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# Wound-healing activity of Polyphenolic fraction of *Strychnos nux vomica* Seeds

**Bhuneshwar Dutta Tripathi[1]*, Nitu Singh[2]**

[*1]Research Scholar, Department of Pharmacy, Oriental University, Indore (M.P), India

[2]Department of Pharmacognosy, Oriental College of Pharmacy and Research Oriental University Sanwer Road, Jakhiya, Indore-453555, Madhya Pradesh, India.

***Corresponding Author**

**Email: tbhunesh@gmail.com**

**ABSTRACT**

Chemical, physical, microbiological, thermal, or immunological damage to the tissue can result in a wound, which is defined as the cellular and anatomic disturbance of a tissue. Healing a wound is the process of restoring a damaged tissue's structure and functionality to something close to its pre-wound state. After an injury, an inflammatory reaction takes place, and the cells below the dermis (the deepest layer of skin) start to produce more collagen (connective tissue). The epithelial tissue, or the outer skin, regenerates later. Inflammation, proliferation, and remodeling are the three phases of wound healing. There is a lot of potential for using plants to treat and mend wounds. Many plant extracts, which contain beneficial compounds like triterpenes, alkaloids, polyphenols, flavonoids, tannins, saponins, anthraquinones, and other biomolecules, promote the recovery of wounds. In the following research, the wound-healing efficacy of Polyphenolic fraction of *Strychnos nux vomica* was evaluated in excision and incision wound models. The parameters studied include rate of wound contraction, period of complete epithelialization, and tensile strength of incision wound. The Polyphenolic fraction of *Strychnos nux vomica* seed was found to possess significant wound-healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction and skin-breaking strength. The present study has demonstrated that the Polyphenolic fraction of *Strychnos nux vomica* seeds have properties that render them capable of promoting accelerated wound-healing activity compared with placebo control.

**Keywords**: Wound, *Strychnos nux vomica*, Polyphenol, Excision wound model, Incision wound model, Wound healing

## Introduction

Chemical, physical, microbiological, thermal, or immunological damage to the tissue can result in a wound, which is defined as the cellular and anatomic disturbance of a tissue. Healing a wound is the process of restoring a damaged tissue's structure and functionality to something close to its pre-wound state. After an injury, an inflammatory reaction takes place, and the cells below the dermis (the deepest layer of skin) start to produce more collagen (connective tissue). The epithelial tissue, or the outer skin, regenerates later. Inflammation, proliferation, and

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remodeling are the three phases of wound healing. Angiogenesis, collagen deposition, granulation tissue creation, epithelialization, and wound contraction are the characteristics of the proliferative phase. Endothelial cells can generate new blood vessels during angiogenesis. Fibroblasts release collagen and fibronectin to create a fresh, temporary extracellular matrix during the development of granulation tissue and fibroplasia. Myofibroblasts then grip the wound margins and compress the wound using a mechanism similar to that of smooth muscle cells as epithelial cells crawl across the wound bed to cover it (Nayak et al., 2007))

For the treatment and management of wounds, it is necessary to identify and formulate plants or chemical entities produced from plants. Numerous herbal products are now being researched in this direction. Over the years, a variety of herbal products have been employed in the care and treatment of wounds. These plants' bioactive phytochemical components, which have specific physiological effects on the human body, are what make them valuable medically. These substances come from a wide range of chemical groups, including alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds (Raina et al., 2008)

*Strychnos nux vomica* Linn often known as nux vomica or poison nut, is a toxic plant with significant medical uses. It belongs to the Loganiaceae family. Commercial cultivation of this plant is practiced around the world, including in the United States, the European Union, Fujian, Guangdong, Guangxi, Hainan, North Australia, Taiwan, and tropical Asia. (Patel et al., 2012). Different parts of this plant, particularly the seeds and bark, have a wide range of uses in traditional and folk remedies around the world. Due to the concentration of very toxic alkaloids, particularly strychnine, this plant is known to be extremely toxic to humans and the majority of domestic animals. Since it has a variety of clinical uses in traditional Chinese and Indian medicines (such as Ayurveda, Unani, and Homeopathy) and Indian medicine at low doses (Daniel, 2006).

