

ACUTE TOXICITY STUDY AND WOUND HEALING EFFECT OF THE ACETONE FRACTION OF *Tetrapleura tetraptera* FRUIT IN EXCISION WOUND MODEL.

ABSTRACT

Aims: The study was aimed at determining the lethal dose and evaluates the wound healing effect of the fruit of *Tetrapleura tetraptera*. **Place and duration of study:** The Department of Clinical Pharmacy, Faculty of Pharmacy, University of Uyo, Nigeria, between July and September, 2014. **Methodology:** Miller and Tainter method was adopted for acute toxicity testing of the plant's fruit while the Excision wound model were used to evaluate the wound healing activity with the progressive wound closure rate calculated via the wound width and number of days for complete epithelisation was determined. **Results:** The acute toxicity of the aqueous fraction of *Tetrapleura tetraptera* dry fruit was calculated to be 10,000mg/kg body weight = 10.0g/ kg body weight. Topical administration of the Acetone fraction with 0.4g/ml and 0.6g/ml doses produced significant decreases ($P=0.05$) in wound width by $0.29\pm0.07\text{cm}$ and $0.47\pm0.09\text{cm}$ respectively in comparison with the $0.10\pm0.03\text{cm}$ of rats treated with 0.2g of Neobacin powder on the first 12 days after excision. On day 15, 0.4g/ml had a non-significant decrease of $0.06\pm0.04\text{cm}$ when compared to the 0.2g Neobacin powder with 0.02 ± 0.02 wound width. **Conclusion:** The Acetone fraction of the dry fruit of *Tetrapleura tetraptera* was not effective on wound healing thus further work should be done using other extracting solvents like Aqueous, Methanol etc. to ascertain the wound healing effect of the dry fruit of *Tetrapleura tetraptera* claimed by the traditional medicine.

24

25 **KEYWORDS:** Acetone fraction, lethal dose, *Tetrapleura tetraptera*, Excision wound,

26 wound healing, epithelisation

27

28 INTRODUCTION

29 There is an increasing interest in finding herbal extracts with wound healing efficacy
 30 although the use of such extracts for treating cuts and wounds is a common practice in
 31 traditional medicine. Wound infection resulting from the impaired immunity and
 32 exposure or poor hygiene is one of the most commonly encountered and clinically
 33 important impediments to wound healing. The injured skin remains vulnerable to
 34 invasive microbial infections of all kinds subsequent development of wound sepsis until
 35 complete epithelial repairs has occurred [1]. Injury becomes infected, because the
 36 wound area is an ideal medium for the multiplication of the infecting organism. Topical
 37 antimicrobial therapy is one of the most important methods of wound care [2].

38 *Tetrapluera tetraptera* is a species of flowering plant in the pea family native to Western
 39 Africa. The tree has many uses and its sweet fragrance is highly valued, its fruit and
 40 bark is used to spice dishes and for medicinal purposes. The fruit is used to prepare
 41 soup for mothers from the first day of birth to prevent postpartum contraction [3]. The
 42 plant has many traditional uses mainly in the management of convulsion, leprosy,
 43 inflammation and rheumatic pains, schistosomiasis, asthma and hypertension [4]. The
 44 major constituents are tannins, flavonoids and saponins [5].

Tannins act as free radical scavengers, triterpenoids, and flavonoids promote wound healing due to their astringent and antimicrobial property, and saponins due to their antioxidant and antimicrobial activity, which appear to be responsible for wound contraction and elevated rate of epithelialisation. Flavonoids also possess potent antioxidant and free radical-scavenging effect, enhancing the level of antioxidant enzymes in granuloma tissue [6]. Sterols and polyphenols are also responsible for wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation, thereby reduce cell necrosis and improving vascularity [7]. Several types of injuries like burn, wounds, and skin ulcers usually generate superoxides and lipid peroxidation through the activation of neutrophils. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage, and by promoting the DNA synthesis. Thus, an intervention into any one of these phases by drugs could eventually lead to either promotion or depression of the collagenation phase of healing [8].

