



## Analgesic and Wound Healing Effect of Methanolic Leaves Extract of *Pennisetum Pedicellatum* in Wistar Albino Rat

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**Abstract:** *Pennisetum pedicellatum* is an aggressive weed, used for treatment of wounds, pain related condition, eye and parasitic infections among the traditional healers in Kebbi State, Nigeria. This study was undertaken to validate the analgesic and wound healing effect of *P. pedicellatum* using tail flick method and excision wound models respectively in wistar albino rats. The result revealed that, methanolic leaves extract of *P. pedicellatum* and standard drug Aspirin exhibited significant ( $p < 0.001$ ) increase in pain reaction time (PRT) when compared with the control group. *P. pedicellatum* leave extract (PPLE) (200-400mg/Kg) demonstrated no significance ( $p > 0.05$ ) in PRT when compared with Aspirin treated group. The wound contraction effect of PPLE ointments was significantly increased when 10% ( $P < 0.05$ ) and 50% w/w ( $P < 0.001$ ) from the 4<sup>th</sup> to 16<sup>th</sup> day of application. However, percentage (%) wound contraction on 16<sup>th</sup> day was highest in rats treated with 50%v/v PPLE (100%) > Penicillin (98.7%) > 10% PPLE (92.3%) > control > (85.7%) and Base (82.3%) respectively. Epithelialization period was significantly ( $P < 0.05$ ) decreased in Penicillin and PPLE treated groups compared with the control group. Phytochemical screening of PPLE revealed the presence of phenols, tannins, alkaloids, saponins, anthraquinones and cardiac glycosides. In conclusion, PPLE exhibited analgesic and wound healing activity which was comparable to the standard drug Aspirin and penicillin respectively. This validates the folkloric medicinal use of this plant by the indigenous people of Kebbi State.

**Keywords:** *Pennisetum Pedicellatum*, analgesic or wound healing, excision wound, epithelization period.

### 1. INTRODUCTION

An analgesic or painkiller is any member of the group of drugs used to achieve analgesia or relief from pain. Pain is a highly unpleasant physical sensation and emotional experience associated with actual or potential tissue damage. Due to its frequent occurrence, pain is a public health problem with considerable socioeconomic effects. It is an indication of several illnesses and it is predicted that about 80–100% of the population will experience, for example, back pain once in life [1]. Though several analgesics drugs exist on the shelves, current drug therapy is related to certain adverse effects such as gastrointestinal irritation, bronchospasm, fluid retention, and extension of bleeding time [2,3].

A wound is defined as the disruption of the cellular and anatomic continuity of a tissue; which may be created by physical, chemical, thermal, microbial or immunological insult to the tissue [4]. Wound care and maintenance involve a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical and systemic antimicrobial agents and healing drugs [5]. Dermal wounds are often caused by surgery, trauma, and chemicals or as a result of diseases [6], deliberately created dermal wounds can be incisional, where by the wound is brought about by surgical cutting into the skin with a scalpel or excision wound created when part of the skin is cut off [7]. The process of tissue repair after an insult to the tissue (wound) is called 'wound healing' [8]. Wound healing is an intricate process in which usually the skin repairs itself [9]. Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers [10].

In Nigeria several medicinal of plant(s) extracts or pastes are used by various tribes and folklore traditions for treatment of cuts, wounds, and burns. The use of traditional medicinal plants with analgesic and wound healing effects has recently gained popularity worldwide because of their natural

origin and fewer side effects [11]. Therefore, this study was undertaken to validate the traditional use of *P. pedicellatum* leaves for the treatment of pain and wound healing in Kebbi State, Nigeria.

## **2. MATERIALS AND METHODS**

### **2.1. Plant Material**

Fresh plant sample of *Pennisetum Pedicellatum* was collected in the month of May, 2017 at Aliero, Kebbi State, Nigeria. The authentication by Dr. Dharmendra Singh at Biological Science Department, Faculty of Science, Kebbi State University of Science and Technology Aliero, Kebbi State and a voucher specimen (V. No: 518A) was deposited.

### **2.2. Drugs**

Penicillin and Aspirin was purchased from Hamdala Pharmaceutical Limited, Birnin Kebbi, Kebbi state, Nigeria. And all other chemicals used in this research work were of analytical grade.

### **2.3. Preparation of Extract**

The collected *Pennisetum Pedicellatum* leaves were air dried under shade and pulverized to small pieces using mortar and pestle. Five hundred gram (500g) was weighed and soaked in 1.2L of 95% methanol. The mixture was kept at room temperature for 72h, and was filtered. The filtrate was evaporated to dryness at 45°C in a drying cabinet.