The dried seeds contain 2.6-3% alkaloids of which strychnine (1.25-1.5%) and brucine (1.7%) are the major active constituents. Besides, various minor alkaloids, phenols and organic acids such Chlorogenic acid, catechol, p-hydroxyphenylacetic acid, caffeic acid, and p-hydroxybenzoic acid are also found in the seeds of nux vomica (Maji and Banerji, 2017).

Polyphenols have ability to scavenge free radicals and possess anti-oxidant properties that are known to lessen lipid peroxidation, which is known to lessen cell necrosis and increase vascularity, sterols and polyphenols are responsible for wound healing (Mittal et al.,2021).

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So, In the following research work an attempt is made to establish wound healing activity of Polyphenol Fraction of *Strychnos nux vomica* in different models in rats of wound healing.

**Materials and methods**

**Collection and Authentication of Plant Material**

The *Strychnos nux-vomica* L. seeds (Fig.1) were collected from the local market of Indore in the month of May. The Seed of *Strychnos nux vomica* was validated by botanist, Professor and Head, Botany Department, Janta PG College, APS University, Rewa, M.P., recognized the plant and provided a herbarium specimen for future reference. Voucher Specimen No. is J/Bot.CFWF-012

**Figure 1. Seeds of *Strychnos nux vomica***

**Extraction of polyphenol rich fractions**

Using a Soxhlet system, the seed powder of *Nux Vomica* (1 kg) was extracted with petroleum ether (2 ltr). The marc was air dried before being extracted with methanol. The methanol extract was dried using a rotary vacuum evaporator under lowered pressure. By boiling the methanol extract for 30 minutes at 50° with constant stirring, it was evenly dispersed in water and partitioned with 200ml of ethyl acetate. There were five more iterations of this process. Under reduced pressure, all of the ethyl acetate fractions were combined and concentrated. The resulting ethyl acetate fraction was high in polyphenols (Farnsworth,1966). It was labelled as Polyphenol fraction (PF)

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**Procurement and selection of animals:**

The Oriental University's Central Animal House, Department of Pharmacy in Indore provided Wistar albino rats of either sex weighing 150–250 gm. The rats were stabilized for a week before being housed in regular room temperature circumstances with a usual light-dark series. They were fed a standard pellet feed and provided unrestricted water during the study. The animals were handled carefully to reduce stress, which might result in an increase in adrenal production. The Institutional Animal Ethical Committee of the Oriental College of Pharmacy and Research approved all animal experimentation (IAEC). The CPCSEA registration number is IAEC/2019-20/RP-24.

**Acute dermal toxicity of Fractions**

Acute dermal toxicity testing was also done in accordance with OECD (2002.Adopted:2017). Three female rats with normal skin texture for each fraction were chosen at random, placed in isolated cages, and given a week to get acclimated to the lab environment before the test. The dorsal portion of the trunk had about 10% of its body surface area fur removed 24 hours before the investigation. The shaved area received a uniform application of the 10% extract ointment of the Polyphenol fraction at a limit test dose of 2000mg/kg for 24 hours. Rats were housed individually throughout the exposure period. After the exposure period, the remaining test chemical was removed, and for the following 14 days, the rats were monitored daily for the emergence of any unfavourable skin reactions. The erythema and edema-defined reactions were assessed and graded in accordance with the (OECD 404, (2002).

M.F stands for Master formula and R.F. stands for Reduced Formula. The 20 g of Simple ointment base was made by melting 1 g of hard paraffin in a beaker over water. After removing from melting, the remaining ingredients, including cetostearyl alcohol (1 g), white soft paraffin (17 g), and wool fat (1 g), were added in descending order of melting point. Over a water bath, all of the ingredients were melted while being constantly stirred until they were homogeneous. After being taken off the heat, the liquid was swirled until it had cooled. Polyphenolic Fraction (2000mg) was combined into portions of simple ointment base to create 10%(w/w) ointments using levigation. The remaining simple ointment base was added gradually and properly blended. In order to apply the extract ointment topically throughout the experiment, it was then transferred to a clean container [Ansel and Popovich,1985].