A wound is a type of injury in which the skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or

67 over healing. Therefore the aim of treating a wound is to either shorten the time required
68 for healing or to minimize the undesired consequences [9]

69 The traditional medicine practitioners and rural dwellers have claimed that *Tetrapluera*
70 *tetraptera* taub are used to treat wounds, it has to been studied to ascertain the
71 authenticity of this claim scientifically with different fractions of the fruit's plant. Recently,
72 the acqueous fraction of *Tetrapluera tetraptera* fruit was studied by [10] and it proves
73 the authenticity of this claim scientifically. The aim of this study is to investigate the
74 wound healing effect of the acetone fraction of the plant.

75 **MATERIAL AND METHODS:**

76 **Collection and identification of plant**

77 The plant material was collected at Ibiaku Itam in Itu Local Government area of Akwa-
78 Ibom state, Nigeria in October, 2012. The plant was identified by Dr (Mrs) Eshiet, a
79 botanist of the department of Botany and Ecological studies, Faculty of Science of the
80 University of Uyo, Nigeria and deposited at the Pharmacognosy and Natural Medicine
81 department's herbarium, Faculty of Pharmacy, University of Uyo, Nigeria with a voucher
82 number –UUH No:42 (F) University of Uyo, Nigeria.

83 **Extraction of plant material**

84 The dried fruit of *Tetrapleura tetraptera* was washed, air-dried and ground into powder.
85 300g of the powdered fruit was macerated cold with 1.8L of Acetone for seventy –two
86 hours (72 hours) at room temperature, the extract was filtered and the filtrate was
87 concentrated in vacuum at 40⁰ c to remove any dissolved, suspended or adhered

88 particles and stored in the refrigerator. The yield of the dried extract fraction was
89 54.50g.

90 **Animals**

91 Swiss albino mice (20-25g) and albino wistar rats (180-270g) of both sexes obtained
92 from the animal house of the department of Pharmacology and Toxicology, Faculty of
93 Pharmacy, University of Uyo, Nigeria were used. They were kept in clean cages and
94 maintained under standard laboratory conditions (Temperature; $23\pm 1^{\circ}$ C, Relative
95 humidity; 50-60%, and a 12/12h light/dark cycle) and were allowed free access to
96 standard diet (Bendel feed and flour mill Ltd, Benin, Nigeria) and water *ad libitum*. All
97 authors hereby declare that the “Principles of Laboratory animal care” (NIH publication
98 No. 85-23, revised 1985) were followed while the Faculty of Pharmacy, University of
99 Uyo, Nigeria’s ethical committee examined and approved the use of the animals and all
100 the experiments.

101 **Acute toxicity studies**

102 The method of Miller and Tainter [11], was adopted for acute toxicity testing. Swiss
103 albino mice were starved of food but allowed access to water *ad libitum* 24 hours before
104 the experiment. The acetone fraction was administered intraperitoneally in a dose range
105 of 7000-12500mg/kg and the animals were randomly selected into groups of six each.
106 The mice were observed for physical signs of toxicity and death, the numbers of deaths
107 per group within 24 hours were recorded and the median lethal dose (LD_{50}) calculated
108 as the square root of the geometrical mean of the maximum dose that did not kill any
109 animal and the minimum dose that killed all.

Experimental design of wound healing effect

Excision wound model were used to evaluate the wound healing activity of *Tetrapleura tetraptera* in rats thus: The dorsal skins of the rats were shaved, and the rats were divided into four groups of five (5) animals each. Under Xylocaine 5% anaesthesia, the animals were depilated on the Para vertebral area prior to wound creation and area of 10mm x10mm skin in its full thickness was excised; Modified [12]. Animals of group one were topically treated with the distilled water (Negative control-NC), group two were topically treated with 0.4g/ml of the acetone fruit fraction of *Tetrapleura tetraptera*, group three were topically treated with 0.6g/ml of the fraction and group four were topically treated with 0.2g Neobacin powder as the positive control (PC). Wound healing property was checked by measurement of wound area and Period of epithilization.

