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Assessment of Biological Activities: A Comparison of *Pergularia daemia* and *Jatropha curcas* Leaf Extracts

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Research Article

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ABSTRACT

The present study was aimed to compare the wound healing, free radical scavenging and cytotoxicity potentials of *Jatropha curcas* and *Pergularia daemia* leaf extracts. Quantitative analysis for some phytochemicals; flavonoids, phenols, glycosides, tannins, saponins and alkaloids were carried out using standard methods. Herbal ointments containing 50% (w/w) methanol leaf extracts of *Jatropha curcas* and *Pergularia daemia* were formulated. Excision wound measuring 7x7 mm^2 was created and the ointment applied topically on the wounded area which was measured at intervals of 4 days. Blank ointment (paraffin base) served as the negative control while Povidone iodine ointment served as the standard treatment. On the 16^{th} day, rats treated with the standard drug (Povidone iodine) showed 82.1% wound closure; *J. curcas* -treated rats showed 91.3% wound healing while *P. daemia* treated rats exhibited 97.2% wound closure, indicating an efficacy of the formulations. The ointment formulated with *P. daemia* leaf extract had the best wound healing potential with very minimal scar formulation. The phytochemical screening revealed that the leaves of *J. curcas* and *P. daemia* contain tannins, alkaloids, phenols, flavonoids and glycosides. *J. curcas* had appreciable amount of saponins, however, saponins were not detected in *P. daemia*. The LC_{50} values for *J. curcas* and *P. daemia* were 586.79 µg/ml and 344.26 µg/ml, respectively while that of the standard (Potassium dichromate) was 62.52 µg/ml. The crude methanol extracts of *J. curcas* and *P. daemia* possessed free radical scavenging activities with IC_{50} of 90.83 and 214.16 µg/ml, respectively while that of the standard quercetin was 50.71 µg/ml. The results obtained in this study strongly support the verbal claims on the use of these plants for wound healing. It also indicates that *J. curcas* and *P. daemia* are potential sources of natural antioxidants and are relatively safe for the purposes utilized.

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Keywords: Pergularia daemia; Jatropha curcas; phytochemicals; wound healing; cytotoxicity; antioxidant;

1. INTRODUCTION

Plants and plant products have been used as sources of medicine since time immemorial and it is almost universal among non-industrialized societies (Edgar et al., 2002). According to World Health Organization, more than 80% of the world's population, mostly in developing countries depend on traditional plant based medicines for their primary health care needs, and has recommended that plants use as medicine should be encouraged especially in places where access to conventional treatment is not adequate (WHO, 1980).

In certain African Countries, up to 90% of the population still relies exclusively on plants as source of medicine (Hostettmann et al., 2000). In 2004, the U.S. National Centre for complementary and Alternative Medicine of the National Institutes of Health began funding clinical trials into the effectiveness of herbal medicine. For this reason, various medicinal plants have been studied using modern scientific approaches which have shown that due to their various biological components, many of these medicinal plants posses a number of properties such as anti-diabetic, antioxidant, anticancer, anti-inflammatory effects, etc. and can be used to treat a wide range of various diseases. The medicinal properties of plants are due to the presence of certain specific substances, referred to as bioactive principles or Phytochemical which may be stored in organs like roots, leaves, stem bark, fruits and seeds (Ashok et al., 2006).

Many herbs have shown positive results in-vitro, animal models, or small-scale clinical tests (Srinivasan, 2005).

Plants and their extracts have immense potentials for the management and treatment of wounds. The phytomedicine for wound healing are not only cheap and affordable, but are also safe as hypersensitive reactions are rarely encountered. These natural agents induce healing and regeneration of tissues by multiple mechanisms. However, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before recommendation for the healing of wounds. The use of the leaf extracts of *J. curcas* and *P. daemia* in skin infections and wound treatment in our local community has inspired this present investigation. *J. curcas* leaves can be used as tea against malaria; the seeds are used as contraceptives in South Sudan and also used against constipation. The watery sap is put onto fresh cuts and sores at the corner of the mouth, and can also be used as antidotes for venomous stings and bites. Seed oil is applied to soothe rheumatic pain (Heller, 1996).

Pergularia daemia is said to have more magical application than medical application as it posses diverse healing potential for a wide range of illnesses. Some of the Folklore people use this plant to treat Jaundice, as laxative, anti-pyretic, expectorants and also in infantile diarrhea. The leaf latex is locally used as pain killers and for relief from toothache (Hebbar et al., 2010), the sap expressed from the leaves are held to cure sore eyes in Ghana. The plant reduces the incidence of convulsion and asthma. It is used to regulate the menstrual cycle and intestinal functions. The root is useful in treating leprosy, mental disorders, anemia and piles (Hebbar et al., 2010).

