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Determination of Wound Healing Activities of A Simple Ointment Base Formulated from the Stem-Bark Extract of *Jatropha Curcas* Plant

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Abstract: - The study investigated the efficacy of an herbal ointment formulated from *Jatropha curcas* stem-bark extract for wound healing activity. The ointment batches that were made from different concentrations (0.5, 1.0, and 1.5g/10g) of *Jatropha curcas* stem-bark extract were topically applied to the wounds inflicted on thirty (30) albino rats and the rate of wound closure was assessed by the measurement of the wound area. These ointments formulated from *Jatropha curcas* stem-bark extracts caused a significant ($P<0.05$) higher level of wound healing in a dose-related manner in the albino rats. The ointment batch containing the highest concentration of the sample extract (1.5g/10g ointment) exhibited the highest rate of wound closure and healing when compared to the blank ointment-treated. The ability of stem-bark extract of *Jatropha curcas* to wound heal and care indicates that its potential can be properly utilized in the production of commercial ointments for wound care and treatment of skin infections. The dose increase is an added advantage to the healing property of this herbal ointment but preservative potentials remains a challenge. There is also ardent need to apply this formulation on humans as to assess the efficacy.

Keywords: Ointment base, percentage wound closure, gentamycin, Trituration, Medicinal plants.

I. Introduction

The use of trees and shrubs in arid and semi-arid regions is of vital importance for the human population in developing countries. Plants form the main ingredients of medicines in the traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs (Egbunefu, *et al.,* 2022).

The use of the medicinal plant is gaining universal acceptance in view of the tremendous expansion of traditional medicine and growing interest in herbal treatments. Plants are used in medicine to maintain and augment health, physically, mentally and spiritually as well as to treat specific conditions and ailments (Obute, 2005). Medicinal plants are divine gifts to us from Mother Nature who has kept these green remedies in her plant kingdom for mankind to use in fighting death from disease and cure themselves from ailments (Igoli *et al.,* 2005). But, as people moved away from Mother Nature in the name of development, they become more prone to disease, decay and degeneration. Their search for "magic" remedies, resulted in the modern medical system which are characterized by the use of antibiotics, steroids, *etc* leading to increased resistance and rebound manifestation of the same and new diseases (Egbunefu, *et al.*,2021; Igbinosa *et al.*,2009).

By conserving traditional medicinal plants, a country can improve its health sector and reduce poverty (Kamal *et al.,* 2011). Medicinal plants are now in a comeback phase within the last two decades, seeing people shifting their focus back to the forgotten traditional natural green remedies. In the western and developed world, people are returning to herbal drugs. Countries that are endowed with diverse and important medicinal plants can grow these traditional herbs for export to developed countries, earning valuable foreign exchange (Mait *et al.,* 2017). Moreover, as people learn more about the medicinal value of plants it leads to increase awareness and improvement in their health as they are applied (Abdelgadir & Van Staden, 2013; Sharma & Singh, 2012).

In folklore medicine, medicinal plants have been used widely in facilitating the treatment of certain ailments with a high degree of successes (Esimonu *et al.,* 2009). *Jatropha Curcas* commonly known as a physic nut that belongs to the *Eupherbiacea* family happens to be one of such plants made available by Mother Nature and is commonly used as an herbal treatment for some ailments by the people of Igboh in Etche ethnic nationality of Rivers State, Nigeria (Egbunefu, *et al.,* 2020). This has inspired much research which are aimed at validating these claims and discovering mechanisms that possibly explains the potentials of this herb in the treatment of these ailments. It is on this premise that this current study on the determination of wound healing activities of a simple ointment base formulated from the stem-bark extract of *Jatropha Curcas* plant is carried out.

The need for the use of natural products as wound healing ointments cannot be over-emphasized as over 70% of Nigerians live in rural communities without immediate health facilities to assuage the challenges of first aid. The occurrence of impetigo remains a

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challenge especially in the volatile Niger Delta resulting from exposure to crude oil which are usually in the rural settlements where illegal bunker activities are prevalent. These activities may not be abated in the nearest future as the youths have taken this as a means of livelihood. Therefore, there is need for local formulations which are cheaper and easily accessible to the rural inhabitants to combat skin infections emanating mostly from reckless exposure to poorly refined petroleum products.

II. Materials and Methods

2.1 Plant Material

The *Jatropha Curcas* Stem-bark used for the work were obtained from Egwi in Etche Local Government Area of Rivers State, Nigeria, where the plant is normally used as life tree to make fence around homes. The stem-bark were sun-dried and pulverized and stored in an air-tight container for further use. About 250g of pulverized stem-bark sample was extracted (Nwala *et al.,* 2013a). The extract was filtered using whatman's No. 1 filter paper and the filtrate was concentrated to dryness in Vacuo using a rotary evaporator to remove the methanol.