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**Experimental Design:**

These studies were approved by the ethics committee for animal experimentation. The study employed healthy albino rats weighing 150–250g. They were kept on regular food and water available at all times, and they were individually housed. Before and after the trial, the animals were periodically weighed. For the excision and incision model, separate 3 groups of animals, each with 6 rats, were used. Animal dosage is specified in the Table no.1

**Table no.1. Group and treatment of animal models**

| Group | Treatment |
|---|---|
| I (Control) | Simple ointment |
| II(Standard) | Nitrofurazone (0.2%) |
| III(PF) | 200mg/kg bw of Polyphenol fraction |

- **Excision wound model**

Ketamine 50 mg/kg intraperitoneally was used to anaesthetize rats .The dorso-thoracic region was defurred. A 300 $mm^2$ circle mark was created with a permanent marker, and the complete thickness was removed with sterile, sharp scissors. This was the start of the day (day 0) (Nagar et al., 2016). The rats were treated and ointments 10% w/w were used on the beginning of day one, that is, after the wound area had been present for 24 hours. For the duration of the healing process, all ointments were administered to the wound area once daily. Using transparency paper and a permanent marker, measurements were made on days 0, 5, 10, 15, and 24th after injuring the rats to check for wound closure (Kokane et al., 2009). The diameter was then determined for each rat using a millimeter-scaled ruler to calculate the traced area. The proportion of wound contraction was assessed in accordance with the below mentioned formula (Shivhare et al., 2010).

$$\% \text{ Wound Contraction} = \frac{\text{Wound Area on Day 0} - \text{Wound Area on Day n}}{\text{Wound area on Day 0}}$$

Where n= number of days i.e. 0,5th, 10th, 15th, $24^{th}$ (the day the wound in the fraction treated groups including the standard completely healed).

Graph paper was used to measure the size of the wound. For the purpose of counting the number of tiny boxes in each traced area, the wound area was traced on a clear sheet of plastic and maintained on graph paper(1 Box = $1mm^2$).

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The endpoint of complete epithelialization was determined as the number of days necessary for the dead tissue remains to slip off from the wound surface without leaving a raw wound behind, and the number of days required for this was designated the period of epithelialization (Pawar et al., 2013).

- **Incision wound model**

As stated for the excision wound model, rats were given general anaesthesia before having their fur removed. On either side of the vertebral column, one centimetre from the midline, a three-centimetre-long, linear paravertebral incision was performed. On day 0, With chromic catgut and a curve needle, the excised skin was maintained together and stitched at intervals of 1 cm. The ointments were applied as discussed previously, beginning on day one. Experimental animals were divided into different groups and given similar care to excision wound models. For nine days, the ointments were administered topically once each day. The stitches were taken out eight days after the injury. On the tenth day, the tensile strength was assessed in order to gauge the degree of healing. It was calculated using the continuous constant water flow method by considering the weight of water needed to rupture the skin (Fikru et al., 2012).

Comparisons were made between the breaking strengths of the groups treated with fraction ointments and the untreated control and standard ointment groups. On the eleventh day, a tensiometer was used to measure the skin-breaking strength.

- **Determination of Tensile Strength**

A tensiometer was used to measure the tensile strength. The wounding or skin-breaking weight is measured using a tensiometer. On either side of the skin where the incision was made, two of the tension meter's hooks were knotted or secured in place. One side hook was fixed, while the other side hook had a balance pan attached to it, on top of which was set a single empty bottle. The weight of the water pulled the hook as we began to fill the bottle, then the skin around the wound region. The weight of water at which the skin of the injured area ruptured is referred to as the tensile strength or weight of wound breaking strength in $gm/mm^2$ (Mulisa et al., 2015).

% tensile strength of reference/Test =tensile strength of Reference/Test- tensile strength of SO X100

tensile strength of SO

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- **Statistical Analysis**

The results are presented as a mean ± SEM. The facts were statistically evaluated using one-way ANOVAs and Dunnett's t-test. The values were considered to be significant when $p < 0.05$.

## Results

### Acute Dermal Toxicity of Fractions:

No significant clinical change was seen in any of the treated rat groups while evaluating the dermal toxic effect of the polyphenol fraction of *Strychnos nux vomica* seeds in acute dermal toxicity bioassay. Over the course of the 14-day trial period, no erythema or edema was seen in the treated animals. The extract was regarded as safe up to such dose levels because no fatalities were observed up to 2000 mg/kg. Therefore, 200 mg/kg body weight is the safe dosage for experimental use. Drug dosages of 200 mg/kg of body weight were chosen for pharmacological studies.