Measurement of wound area

The progressive wound closure rate was accessed by tracing the wound on day 3, 6, 9, 12 and 15 post wounding using a transparent paper and a permanent marker. The wound areas recorded were measured using a millimetre scale graph paper. Number of days required for filling of scar without any residual raw wound was taken as end point of complete epithelisation. The wound healing effect was determined by the reduction of injured area in relation to initial day and calculated using the formula:

$$W_m = \frac{W_{exp} \times 10}{W_m}$$

Where,

W_m = Initial wound area on day one.

132 W_{exp} = Wound area on day of measurement.

133

134 **STATISTICAL ANALYSIS**

135 Results were expressed as multiple comparison of mean $\pm$ SEM significance was
136 determined using one way analysis of variance (ANOVA) followed by Duncan test. A
137 probability level of less than 5 per cent ($P=0.05$) was considered significant.

138 **RESULT AND DISCUSSION**

139 **RESULTS**

140 **Acute toxicity studies**

141 Acute toxicity studies of the crude extract of *Tetrapleura tetraptera* dry fruit was
142 calculated to be 10000 mg/kg which is 10.0g/kg as seen on Fig.1 and Table 1.

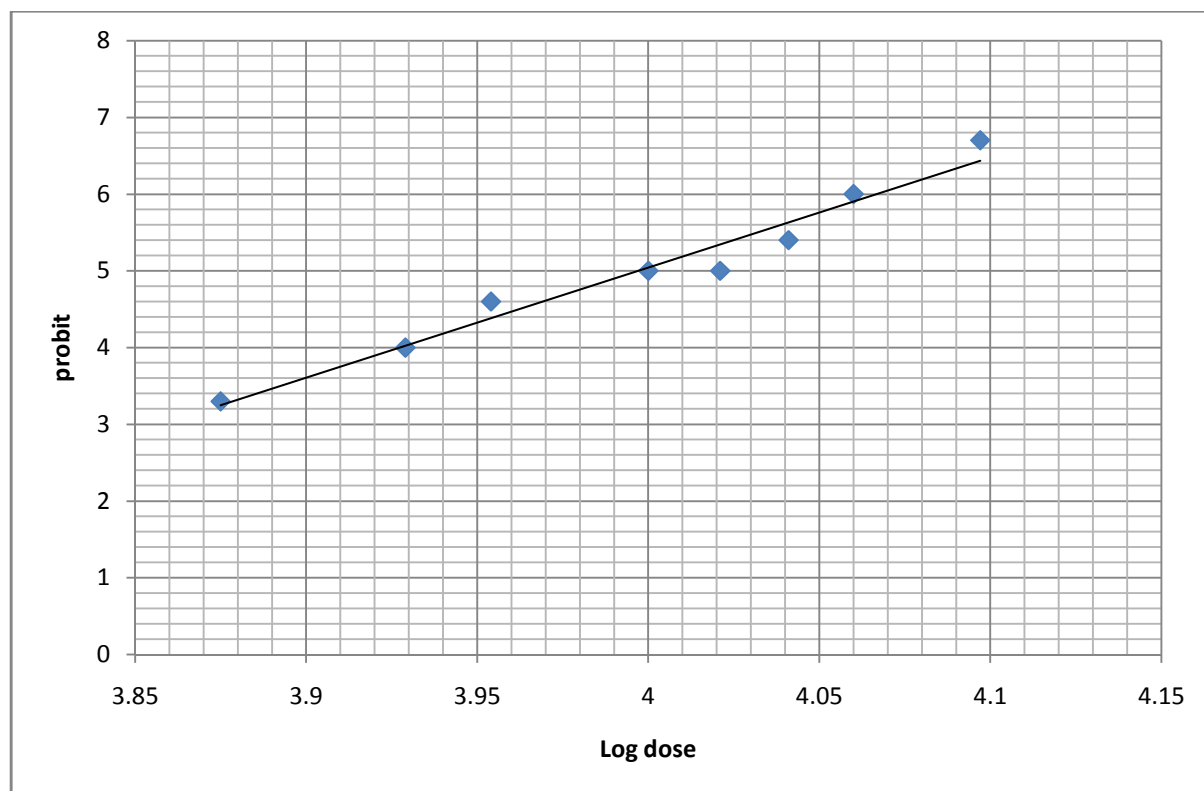


Fig 1: A Graph of Probit versus Log dose indicating a graph of intraperitoneal acute toxicity study of *Tetrapleura tetraptera* fruit crude extract in mice. Dotted lines indicate an LD₅₀ of approximately 10000mg/kg = 10.0g/kg. (Antilog of 4.00 = 10000mg/kg).