2.2 Experimental Animals

Thirty (30) Wistar albino rats (of mass between 200g and 250g) obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria were used for the studies. They were allowed to acclimatize in the laboratory for 4 days before the commencement of the study and were fed with standard livestock feed (Guinea feed Nigeria limited). They were allowed unrestricted access to clean drinking water.

2.3 Preparation of *Jatropha* Extract Ointment

Five batches of the ointments were prepared and used for the study. Batches 1 – 3 contained the three extracts of varying concentration (0.5, 1.0, and 1.5g per 10g of the ointment base). Batch 4 was a commercial gentamycin, gentalek cream, containing 1% gentamycin and was used as a standard drug treatment . The fifth batch (batch 5) was a blank control ointment which was prepared with neither the extract nor the standard drug, but only the blank petroleum Jelly. Each batch contained 10g of blank petroleum, B.P which was weighed into a breaker and then melted in a thermostatic water bath. The required quantities of the extract was weighed, added to the molten ointment base and then homogenized by titration. The ointments were stored in the refrigerator until they were used (Nwala *et al.,* 2013b).

2.4 Infliction of Wounds

A round seal of 21mm diameter was impressed on one side of central trunk depilated and sterilized with ethanol. Excision wound was inflicted on the rats according to methods described by Esimone *et al.,* (2009), under the light ether anaesthesia. Full skin thickness was excised from the marked area to get wound measuring about 347mm^2. After achieving complete homeostasis by blotting the wound with cotton swab soaked in warm saline solution, the animals were placed singly in individual cages. The wounds of the animals were treated topically depending on the group. Groups 1, 2 and 3 were treated with ointments formulated from extracts of various concentrations (0.5, 1.0 and 1.5g of extracts per 10g of ointment base). Group 4 was treated with the standard cream (Batch 4), while group 5 served as the control and was treated with the blank ointment formulation (Batch 5).

The wound area was measured with a translucent paper and thereafter estimated on a 1mm^2 graph sheet every 3 days until epithelialization and complete wound closure was observed. Wound contraction was calculated as a percentage of original wound size.

2.5 Statistical Analysis

The data were analyzed using tables, range, means, percentages, standard deviation and hence standard error (S.E). Also, all the data obtained were subjected to analysis of variance (ANOVA) using computer aided science planning and scheduling system (SPSS) compared using Duncon's multiple range test as reported by Nwala *et al.,*(2013c) at 5% level of significance.

III. Results

The effect of *Jatropha Curcas* Stem-bark extract based ointment on excision wound healing in rats are shown in Table 1. The result on the day 3 of the treatment showed that the wound healing area and percentage wound contraction of rats treated with different dose levels of stem-bark formulated ointment was largest in the group treated with 0.5g/10g ointment which had 253.00±0.87 mm^2 (27.09%). This was significantly ($P<0.05$) different from the groups treated with 1.0g/10g (248.00±0.53mm^2 (28.53%), gentamycin ointment (248.33±0.98mm^2 (28.44%) and blank ointment (248.67 ± 0.67mm^2 (28.34%), except the group treatment with 1.5g/10g ointment which had 250.00 ± 0.87 mm^2 (27.09%).

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On day 6, the wound area of the rats was significantly ($P<0.05$) reduced in the group treated with 1.5g/10g ointment which had 159.00 ± 0.58mm^2 (54.19%), relative (51.44%) and 1.0g/10g with 170.67 ± 1.37mm^2 (51.68%) and blank ointment with 171.00 ± 0.81mm^2 (50.72%). The wound healing area and percentage wound contraction was smaller.

On day 9 were 70.00 ± 0.58mm^2 (79.83%) for the 0.5g extract, 69.33 ± 0.52mm^2 (80.02%) for the 1.0g extract, 51.00 ± 0.99mm^2 (85.30%) for the 1.5g extract and 43.17 ± 1.10mm^2 (87.58%) for the blank ointment of gentamycin and 59.67 ± 0.62mm^2 (82.80%) for the blank ointment. Wound healing area and percentage wound contraction on day 12 ranged from 8.67 ± 0.61mm^2 (97.50%) for the 1.5g extract ointment to 24.83 ± 1.06mm^2 (92.84%) for the 0.5g extract ointment. On day 15, the wound healing area was greatest in the group treated with blank ointment (9.33 ± 0.77mm^2 (97.31%), followed by the group treated with 0.5g ointment (8.67 ± 0.87mm^2 (97.50%), then the group treated with 1.0g ointment (4.00 ± 0.52mm^2 (98.85%) while the group treated with gentamycin ointment (0.50 ± 0.10mm^2 (99.86%) had the least.

On day 18, the rats treated with 1.5g/10g and gentamycin ointments had zero wound healing area and100% wound, closure of 0.00 ± 0.00 mm^2 (100%), while those treated with 0.5g/10g, 1.0g/10g and blank ointments had 3.33 ± 0.52mm^2 (99.04%), 0.67 ± 0.19mm^2 (99.51%) and 4.17 ± 0.44mm^2 (99.80%) respectively. On the least day of the study *i.e* day 21, the group of rats treated with 1.0g/10g, 1.5g/10g and gentamycin ointments had 100% wound closure, while the groups treated with 0.5g/10g and blank ointments had wound healing area of 0.50 ± 0.12mm^2 (99.86%) and 2.33 ± 0.52mm^2 (99.33%) respectively.