### Excision wound model

### Wound Area Measurement

**Table no. 2. Effect of PF of *Strychnos nux vomica* Seeds on wound area in rats**

| Group | Days of wound area measurement in $mm^2$ (mean±SEM) | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 5th | Day 10th | Day 15th | Day 20th | Day 24th |
| I | 300.16±1.29 | 272.13±1.14 | 238.44±3.59 | 146.20±2.11 | 90.26±1.02 | 58.33±1.61 |
| II | 302.05±0.16 | 180.12±1.36 | 98.18±1.23** | 33.28±1.21*** | 8.29±1.21*** | 0.00±0.00 *** |
| III | 301.13±0.17 | 218.42±1.10 | 136.18±2.21 * | 50.01±1.44 ** | 10.17±1.23 *** | 5.13±1.27 *** |

**Values are expressed as mean ± SEM (n = 6), one-way ANOVA. *P < 0.05, **P < 0.01, and***P < 0.001 vs Control**

- **Percentage wound Contraction**

On the basis of wound area measurement in $mm^2$ (Table no.2) the percentage of wound contraction is calculated.

When compared to the control group, the rats given 200 mg/kg bw of Polyphenol fraction had a higher ($p<0.001$) percentage of wound contraction on day $20^{th}$ and $24^{th}$ (Table no. 3 and Figure no. 2) and a shorter period of epithelialization (Table 4). On days 20, the wound contraction in

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the group that received Polyphenol fraction was comparable (P< 0.001) to that of the positive control (NF 0.2%). For 200 mg/kg bw of polyphenol fraction and NF 0.2% ointments, respectively, these were 96.63 and 97.25% on day 20; 98.29 and 100% on day 24.

**Table no.3. Effect of PF of *Strychnos nux vomica* Seeds on percentage wound contraction in rats**

| Groups | Percentage wound contraction | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 5th | Day 10th | Day 15th | Day 20th | Day 24th |
| I | ____ | 9.33 | 20.56 | 51.29 | 69.92 | 80.56 |
| II | ____ | 40.36 | 67.49** | 88.98*** | 97.25 *** | 100*** |
| III | ____ | 27.46 | 54.77* | 83.39** | 96.63*** | 98.29*** |

**Values are expressed as mean ± SEM (n = 6), one-way ANOVA. *P < 0.05, **P < 0.01, and***P < 0.001 vs Control**

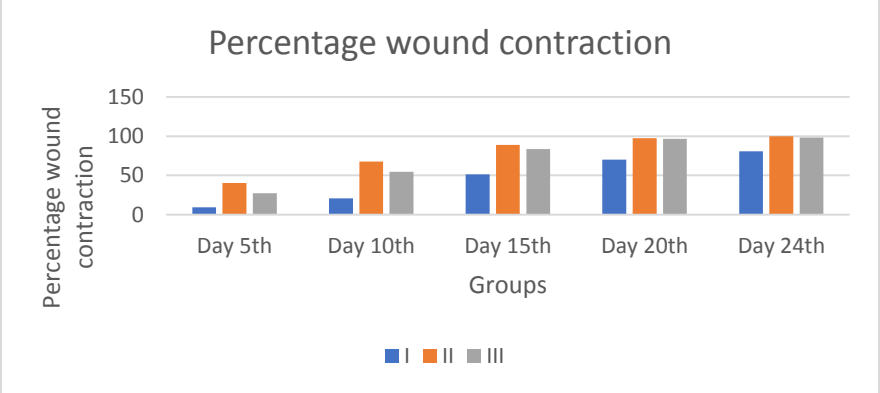


**Figure no.2. Effect of Polyphenol fraction of *Strychnos nux vomica* Seeds on percentage wound contraction in rats**

- **Epithelialization Period** :

**Table no.4. Effect of PF of *Strychnos nux vomica* Seeds on Period of epithelialization in days in rats**

| Groups | **Period of epithelialization in days (mean ± S.E.M)** | **% decrease in epithelialization periods** |
|---|---|---|
| I | 26.32±0.43 | |
| II | 18.27±0.26** | 30.58 |
| III | 19.23±0.16** | 26.93 |