### **2.4. Qualitative Phytochemical Screening of *Pennisetum Pedicellatum* Leaves**

A preliminary qualitative phytochemical screening for the presence of Alkaloids, Saponins, Tannins, Phenol, Flavonoids, Terpenoids, Anthraquinones, Steroids and Cardiac glycosides done using standard analytical methods [12, 13, 14,15].

### **2.5. Experimental Animals**

Adult wistar albino rats of both sexes about (120-170g) were used for this experiment (studies). The animals were purchased from the Animal House Facility at Usmanu Danfodio University, Sokoto. All the animals were kept in well ventilated cages under a standard laboratory condition, at the animal house, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. The rats were allow free access to both growers feed and water. The rats were allowed to acclimatize for the period of two (2) weeks. Weight of each rat was taken before the commencement of the experiment. The animals were maintained according to the guidelines of institutional animal ethics committee while the experiment was conducted in accordance with WHO guidelines for the use of experimental animals.

### **2.6. Analgesic Effect *P. pedicellatum* Leaves Extract**

The method of Kumar and Shankar [16] was adopted with slight modification for this experiment. Twenty (25) albino rats were randomly divided into five groups of five rats each and were fasted overnight with prior to commencement of experiment. The animals were pre-treated 60 minutes before tail immersion with 10ml/Kg between the solution for group I (negative control), 20mg/Kg acetylsalicylate acid (aspirin) for group II (positive control) and 100, 200, 400 mg/Kg of the plant extract for groups III, IV and V respectively. Then about 2-3cm of the tail of each rat was dipped into a water bath containing warm water maintained at a temperature of  $55 \pm 1^\circ\text{C}$  and the time taken for the rats to flick its tail or withdraw it from the warm water known as the pain reaction time (PRT) was recorded for all the rat. The cut off time was recorded at 15 seconds, the latency was evaluated at 0, 30, 60, 90, and 120minutes with 0mins been the initial reading.

### **2.7. Wound Healing Activity**

For excision wound model, animals were divided into five groups each consisting of five animals as follows: group I, was left untreated and considered as vehicle control, group II, served as negative control (ointment base treated), groups III and IV were treated with 10% and 50% (w/w) plant extract ointment, respectively whereas group V, served as standard and was treated with 5% (w/w) penicillin ointment. All the treatments were given once daily. Hairs of the grouped animals were removed from the dorsal thoracic central region of anaesthetised rats and were depilated on the back. One excision wound was inflicted by cutting away 300mm<sup>2</sup> full thickness of skin from a predetermined area; the wound was left undressed to the open environment as percent reduction in wound area. On the

subsequent day, treatments were administered topically once alternative day till the day of complete healing. The area of the wound was measured on 2<sup>nd</sup>, 6<sup>th</sup>, 10<sup>th</sup>, 14<sup>th</sup> and 16<sup>th</sup> day following wounding [17].

### **2.8.Measurement of Wound Contraction and Epithelialization Period**

The wound area (diameter) was measured by tracing the wound with the help of a transparent meter ruler, on 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup>,14<sup>th</sup> and 16<sup>th</sup> days post wounding (DPW) for all groups. Wound contraction was measured every 2nd day until complete wound healing and represented as percentage of wound healing area [18, 19]. Percentage of wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

$$\% \text{ Wound Contraction} = \frac{(\text{Initial wound area} - \text{Specific day wound area})}{\text{Initial wound area}} \times 100$$

Epithelialization period was calculated as the number of days required for falling off the dead tissue remnants (scar) of the wound without any residual raw wound [20, 21].

### **2.9.Statistical Analysis**

Statistical analysis of all the results collected was performed using ANOVA followed by the Tukey-Kramer Multiple Comparisons Test employing statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the P<0.05 were considered as statistically significant.

## **3. RESULTS**

### **3.1.Percentage Yield of *P. pedicellatum* Methanol Extract**

The methanol extraction of 500g of *P. pedicellatum* leaves yielded 12% of extract, which was very powdery and brownish in colour.

### **3.2.Phytochemical Composition of *P. pedicellatum* Leave Methanol Extract**

The qualitative phytochemical screening of PPLE revealed the presence of some secondary metabolites (Table 1).