Table 1: Effect of *Jatropha curcas* Stem-bark Extract based Ointment on Excision Wound Healing in Rats.

Treatment group	wound area in mm^2 (percentage wound contraction in parenthesis)						
	day3	**day 6**	**day 9**	**day 12**	**day 15**	**day 18**	**day 21**
Jatropha Curcas 0.5g/10g of Ointment base	253.00± 00.87^{ac} (27.09)	168.50 ± 069^{abde} (51.44)	70.83 ± 0.58^{ab} (79.83)	24.83 ± 1.06^{a} (92.84)	8.67 ± 0.87^{ac} (97.50)	3.33 ± 0.52^{ac} (99.04)	0.50 ± 0.12^{abcd} (99.86)
Jatropha Curcas 1.0g/10g of Ointment base	248.00 ± 0.52^{bcde} (28.53)	170.67 ± 0.68^{abde} (50.82)	69.33 ± 0.52^{ab} (80.02)	19.00 ± 0.52^{bde} (94.62)	4.00 ± 052^{bc} (98.85)	0.67 ± 0.19^{bcd} (99.31)	0.00 ± 0.00^{abcd} (100.00)
Jatropha Curcas 1.5g/10g ointment base	250 ± 0.84^{abcde} (27.95)	159. 00 ± 0.58^{c} (54.79)	51.00 ± 0.99^{c} (85.30)	8.67 ± 0.61^{c} (97.50)	1.33 ± 0.46^{bcd} (99.62)	0.00 ± 0.00^{bcd} (100.00)	0.00 ± 0.00^{bcd} (100.00)
Gentamycin Ointment (1%)	248.33 ±0.96^{bcde} (28.44)	167.67 ± 1.37^{abde} (51.68)	43.17 ± 1.10^{d} (87.56)	16.33 ± 0.71^{bd} (95.29)	0.50 ± 0.10^{cd} (99.86)	0.00 ± 0.00^{bcd} (100.00)	0.00 ± 0.00^{abcd} (100.00)
Blank Ointment	248.67 ± 0.67^{bcde} (28.34)	171.00 ± 0.81^{abde} (50.72)	59.67 ± 0.62^{e} (82.86)	20.17 ± 0.59^{be} (94.19)	9.33 ± 0.77^{ac} (97.31)	4.17 ± 0.44^{ac} (99.80	2.33 ± 0.52^{e} (99.33)

Wound Area in mm^2 (percentage wound contraction in parenthesis)

Values are means ± standard deviation of triplicate determinations. Means in the same column with different superscript letters were significantly differentiated by the 0.05 level.

IV. Discussion

The wound area and percentage wound contraction of albino rats topically treated with ointment base formulated from stem-bark extract of *Jatropha Curcas* on the day 3 of the study was best in the group treated with the ointment formulated with the 1.0g extract of the stem-bark with the wound area of 248.00 ± 0.52mm^2 and percentage wound contraction of 28.53%. These estimation (wound area and percentage wound closure), continued till day 21 of the study, which happens to be the last day. From the results of the study, it was discovered that the group treated with 1.5g/10g ointment competed favourably with that treated with the standard drug (gentamycin). The study also showed that on day 18 of the treatment, the albino rats treated with both 1.5g/10g and gentamycin had complete wound closure (i.e 100% wound contraction). Compared to the rest treatment groups. It was also discovered that on the last day of the study (day 21), three of the study groups had recorded 100% wound closure with exception of the groups treated with 0.5g/10g ointment and that of blank ointment which had wound areas of 0.50 ± 0.12mm2 and 2.33 ± 0.52mm2 and percentage

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wound closure of 99.86% and 99.33% respectively. The study showed that the stem-bark extracts of *Jatropha Curcas* formulated into an ointment base are good for wound care and healing activities. The results here corroborated with that reported by Esimone *et al*., (2009), Nwala, *et al*., (2013), and Egbunefu, *et al*., (2021) for leaf extracts of *Jatropha Curcas* and that of Okoli, *et al.*, (2008) for the anti-inflammatory activity studies of the roots of *Jatropha Curcas*. Egbunefu, *et al*.,(2021), stated that medicinal plants have great potentials and have been shown to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scaring to the patient. Similarly, Odumegwu *et al*., (2008) has also established that medicinal plants have been in use for many years as topical and internal preparations to promote wound repair. Hence, current researches are devoted to validating their efficacy and uncovering the mechanisms responsible for this activity.

V. Conclusion

This study confirms the effectiveness of the wound healing activity of the extract of *Jatropha Curcas* stem-bark formulated as an ointment. So the stem-bark extract of this wonder plant can be harnessed by pharmacologist for the formulation of ointment that can be used in the treatment of tropical diseases.

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