**Values are expressed as mean ± SEM (n = 6), one-way ANOVA. *P < 0.05, **P < 0.01, and***P < 0.001 vs Control**

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In comparison to the control (simple ointment-treated group), the time it took for complete epithelialization was short in the Polyphenol fraction ointment and nitrofurazone-treated groups. The average duration of epithelialization for the control group, standard drug, and polyphenol fraction, respectively, was 26.32, 18.27, and 19.23%. In comparison to the control group, the Polyphenol fraction 200mg/kg bw treated group had a quicker rate of epithelialization ($P<0.01$) than control (Table no.4). In comparison to the control group, animals treated Polyphenol fraction, and nitrofurazone showed respective decreases in epithelialization periods of 26.93, and 30.58 %.

**Incision wound model**

- **Tensile Strength.**

The rate of collagen production and maturation, which involves the binding of collagen fibres by inter- and intramolecular cross-linking, determines the tensile strength (Fikru et al., 2012). In a study of incision wounds, Polyphenol fraction was found to improve wound breaking strength, which may indicate that these fractions have higher collagen content, cross-linked collagen, and matured collagen.

When compared to the control group, Polyphenol fraction, and standard drug-treated groups all displayed substantial improvements in breaking strength of 35.65, and 41.04 %, respectively (Table no. 5).

**Table No.5. Effect of AF, TF and PF of *Strychnos nux vomica* Seeds on Tensile Strength of groups**

| Group | **Tensile strength in gram (mean ±S.E.M)** | **Percent of tensile strength [%]** |
|---|---|---|
| I | 188.00 ± 5.42 | - |
| II | 265.17 ± 2.29** | 41.04 |
| III | 255.03 ± 1.17** | 35.65 |

**Values are expressed as mean ± SEM (n = 6), one-way ANOVA. *P < 0.05, **P < 0.01, and***P < 0.001 vs Control**

The wound breaking strength is determined by the rate of collagen synthesis and maturation process, wherein there is binding of collagen fibres through inter- and intramolecular cross-linking PF was found to increase wound breaking strength in incision wound study which may indicate increased collagen content, cross-linking collagen, and maturation by PF (Fikru et al., 2012).

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**Discussion**

Wound healing is a highly complex, but orchestrated cascade of events that can roughly be divided into three overlapping phases—inflammation, granulation tissue formation, and remodeling of the extracellular matrix. These events involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. Immediately after injury, there is clot formation and the earlier phases of wound repair involve inflammation and synthesis of ground substance. Generally, polyphenols are known to have high antioxidant activity, providing protection against ROS through the neutralization of free radicals by donating an electron or a hydrogen atom. Moreover, some polyphenols have also antimicrobial activity against certain bacteria present in infected chronic wounds (Guimaraes et al., 2021). The polyphenols and organic acids present in *Strychnos nux vomica* are Chlorogenic acid, Catechol, p-hydroxyphenylacetic acid, caffeic acid, and p-hydroxybenzoic acid (Maji and Banerji, 2017). Chlorogenic acid have great antioxidant properties to promote wound healing in chronic wounds (Guimaraes et al., 2021). Caffeic acid (CA), has antimicrobial, anti-inflammatory, antioxidant, anxiolytic, and antitumor properties. The treatment of skin incisions with CA progressively increased the levels of collagen-like polymers. In a vitro study of NIH 3T3 fibroblasts, CA was found to exert antioxidant and anti-inflammatory effects by inhibiting ROS generation and releasing arachidonic acid and prostaglandin 2 (PGE-2) ( Melguizo-Rodríguez et al.,2021). The wound-healing property of the polyphenol fraction of *Strychnos nux vomica* may be attributed due to the presence of Chlorogenic acid and caffeic acid in the fraction and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents.

**Conclusion**

The present study has demonstrated that the Polyphenolic fraction of *Strychnos nux vomica* seeds have properties that render them capable of promoting accelerated wound-healing activity compared with placebo control. Wound contraction and increased tensile strength support further evaluation of Polyphenolic fraction in the topical treatment and management of wounds

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**Conflict of interest**:

The authors have declared that there is no conflict of interest associated with this publication.

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