Table 1: Acute toxicity determination of the Crude extract of *Tetrapleura*

***tetraptera* dry fruit.**

Group	Dose	Log Dose	24h Mortality	% Mortality	Probit	Probit (Approx)
1.	7000	3.845	0/6 ^a	04.7	3.2721	3.3
2.	7500	3.875	0/6 ^a	04.7	3.2721	3.3
3.	8000	3.903	0/6 ^a	04.7	3.2721	3.3
4.	8500	3.929	1/6	16.6	4.0299	4.0
5.	9000	3.954	2/6	33.3	4.5684	4.6

6.	9500	3.978	2/6	33.3	4.5684	4.6
7.	10000	4.000	3/6	50.0	5.0000	5.0
8.	10500	4.021	3/6	50.0	5.0000	5.0
9.	11000	4.041	4/6	66.6	5.4289	5.4
10.	11500	4.060	5/6	83.3	5.9661	6.0
11.	12000	4.079	6/6*	95.8	6.7279	6.7
12.	12500	4.097	6/6*	95.8	6.7279	6.7

150 Key: ^a = 0% Mortality and * = 100 % Mortality.

151 Specifically derived by the method of Miller and Tainter [11] in which 0.25 was added to
152 zero mortality and subtracted from 100% mortality.

153

154 **Wound healing effect**

155 Wound healing effect of the acetone fraction of *Tetrapleura tetraptera* fruit extract
156 showed no significant difference ($P = 0.05$) between groups 1, 2, 3, and 4 on days 3, 6
157 and 9 as shown on table 2. Acetone fruit extract of *Tetrapleura tetraptera* in groups 1, 3
158 and 4 showed a significant decrease in wound healing in comparison with group 2
159 (Neobacin Powder 0.2g) on day 12. Day 15 also indicated a significant decrease in
160 wound healing in group 1 and 4 when compared to group 2 while group 3 showed a
161 non-significant decrease compared to group 2 (Table 2).

162

163

164 **Table 2: Effect of various Doses of Acetonic Extract of Tetrapleura**

165 **tetraptera dry fruit on Excision Wound Healing in Rats.**

166

167

Group	Treatment	DAY 1	DAY3	DAY 6	DAY 9	DAY 12	DAY 15
1	Negative Control (1ml of distilled water)	1cm	0.88±0.80	0.72±0.09	0.44±0.09	0.28±0.07*	0.12±0.04*
2	Positive Control (0.2g of neobacin powder)	1cm	0.96±0.04	0.70±0.05	0.34±0.05	0.10±0.03	0.02±0.02
3	0.4g/ml of T. <i>tetraptera</i>	1cm	0.88±0.02	0.78±0.03	0.44±0.05	0.29±0.07*	0.06±0.04
4	0.6g/ml of T. <i>tetraptera</i>	1cm	0.89±0.01	0.74±0.02	0.56±0.08	0.47±0.09*	0.14±0.09*

168 Values are expressed as Mean ± SEM (n=5), significant at * $P=0.05$ when compared to
 169 positive control.

170

171

172 **DISCUSSION**

173 The mean lethal dose (LD50) was calculated to be 10000mg/kg = 10.0g/kg. The
 174 physical signs of toxicity included excitation, paw-licking, decreased motor activity.
 175 Others were increased respiratory rate, convulsion and death. According to the OCED
 176 protocol [13]. Acetone fraction of *Tetrapleura tetraptera* can be classified as non toxic

177 since the limited dose of an acute toxicity is generally considered to be 5.0 g/kg bw [14,
178 15].

