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Phytochemical Screening and wound healing activities of Extracts of Jatropha Curcas leaf formulated in a Simple ointment Base

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ABSTRACT: *This study investigated a herbal ointment containing Jatropha curcas leaf extract for wound healing activity. The ointment batches containing different concentrations (0.5, 1.0 and 1.5g/10g) of J. curcas extract was applied topically on the wounds inflicted on rats and the rate of wound closure assessed by wound area measurement. These ointments formulated from extracts of J. curcas caused significantly ($p<0.05$) higher rate of wound healing in a dose related manner in rats. Application of the ointment batch containing the highest concentration of the sample extract (1.5g/10g ointment) showed the highest rate of wound healing and closure when compared to the blank ointment treated group. Phytochemical studies revealed that the methanol extract of J. curcas leaf contains flavonoids, saponins, tannins, alkaloids and glycosides. The ability of extract of J. curcas in wound care and healing suggests that its potentials can be harnessed in production of commercial ointments for wound healing and skin infections.*

KEYWORDS: *Herbal ointment, Jatropha curcas, Phytochemical screening, Wound healing.*

I. INTRODUCTION

Several plants exist with very high nutritive and medicinal values and yet remain unexploited for human and animal benefits. Plants are very vital because they are a fundamental part of life on earth, which generates the oxygen food, fibres fuel and medicine that allow humans and other higher life forms to exist. It is no small wonder then that green leaves are so very extremely beneficial to health [1]. In addition to using green leaves as food, specific green leaves makes excellent natural medicines. Leaves generally are very cleansing, healing, smoothing and revitalizing as well as nourishing [2]. There are considerable documentations of traditional medicinal uses of various parts of the jatropha plant. Only some of these uses have been verified by research. Preparations of all parts of the plant, including seeds, leaves and stem-bark, fresh or as a decoction, are used in traditional medicine and for veterinary purpose [3]. Esimone *et al*., [4], reported that the roots, stems, leaves, seeds and fruits of *J. curcas* have been widely used in traditional folk medicine in many parts of West African. Some of these plants employed in traditional medicine for wound healing or care like *J. curcas* may either promote direct wound repair or exhibit anti-microbial and other related properties which are beneficial in overall wound care [5]. Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds involves the activity of intricate networks of blood cells, cytokines and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue [6].

Jatropha curcas commonly known as physic nut belongs to the Euphorbiaceae family. It is a multipurpose tree and is of significant economic importance. The plant grows quickly; survives in poor stony soils and is resistant to drought and diseases as well as reaches height of 3-8m, and can be grown on waste lands, or barren marginal agricultural lands where no irrigation facility is available.

J. *curcas* does not compete with conventional food or feed crops for land and water, and thus could be an ideal choice to make use of vast land resources that are presently under-utilized [7].

Extracts from the leaves and stem of *Jatropha curcas* plant popularly called *Anyaso-obara* or *Ogwo-nma* by the local people of Igbo in Etche Local Government Area of Rivers State, Nigeria are used to treat wounds and sores.

This present study was carried out to assess the wound healing properties of the methanol extract of *Jatropha curcas* leaf formulated in a simple ointment base as part of the exploration for new and novel bio-active compounds.

II. MATERIALS AND METHODS

2.1. Plant Materials

The *Jatropha curcas* leaves (fig. 1, below), used for the work were obtained from Igbo in Etche Local Government Area of Rivers States, Nigeria, where the plant is normally used as life tree to make fence that demarcate plots of land. The leaves were sun-dried and pulverized and stored in an air-tight container for further

use. About 250g of the pulverized leaf sample was extracted with methanol [8]. 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.

Figure 1: Jatropha curcas plant

2.2. Experimental Animals

Thirty (30) white albino rats (of mass between 200 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 research laboratory for 4 days before the commencement of the study and were fed with standard livestock feed (Guinea Feed Nigeria Limited). The animals were allowed unrestricted access to clean drinking water.

2.3. Preparation Of Jatrapha Extract Ointments

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. Batch 5 was a blank control ointment and was prepared with neither the extract nor the standard drug, but only the blank petroleum jelly. For each batch, 10g of blank petroleum jelly B.P was weighed into a beaker 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 trituration. The ointments were stored in the refrigerator until they were used.

2.4. Infliction Of Wounds

A round seal of 21mm diameter was impressed on one sides of central trunk depilated and sterilized with ethanol. Excision wound was inflicted on the rats according to methods described by Esimone *et al*., [4] under 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 socked in warm saline, 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 at 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 day until epithelialization and complete wound closure was observed. Wound contraction was calculated as a percentage of original wound size.

2.5. Phytochemical Analysis Of The Plant Extracts

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, flavonoids, alkaloids and glycosides in accordance with AOAC [9] and Makker *et al*., [10] with little modifications.

2.6. Statistical Analysis

The data were analyzed using tables, range, means, percentages, standard deviation and hence standard error (SE). Also, all the data obtained were subjected to analysis of variance (ANOVA) using computer aided science planning and scheduling system (SPSS) compared using Duncan's multiple range test [11] at 5% level of significance.

III. RESULTS

The results are presented in tables 1 and 2 below. Phytochemical studies showed that the methanol extract of *J. curcas* leaves contains saponins, tannins, flavonoids, alkaloids and glycosides. The glycoside value of *J. curcas* was higher (36.18 ± 0.46) than the other extracts, followed by the saponins (15.73 ± 0.42%).

