plant extract, respectively. **Results:** The percentage yield of the extract was 49.80% w/w dry matter. The phytochemical analysis revealed several bioactive constituents including glycosides, tannins, alkaloids, perpenoids, saponins, steroids, proteins, and carbohydrates. The LD_{50} was beyond our experimental limit and was not determined. Increased concentrations (5, 20, and 50 mg/mL w/v) of the extract had significant (ANOVA, P<0.05) healing effect on the incision wounds giving rise to 125%-140% while treatment with Neobacin resulted in 150% healing effect on the third treatment regimen compared to the control (100%). **Conclusions:** These data indicate that *Napoleona vogelii* leaf extract contains potent bioactive compounds containing wound healing activity, substantiating its use as a wound healer in folkloric medicine.

1. Introduction

A break in the cellular and anatomical architecture of body tissue including the skin, mucus membrane, deeply lying tissues or surface of internal organs ranging from incision, laceration, abrasion, puncture, and closed wounds such as contusion, hematoma and crush injuries is termed wound[1,2]. It may result from traumatic injuries, metabolic disturbances, and long standing debilitating systemic conditions such as diabetes and hyperglycemia[3-5]. Naturally, wound healing is slow and sometimes may become chronic with a long clinical course thereby resulting in a constant release of inflammatory modulators that cause pain and swelling[5]. Chronic wound may become infected with micro-organisms and this may result in delay in the wound healing, septicemia, organ failure and death in severe conditions[6].

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Regardless of how wound is created, healing brings about interaction of cellular and biochemical body machinery to restore disrupted or damaged body tissues[7]. Wound healing is accomplished by inflammation, proliferation, differentiation, migration, organization, and remodeling of cells in and around the vicinity of the injury. These cells include endothelial, fibrocytes, platelets, and mesenchymal cells under the control of growth factors and cytokines that are subsequently anchored by extracellular matrix[8]. This coordinated series of processes involve movement and infiltration of cellular elements, collagen synthesis and remodeling, angiogenesis, epithelization, production of glycosaminoglycans, and proteoglycans to restore the disrupted tissue. Subsequently, there is scar formation with fibrous union of the edges of the wound that eventually merge with the surrounding tissues. Several factors contribute to delay in wound healing. These include complications resulting from contaminated infective microorganisms, anti-inflammatory agents, nutritional deficiencies, inadequate blood supply, inappropriate movement of the disrupted parts of the body as well as interposed foreign materials such as surgical sutures[7]. Debilitating diseases such as diabetes interferes and delays wound healing. To avoid complications and delay in wound healing, therapeutic intervention using synthetic and or natural wound healing therapeutic agents is necessary to accelerate and accomplish healing[5].

Bio-active substances in plants have shown remarkable potential and tremendous benefit in accelerating wound healing. These substances owe their medicinal activity to the presence of the constituent secondary metabolites such as terpenoids, alkaloids, tannins, steroids, glycosides, phenolics, saponins, and flavonoids that are contained in their leaves, stems, barks, fruits, seeds, roots and flowers[9]. These metabolites differ among the plant community and provide tremendous reservoir of various chemical substances that are potential therapeutic agents[10]. Over 50 plants have been reported to have wound healing activity in traditional African medical practice including *Chromolaena odorata*, *Psidium guajava*, *Vernonia amygdalina*, and *Napoleona vogelii* (*N. vogelii*)[1,2,7,9,12].

N. vogelii (Family: Lecythidaceae) is tropical flora that is widely distributed in the coastal regions of West African countries including Nigeria. This medicinal shrub is known locally in various communities by different names. In the south-eastern part of Nigeria, it is called Odure, Akpuruke or Mkpodu where it is used in the treatment of ulcers, stomach aches, and diarrhea[9]. Previous report showed that the extract from this plant has antimicrobial activity against bacterial agents[9]. Preparation from the bark of *N. vogelii* has been used in the management of cancer in the western part of Nigeria[11]. Topical preparation from the stem bark has been used and found beneficial in the treatment of dermatosis, ingested to treat sexual asthenia, and extract from the leaves has been used in the treatment of external wounds[9]. However, the pharmacological basis of using *N. vogelii* leaf extract in wound treatment is unknown. The objective of this study is to evaluate the wound healing potential of *N. vogelii* leaf extract and elucidate the rationale behind its use in the treatment of wound in folkloric medicine using a rat model of skin wound healing.

2. Materials and methods

2.1. Plant and extraction

The plant was procured from herbal section of Owerri main market, Owerri, Imo State, Nigeria and was identified by Pharm. Osuala, a pharmacognisist in the Department of Pharmacology and Toxicology, Madonna University, Elele, River State, Nigeria. The leaves were removed, washed, and dried by exposing it to natural sunlight for 4 d. Subsequently, the sun-dried leaves were chopped into tiny bits using an electric blender and thereafter pulverized into powder with a mortar and a pestle.