179 There is an increasing interest in finding herbal extracts with wound healing efficacy
180 although the use of such extracts for treating cuts and wounds is a common practice in
181 traditional medicine [1]. Wound healing effect of the acetone fraction of *Tetrapleura*
182 *tetraptera* fruit extract was not enhanced up to the 12th day as it showed no significant
183 difference between the negative control rats and the fraction treated groups, this was in
184 contrast to the report [16, 17] that reported on the aqueous extracts possession of anti-
185 inflammatory and hypoglycaemic properties. This could be due to the portraying sign of
186 toxicity to wound area and thus resulting in expansion rather than reducing the width of
187 the wound.

188 Acetone fruit fraction of *Tetrapleura tetraptera* in groups 1, 3 and 4 showed a significant
189 decrease in wound healing in comparison with the rats in group 2 treated with Neobacin
190 Powder on day 12. Day 15 also indicated a significant decrease in wound healing in
191 groups 1 and 4 when compared to group 2. This finding contradicts Badu and Effiong's
192 [17, 10] reports on aqueous fruit fraction of the plant. The reason could be traced to the
193 poor extractive property and toxic nature of acetone or may be due to the fact that the
194 necessary phytochemical components or nutrients of the plant responsible for
195 management and treatment of wounds are more soluble in polar solvents (eg; Water)
196 rather than non-polar solvents like acetone, hence not suitable for wound healing.

197 The effect of acetone fraction of the plant confirmed that the plant extract has dose
198 limitation effect, thus at high doses, the extract may have induced severe tissue
199 damage and even lead to neoplastic transformation, which further impede the healing

process by causing damage to cellular membranes, DNA, proteins, and lipids as well as reported by [18]. Also, oxidative stress plays an important role in impaired wound healing. This oxidative stress may cause damage to the growing tissue at the repair site towards cellular proliferation, granular tissue formation and epithelisation [19], thus the negative result of the acetone fraction of *Tetrapleura tetraptera* fruit extract.

Increased cellular proliferation may be due to the mitogenic activity of the positive control, which might have significantly contributed to the healing process of this group. The plant *Portulaca oleracea* containing the tannins possesses wound healing activity [20, 18] as do *T. tetraptera* thereby suggesting the mechanism of action of the extract effect on wound. The less effective nature of the acetone extract could be traced to the poor extraction property of less polar solvent compared to more polar solvent, thus the hampered wound healing effect of the fraction. It was reported that the dry fruit of *Tetrapleura tetraptera* constitutes of Zinc, Calcium, Iron, Magnesium, Pottasium, Vitamin C, and Phosphorus [21] and saponins, tannins, sugar, glysocides, steroids. Triterpenes [22] which are found to be effective in wound healing. This could be attributed to the report that the process of wound healing is promoted by several herbal extracts, which are composed of active agents like triterpenes, alkaloids, flavonoids, tannins, saponins, anthraquinones, and other biomolecules [23, 19].

CONCLUSION

In conclusion, the acetone fraction of the dry fruit of *Tetrapleura tetraptera* is not effective on wound healing. The high dose treatment even indicated that the plant has a limit dose treatment for wound healing at which it was toxic, thereby increasing the

wound surface area. Thus, it is recommend that further work should be done using other extracting solvents like Water, Methanol etc., to ascertain the wound healing effect of the dry fruit of *Tetrapleura tetraptera*.

225

226 REFERENCES:

227 Odimegwu DC et al. Wound healing and antibacterial activities of the extract of *Dissotis*
228 *theifolia* (Melastomataceae) stem formulated in a simple ointment base, *Journal of*
229 *Medicinal Plants Research*. 2008: 2(1): 11–16.

230 Esimone CO et al. Cutaneous wound healing activity of a herbal ointment containing the
231 leaf of *Jatropha curcas* L. (Euphorbiaceae). *International Journal of Applied Research*
232 *in Natural Products*. 2009: 1(4): 1-4.

233 Nwawu JI ; Akali PA. Anticonvulsant activity of the volatile oil from the fruit of
234 *Tetrapleura tetraptera* J. *Ethnopharmacol*. 1986: 18: 103-107.

235 Ojewole JA; Adesina SK. Mechanism of the hypotensive effect of Scopoletin isolated
236 from the fruit of *Tetrapleura tetraptera* plant med. 1983: 49:46-50.