**Table1.** *Phytochemical Composition of Pennisetum pedicellatum leaves extract (PPLE).*

| <b>Phytochemicals</b> | <b>Results</b> |
|-----------------------|----------------|
| Phenol                | +              |
| Flavoniods            | -              |
| Tannins               | +              |
| Alkaloids             | +              |
| Terpenoids            | -              |
| Steroids              | -              |
| Saponins              | +              |
| Antraquinones         | +              |
| Cardiac glycosides    | +              |

**Key:** (+): present, (-): Not detected

### **3.3.Effect of PPLE on Tail Flick Response in Rats**

The pretreatment of rats with PPLE (100mg/kg) caused a significant (p< 0.05) increase in pain reaction time while at 200-400mg/kg, the extract exhibited an extremely significant (P<0.001) increase in PRT when compared to the control respectively (Table 2). All the extract treated groups exhibited a dose-dependent increase in pain inhibition. The analgesic effect of the standard drug, Aspirin treated group were comparable to the extract treated groups receiving 200-400mg/kg.

### **3.4.Effect of PPLE on Percentage Wound Contraction and Epithelialization Period**

The wound healing activity of PPLE ointments 10% and 50% w/w significantly (P< 0.001) increased wound contraction, in rats from the 4th day onwards. However, percentage (%) wound contraction at 16<sup>th</sup> day was highest in rats treated with 50% w/w PPLE (100%) > Penicillin (98.7%) > 10% PPLE (92.3%) > control > (85.7%) and Base (82.3%) respectively as shown in (Table 3). Epithelialization period was significantly (p<0.01 and P< 0.001) reduced in Penicillin and PPLE treated groups when compared with the control group respectively (Table 4).

**Table2.** Effects of *P.pedisellatum* Extract on Tail Flick Response in Rats

| Treatment group        | Dose (mg/kg) | Mean PRT±SEM (sec) |                |                 |                 |                 |
|------------------------|--------------|--------------------|----------------|-----------------|-----------------|-----------------|
|                        |              | 0min               | 30mins         | 60mins          | 90mins          | 120mins         |
| Control                | 10ml/kg      | 0.950±0.0646       | 1.00±0.00      | 1.025±0.0250    | 1.025±0.0250    | 1.050±0.02887   |
| <i>P. pedicellatum</i> | 100mg/kg     | 3.125±0.1250***    | 4.00±0.7071*** | 3.250±0.2500*** | 3.000±0.4082**  | 2.250±0.2500*   |
| <i>P. pedicellatum</i> | 200mg/kg     | 3.250±0.1443***    | 4.00±0.2449*** | 5.525±0.2136*** | 4.900±0.227***  | 3.725±0.1548*** |
| <i>P. pedicellatum</i> | 400mg/kg     | 4.325±0.1601***    | 5.93±0.2175*** | 5.250±0.3227*** | 4.700±0.3391*** | 4.075±0.3250*** |
| Aspirin                | 100mg/kg     | 3.250±0.1443***    | 5.38±0.3750*** | 5.525±0.2056*** | 5.775±0.1315*** | 3.650±0.2363*** |

Each value is expressed in Mean ± SEM (n=5) \*p<0.05 shows a significant difference in reaction time when the individual treated groups were compared with control, \*\*p<0.001 shows a very significance difference in reaction time when the individual treated groups were compared with control and \*\*\*p<0.001 shows an extremely significance difference in reaction time when the individual treated groups were compared with control, PRT: Pain reaction time, SEC: second, SEM: standard error of mean. One way ANOVA followed by Tukey-Kramer Multiple Comparisons Test.