Using a weighing balance, 150 g of the powdered leaf was then added into 1 500 mL of methanol at room temperature for 48 hr with intermittent agitation. The crude extract was filtered into a flat-bottom flask to remove the coarse residue and concentrated using a rotary evaporator. Approximately, 340 mL of the concentrated supernatant was evaporated to dryness at 40 ℃ to yield the dry powdered extract. The percentage yield was calculated as the percentage of the weight of extract to the weight of the powdered material [(wt of extract×100)/wt of powdered material]. The extract was then subjected to phytochemical analysis for the presence and or absence of various constituent bio-active compounds according to previous report[13]. The compounds analyzed for included terpenoids, alkaloids, tannins, steroids, glycosides, phenolics, saponins, and flavonoids.

2.2. Formulation of N. vogelii extract into topical gel application

Topical gel of *N. vogelii* was formulated according to Ofori-Kwakye *et al*(2011)[14]. Briefly, using pestle and mortar 50, 100, and 150 mg of the powdered leaf extract were incorporated in 10, 5, and 3 mL of petroleum jelly producing 5, 20, and 50 mg/mL w/v, respectively. Similarly, 2 g of the reference drug (positive control), Neobacin (5 mg Neomycin

as sulfate plus 250 IU bacitracin as zinc bacitracin, Drugfield Pharmaceuticals, Sango-Otta), was incorporated in 5 mL of petroleum jelly to produce a topical gel of 400 mg/mL w/v. Petroleum jelly alone was used as the topical preparation for the negative control group.

2.3. Animal experimentation

The experimental protocols were approved by the ethics committee of animal care and use of Madonna University, Elele, River State, in agreement with the guidelines stipulated for the care and use of laboratory animals in research and teaching of the University. All the chemicals and solvents used in this study were of standard and analytic grade and solutions were freshly prepared prior to experimentation.

2.3.1. Albino rats

Albino rats (n=25) of both sexes weighing approximately 150-230 g were procured from the University of Nigeria, Nsukka, Nigeria, laboratory animal holding. The animals were housed at room temperature [(27±2) ℃], 12:12 light/dark, in clean aluminum cages in the animal house of Department of Pharmacology and Toxicology, Madonna University for 24 d acclimation prior to experimentation. The animals were fed *ad-libitum* with standard grower's mash and were provided free access to portable drinking throughout the duration of the experiment.

2.3.2. Acute toxicity test

Prior to experimentation, the lethal dose50 (LD_{50}) of the extract on the test animals was determined in two stages according to Lorke (1983)[15]. In the first stage, three groups (n=15) of Albino rats with 5 animals in each group were treated (*per os*) with increasing concentrations of 10, 100, and 1 000 mg/kg bw of the crude leaf extract for 24 hr with continual monitoring of the animals for mortality or non-mortality. Counts were made of the number of animals that were dead or alive. The result of the test subsequently informed a further exposure of three more groups (n=15) of animals to higher concentrations of 1 500, 3 000, and 5 000 mg/kg bw of the extract, respectively, in the second stage of the acute toxicity test as in stage one above.

2.3.3. Wound healing experiment of the methanol leaf extract of *N. vogelii*

To investigate the wound healing effect of the methanol leaf extract of *N. vogelii*, five groups of albino rats with five animals in each group were used. The trunks of the rats were thoroughly shaved with sterile razor blade, disinfected with methylated spirit (94% ethanol+1% methanol), and 1.5 cm incision wound was created on the skin using surgical blade under anesthesia (diethyl ether). The extract, positive (Neobacin), and negative (petroleum jelly alone) control drugs, formulated as gels, were topically applied at two day interval for three treatment regimen on the incision wound and the surface areas of the wounds were measured daily as healing is accomplished and the wound is closed. Three groups of the animals were treated with increasing concentration (5, 20, and 50 mg/mL w/v) of the extract; the positive control (group four) was treated with 400 mg/mL w/v of Neobacin while the negative control (group five) was treated with petroleum jelly alone.

2.4. Statistical analysis

The statistical analysis was done using One-way Analysis of Variance (ANOVA). Post hoc analysis was done with the least square difference (LSD) test. All data were reported as means±SEM with a level of significance of P<0.05.

3. Results

3.1. Phytochemical analysis of the *N. vogelii* leaf extract

The percentage yield the leaf extract was 46.80%. The phytochemical analysis of the leaf extract revealed a number of relative amounts of bioactive constituents including, tannins, saponins, glycosides, terpenoids, steroids, flavonoids, alkaloids, resins, proteins and carbohydrates (Table 1).

Table 1

Phytochemical analysis of *N. vogelii* methanol leaf extract indicating the presence of relative amounts of constituent bio-active substances present in the extract.

Phytochemical constituents	Relative presence
Tannins	++++
Saponins	++++
Glycosides	++++
Terpenoids	++++
Steroids	++++
Flavonoids	++
Alkaloids	++
Resins	+
Acidity	+
Proteins	+++
Carbohydrates	+++

Plus (+) sign indicates the presence of the constituents in very high (++++), high (+++), moderate (++) and small (+) concentrations, respectively.