In the wound healing study, the extracts of *Jatropha curcas* leaf formulated ointments were able to effectively, dose dependently reduce and also effected complete wound closure within the stipulated time of the work. The groups treated with 1.5g/10g of ointment base and those treated with the gentamycin cream were able to effect total wound closure on/before the 18th day of the studies, while those treated with 0.5/10g ointment base, and 1.0g/10g ointment base effected complete wound closure on the experimental animals on/before the last day of the experiment (day 21). Small size of the excision wound (2.33 ± 0.52mm^2) was still found on the trunk of the group treated with the control ointment (blank ointment) as at the last day. That was the only group that still had the excision wound till the last day of the study, though the size of the wound was very insignificant compare to the original size of the wound.

Table 1: Phytochemical constituents of methanol extracts of *J. curcas* leaf

Composition	Leaf Extract
Flavonoids (%)	0.30 ± 0.06^{a}
Tannins (%)	1.10 ± 0.18^{a}
Saponins (%)	15.73 ± 0.42^{cd}
Alkaloids (mg/100g)	15.17 ± 0.13^{cd}
Glycosides (mg/100g)	36.18 ± 0.46^{e}

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

Table 2: Effect of *Jatropha curcas* leaf extract based ointment on excision wound healing in rats. Wound area in mm^2 (percentage wound contraction in parenthesis)

TREATMENT GROUP	WOUND AREA IN mm^2 (PERCENTAGE WOUND CONTRACTION IN PARENTHESIS)						
	DAY 3	DAY 6	DAY 9	DAY 12	DAY 15	DAY 18	DAY 21
Jatropha curcas 0.5g/10g of ointment-base.	240.00± 1.13^{ab} (30.84)	167.00± 0.52^{ad} (51.87)	59.83± 0.48^{abe} (82.90)	18.67± 0.63^{de} (94.62)	7.83± 0.48^{ae} (97.74)	2.50± 0.52^{a} (99.28)	0.00± 0.00^{abcd} (100.00)
Jatropha curcas 1.0g/10g of ointment-base.	235.33± 0.54^{ab} (32.18)	174.00± 0.40^{be} (49.86)	59.83± 0.87^{abe} (82.76)	15.00± 0.58^{bd} (95.68)	2.33± 0.65^{bcd} (99.33)	0.17± 0.06^{bcd} (99.95)	0.00± 0.00^{abcd} (100.00)
Jatropha curcas 1.5g/10g of ointment-base.	247.67± 1.54^{de} (28.63)	152.33± 1.35^{c} (56.09)	39.67± 0.62^{cd} (88.57)	4.00± 0.52^{c} (98.85)	0.67± 0.27^{bcd} (99.81)	0.00± 0.00^{bcd} (100.00)	0.00± 0.00^{abcd} (100.00)
Gentamycin ointment (1%)	248.33± 0.77^{cde} (28.44)	167.67± 0.39^{ad} (51.68)	43.17± 0.68^{cd} (87.56)	16.33± 0.59^{abd} (95.29)	0.50± 0.12^{bcd} (99.86)	0.00± 0.00^{bcd} (100.00)	0.00± 0.00^{abcd} (100.00)
Blank ointment	248.67± 0.68^{cde} (28.34)	171.00± 0.58^{bde} (50.72)	59.69± 0.96^{abe} (82.86)	19.84± 0.42^{ae} (94.19)	9.33± 0.79^{ae} (97.31)	4.17± 0.46^{e} (98.80)	2.33± 0.52^{e} (99.33)

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

IV. DISCUSSION

The wound area and percentage wound contraction of rats topically treated with *J. curcas* leaf extract based ointments on excision wound healing on the 3rd day of the study was best in the group treated with ointment made from 1.0g/10g ointment base with wound area of $235.33 \pm 0.54 mm^2$ and percentage wound contraction of 32.18%. The estimation of the wound area and percentage wound closure continued every 3 days till the last day of the study (day 21). It was found that the group treated with the highest concentration of extract (i.e. 1.5g/10g ointment base) competed favourably with the group treated with the standard drug (gentamycin). On day 18 of the treatment, the rats in these two groups had observed complete wound healing (100.00% wound closure) than the other treatment groups. But before the last day of the study (day 21), the remaining treatment groups with the exception of the group treated with the blank ointment also showed complete wound healing (100.00% wound contraction). The study showed that the leaf extracts of *J. curcas* formulated into ointment base is good for wound care and healing activities. The result obtained here was almost similar to that reported by Esimone *et al*., [4] for leaf extract *of J. curcas*. The anti-inflammatory activity of the roots of j. curcas has been demonstrated in rodents [12]. 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 scarring to the patient [13].

Traditionally, medicinal plants have been used for many for many years as topical and internal preparations to promote wound repair. Some of these plants owe their effects to direct effect on the wound healing processes and some to their anti-inflammatory and anti-microbial properties. A combination of these properties is also possible in some of the medicinal plants used in wound care [14]. Wound healing is a natural process of regenerating dermal and epidermal tissue. Whenever there is a wound, a set of overlapping events takes place in predictable fashions to repair the damage [15]. The process has been conveniently categorized into phases such as the inflammatory, proliferative, remodeling phases [15].

Although, wound treatment with the ointment containing the extract of *J. curcas* showed a pre-healing activity, the exact step and mechanism in wound repair processes affected by the extract was not established.

The phytochemical studies showed that the methanol extract of *J. curcas* contain flavonoids, saponins, tannins, alkaloids and glycosides. Some of these phytochemicals are known to have anti-inflammatory, anti-infective and pro-wound healing activities which could be responsible for the results of these studies [16].

V. CONCLUSION

This study shows that extract of *J. curcas* leaf has wound healing effect when formulated as ointment, so the pharmacologist can harness the potentials in the extracts of this plant in the formulation of ointments for the treatment of topical diseases.

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