237 Achi OK. Composition and Antibacterial activities of *Tetrapleura tetraptera* taub. pod
238 extracts. *Research Journal of Microbiology*. 2006: 1: 416-422.

239 Shenoy C et al. Preliminary phytochemical investigation and wound healing activity of
240 *Allium cepa* Linn (Liliaceae), *International Journal of Pharmacy and Pharmaceutical*
241 *Sciences*, 2009: 2(2):167–175.

242 Baravkar AA et al. Pharmaceutical and biological evaluation of formulated cream of
243 methanolic extract of *Acacia nilotica* leaves, *Research Journal of Pharmacy and*
244 *Technology*. 2008:1(4):481–483.

245 Umadevi P et al. Antidiabetic and hypolipidemic effect of *Cassia auriculata* in alloxan
246 induced diabetic rats, *International J of Pharmacology*. 2006: 2: 601-607.

247 Myers KA et al. *Principles of Pathology in Surgery*, 1st edn. Blackwell scientific
248 publications: London. 1980: 58-82.

249 Effiong GS et al. Effect of aqueous extract of *Tetrapleura tetraptera* on excision wounds
250 in albino rats. *Int. Res. J. Plant Sci*. 2014:5(4):57-60

- 251 Miller LC; Tainter ML. Estimation of LD₅₀ and its errors by means of log probit graph
252 paper. Proc. Soc. Exp. Biol. Med.1994;57: 261-264.
- 253 Suguna L et al. Effects of Centella asiatica extract on dermal wound healing in rats. Ind.
254 J.Exp.Biol. 1996;34: 1208-1211.
- 255
256 OECD; The Organisation of Economic Co-operation and Development (OECD): The
257 OECD guideline for testing of chemical: 420 Acute Oral Toxicity, OECD, Paris. 2001:1-
258 14.
- 259
260 Assam A et al. In vitro antibacterial activity and acute toxicity studies of aqueous
261 methanol extract of Sida rhombifolia Linn. (Malvaceae). BMC Complementary and
262 Alternative Medicine. 2010: 10:40.
- 263 Nofal SM et al. Anti-Diabetic Effect of *Artemisia Judaic* Extracts. *Res. J. Med. And*
264 *Medical Sci.*, 2009: 4(1): 42-48.
- 265 Ojewole JAO; Adewunmi CO. Anti-Inflammatory and hypoglycaemic effects of
266 *Tetrapleura tetraptera* (taub.) (Fabaceae) fruit aqueous extract in rats. J.
267 Ethnopharmacol. 2004: 95: 177-182.
- 268 Badu M et al. Antioxidant activity of methanol and ethanol/water extracts of *Tetrapleura*
269 *tetraptera* and *Parkia biglobosa*. *Int. J. Pharm. Bio Sci.* 2012: 3(3): 312 – 321.
- 270 Kerstein, MD. Factors affecting wound healing. Adv. Wound care. 2007: 10:30 – 36.
- 271 Rashed AN et al. Simple evaluation of the wound healing activity of a crude extract of
272 *Portulaca oleracea* L. growing in Jordan. J Ethnopharmacology. 2003: 88:131-136.
- 273 Adewunmi CO. Aridan- success in fighting Bilharzia the natural way.

- 274 Research Journal of Agricultural and Biological Sciences. 2011:1:1 – 9.
- 275
- 276 Aderibigbe *et al.* Neuropharmacological evaluation of Aridan, a glycoside isolated from
- 277 *T. tetrapleura* fruit in mice. Discovery and Innovation. J. Boil. Sci; 2006: 7:1520-1524.
- 278 Akin-Idowu PE et al. Chemical Composition of the Dry Fruit of *Tetrapleura tetraptera*
- 279 and its Potential Impact on Human Health. Journal of Herbs, Spices & Medicinal Plants.
- 280 2011: 17(1):52-61.
- 281 Aladesanmi JA. *Tetrapleura tetraptera*: Molluscicidal activity and chemical constituents:
- 282 A review. Afr. S. Trad. Complementary Alternative med. 2007: 4: 23-36.
- 283