3.2. Acute toxicity test

There was no mortality in the first as well as in the second stage of acute toxicity test. All the animals exposed to increasing concentrations of the leaf extract up to highest concentration of 5 000 mg/kg bw used in this lethality test survived. Therefore, the LD_{50} of the extract was not deduced.

3.3. Wound healing effect of the methanol leaf extract of N. vogelii

In the group of animals that had incision wounds and were topically treated with petroleum jelly alone, healing occurred but at a slow rate throughout the first, second and third treatment regimen. Topical treatment of the incision wound with 400 mg/mL w/v Neobacin resulted in wound healing and a significant ($P<0.05$) reduction in wound area from the second treatment regimen compared with the negative control.

Treatment with *N. vogelii* leaf extract incorporated in gel showed significant ($P<0.05$) wound healing activity in all the concentrations used in the study. Increased concentrations (5, 20, and 50 mg/mL w/v) of the extract resulted a significant ($P<0.05$) wound healing effect on the incision wound giving 140%, 125% and 140% of healing effects at the third treatment regimen, respectively. Topical treatment with Neobacin resulted in 150% healing effect at the third treatment regimen compared to the negative control (100%) group (Table 2). Comparably, there was an increase in wound healing of approximately 30%-60% by the extract relative to natural (negative control) healing process.

4. Discussion

Interestingly, there was no mortality in all the animals treated with *N. vogelii* leaf extract in both the acute toxicity test and the actual wound healing experiment. The acute toxicity test showed that the leaf extract was relatively less toxic with an LD_{50} exceeding 5 000mg/kg bw. This indicates that the plant extract is well tolerated at high concentrations and therefore has a very high margin of safety. This test is necessary to elucidate the safety and efficacy of the plant extract[16]. The methanol extract of *N. vogelii* leaves demonstrated wound healing activity and caused a significantly reduction in the wound area at the third treatment regimen. The wound healing effect of the extract was comparable with that of the reference drug, Neobacin, indicating that the extract has potent ingredient(s) that accelerate wound healing. Comparatively, the extract relatively accelerated wound healing significantly in the treated animals than the natural healing process (negative control).

The wound healing potential of the plant extract was re-enforced by the phytochemical analysis of the leaf extract which revealed the presence of several bioactive and therapeutic metabolites including glycosides, alkaloids, saponins, tannins, terpenoids, steroids, flavonoids, resins, protein and carbohydrates. Several mechanisms have been suggested to be involved in the wound healing activity of *N. vogelii*, as a result of these constituent metabolites that accelerate and promote the natural healing process[17]. The anti-microbial and astringent properties of the tannins, flavonoids, saponins, glycosides and terpenoids were suggested to play critical role in the wound healing process by increasing the rate of wound contraction, epitheliazation and prevention of secondary bacterial infection that would have complicated and delayed wound healing[18-22]. Being anti-oxidants, saponins, sterols, flavonoids, and tannins reduced lipid peroxidation thereby preventing cell necrosis, promoting tissue vascularity and local microcirculation, increasing strength and viability of collagen fibrils in the wound area[18,19,20,23]. These bio-active components are potent effectors of one or several stages in the healing process that include disinfection, anti-inflammation, cell proliferation, synthesis of tissue ground substances and

Table 2

Wound healing effect of the methanol leaf extract of *N. vogelii* after three consecutive treatment regimen of two days apart of the rats inflicted with incision wound and treated with petroleum jelly (0 mg/mL w/v, negative control), Neobacin (400 mg/mL w/v, positive control) and 5-50 mg/mL w/v of leaf extract of *N. vogelii*. These treatments resulted in 100%-150% wound healing effects.

Treatment groups (mg/mL w/v)	Initial wound	First treatment	Second treatment	Third treatment	% healing
0	1.500 ± 0.000	1.250 ± 0.050	0.870 ± 0.090*	0.750 ± 0.190*	100
Neobacin	1.500 ± 0.000	1.200 ± 0.240	0.350 ± 0.050**	0.010 ± 0.009***	150
5	1.500 ± 0.000	0.850 ± 0.050*	0.350 ± 0.190**	0.130 ± 0.070**	140
20	1.500 ± 0.000	1.000 ± 0.240	0.650 ± 0.190*	0.350 ± 0.050**	125
50	1.500 ± 0.000	0.870 ± 0.090*	0.450 ± 0.050**	0.150 ± 0.050**	140

Data are means±SEM. Values without asterisks (*) are not significantly different from the control (ANOVA, $P<0.05$).

organo-glycans, angiogenesis and providing rich enabling environment that promote normal healing process[24].

Conclusively, the present data suggest that *N. vogelii* leaf extract contains bio-active compounds that are active as wound healing agents. Further studies are suggested to determine the LD_{50} of the methanol leaf extract of this shrub, and to use different solvent of varying polarities in the extraction process. This will help to decipher the best solvent for maximum yield prior to separation of the consistent phytochemical compounds for identification of the metabolite(s) that harbor the active agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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