**Table3.** Wound contraction effect of *P. pedicellatum* methanol leaves extract in albino rats.

| Treatment Days | Wound Area (mm <sup>2</sup> )±SEM (% Wound contraction) |                          |                           |                            |                            |
|----------------|---|--------------------------|---------------------------|----------------------------|----------------------------|
|                | Control   | Normal base              | 10% Extract               | 50% Extract                | Penicillin                 |
| Day 2          | 333.25±2.358<br>(5.6)                                   | 347.25±3.568<br>(3.8)    | 324.50±2.102**<br>(8.7)   | 303.25±1.493***<br>(13.3)  | 336.75±1.250*<br>(6.0)     |
| Day 4          | 307.50±0.9574<br>(13.0)                                 | 294.75±2.250<br>(20.0)   | 288.50±2.500**<br>(25.5)  | 223.00±1.958***<br>(36.3)  | 295.50±1.041**<br>(20.4)   |
| Day 6          | 249.50±2.102<br>(28.7)                                  | 247.00±1.414<br>(28.0)   | 191.50±4.272***<br>(43.7) | 116.75±2.394***<br>(67.9)  | 154.50±2.102***<br>(55.0)  |
| Day 8          | 143.00±0.4082<br>(60.97)                                | 140.00±0.4082<br>(61.61) | 82.750±2.056***<br>(77.8) | 37.000±0.7071***<br>(87.6) | 80.000±0.8165***<br>(78.5) |
| Day 10         | 101.00±1.581<br>(71.90)                                 | 102.00±1.225<br>(71.86)  | 45.750±1.436***<br>(85.5) | 20.000±0.4082***<br>(95.9) | 33.250±1.702***<br>(87.7)  |
| Day 12         | 77.000±1.871<br>(76.40)                                 | 74.000±1.633<br>(78.70)  | 30.500±2.466***<br>(88.1) | 10.500±0.6455***<br>(98.5) | 13.250±1.377***<br>(96.1)  |
| Day 14         | 67.750±0.6292<br>(79.82)                                | 59.000±2.309<br>(81.16)  | 28.000±0.000***<br>(90.3) | 6.000±0.000***<br>(99.6)   | 11.500±0.2887***<br>(98.3) |
| Day 16         | 45.500±0.2887<br>(85.70)                                | 43.000±0.000<br>(83.20)  | 27.000±0.000***<br>(92.3) | 3.000±0.000***<br>(100)    | 8.500±0.2887***<br>(98.7)  |

Each value is expressed in Mean ± SEM of five rats per group; \*P <0.05, \*\*p<0.01, \*\*\*p<0.001 vs. control, One way ANOVA followed by Tukey-Kramer Multiple Comparisons Test.; % wound contraction is given in parentheses.

**Table4.** Effect of Methanol Extract of *Pennisetum pedicellatum* on Epithelization Period.

| Groups                       | Treatments (w/w) | Period of Epithelization (Days) |
|------------------------------|------------------|---------------------------------|
| Control                      | Untreated        | 25±0.58                         |
| Normal base                  | 100%             | 25±0.67                         |
| <i>PenesetumPedicillatum</i> | 10%              | 22±0.67*                        |
| <i>PenesetumPedicillatum</i> | 50%              | 19±0.58***                      |
| Penicillin ointment          | 10%              | 22±0.33*                        |

Each value is expressed in Mean±SEM (n=5) \*p<0.05 and \*\*\*p<0.001 shows significant and an extremely significant difference in epithelization when compared with control respectively using Tukey Kramer Multiple Comparisons Test. PRT=Pain reaction time.

#### 4. DISCUSSION

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Derivative of acetylsalicylic acid known as aspirin is used as a pain killer. The main mechanism of aspirin action is by inhibition of both forms of cyclooxygenase enzyme (COX) which is nonselective and permanent. Analgesics relieve pain as a symptom, without affecting its cause [22]. Plant-derived molecules such as morphine, nicotine, quinine, steroidal, and many other extracted natural products possess analgesic activity [23, 24]. The

profound analgesic activity of PPLE in the present study may be due to interference of its active principle(s) with the release of pain mediators.

Wound healing involves the dynamic process of multiple biochemical consequences towards restoration of the damaged cellular structure to its regular and original state [25]. A classical or usual cascade of wound healing involves three sequential and overlapping phases: inflammation, proliferation, and remodeling [26]. Topical application of prepared 10% and 50% w/w PPLE ointments improved the wound healing effect in excision wound model in rats. Recent studies suggested the valuable role of flavonoids, triterpenoids, and tannins in promoting the wound healing by multiple mechanisms, for example, wound contraction, increased rate of epithelialization, and prevention of secondary bacterial infection that would have complicated and delayed wound healing [27,28].

In this present study, pain and wound healing potency of PPLE may be attributed to its tannins, anthraquinones, saponins, cardiac glycosides, phenols and alkaloids content owing to their astringent, anti-inflammatory, and antimicrobial activity.

## **5. CONCLUSION**

*Pennisetum pedicellatum* is a traditional medicinal plant used in Kebbi State for the treatment of wound. The results of the present study revealed that the methanolic leaves extract of *P. pedicellatum* possess analgesic and wound healing effect. This validates its folkloric medicinal claim of Kebbi State indigenes. Further studies are in-process to elucidate the mechanism of analgesic action and isolation of the bioactive compound(s) responsible for its pharmacological effects.

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