2. MATERIAL AND METHODS

2.1 Collection of Plant Materials

Pergularia daemia leaves were collected in October, 2010 from the netted fence of Kogi State University Stadium while *Jatropha curcas* leaves were collected in November, 2010 from Dolphin Complex which is situated directly behind "New Age" Digital Photo Studio beside One Nigeria Restaurant and Bar, Anyigba. The leaves were properly raised with tap water to remove dirt and were later air dried for three week. The dried leaves were pulverized using an electric blender to obtain a powdered form which was then stored in an airtight container until required for analysis.

2.2 Phytochemical Composition Analysis

The total phenol composition was determined using the method described by A.O.A.C (1950). The total flavonoids were quantified using the aluminum chloride colorimetric method which was modified from the procedure reported by Woisky and Salatino (2009). 10mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 mg/ml. The diluted standard solutions (0.5ml) were separately mixed with 1.5ml of 95% ethanol, 0.1ml of 10% ammonium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water.

After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5ml of ethanol extracts were reacted with aluminum chloride for determination of flavonoid content. The flavonoid content was calculated using the formula:

$$\text{Flavonoid (mg/g)} = \frac{7.000 \times \text{Absorbance (sample)}}{1.4}$$

2.3 Tannins

The tannic acid in the powdered leaves of *J. curcas* and *P. daemia* was determined according to the method of Akinmutimi (2006). A portion (2g) of each sample was weighed into separate beakers. Each was soaked with solvent mixture (80 ml of acetone and 20 ml of glacial acetic acid) for 4 hours, after which the filtrates were removed. The samples were filtered through a double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 10 ppm to 50 ppm. The absorbance of the standard solution as well as that of the filtrates was read at 250 nm on spectrophotometer and the percentage tannin calculated.

The gravimetric method of Harbone (1980) was used in the quantification of alkaloids. A portion (1g) of each sample was weighed and dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4 hours before filtration. The filtrate was evaporated to quarter of its original volume and concentrated NH_4OH solution. The precipitate in the filter paper was dried in the oven at 60^0C for 30 minutes and was re-weighed. By weight difference, the weight of alkaloid was determined and expressed as a percentage of the sample weight analyzed.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

Where:

W = Weight of sample
W_1 = Weight of empty filter paper
W_2 = Weight of filter paper + precipitate.

Alkaline picrate method as described by Onwuka (2005) was employed for the determination of cyanogenic glycosides. A portion (5g) of each sample was grinded into paste and dissolved in 50 ml distilled water in a corked conical flask. The extraction was allowed to stay overnight (12 hrs). The sample was filtered and the filtrate was used for the cyanide determination. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate was added and incubated in a water bath for 5 minutes than the absorbance was read at 490 nm, the absorbance of the blank containing only 1 ml distilled water and 4 ml alkaline picrate solution was also read. The cyanide content was extrapolated from a cyanide standard curve. The cyanide content was calculated using the formula:

$$\text{Cyanide (Mg/g)} = \frac{\text{Absorbance X Gradient factor X Dilution factor}}{\text{Weight of Sample}}$$

The spectrophotometer method of Brunner (1984) was used for the estimation of Saponins in the plant samples. A portion (1g) of finch, ground sample was weighed into a 250 ml beaker and 100 ml of ethanol was added. The mixture was vortexed on a mechanical shaker 5 hrs to ensure uniform mixing. Therefore, it was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40% solution of magnesium carbonate was added. The mixture obtained with magnesium carbonate was again filtered to obtain a clear, colourless solution. 1 ml of the colourless solution was pipette into a 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water and was allowed to stand. 0-10 ppm standard saponin was prepared from saponins stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$. The absorbance of the sample as well as standard saponin solution was read after colour development on a spectrophotometer at a wavelength of 380 nm.

$$\% \text{ Saponin} = \frac{\text{Absorbance of sample X Gradient factor X Dilution factor}}{\text{Weight of Sample}} \times 100$$

2.4 DPPH Radical Scavenging Activity Test

The free radical scavenging activities of *J. curcas* and *P. daemia* leaf extracts were determined using the modified method of Blois (1985). To 1 ml of different concentrations of extracts (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) and standard (quercetin) in test tubes was added 1 ml of 0.3 mm DPPH (2, 2-diphenyl – 1 – picryl hydrazyl) in methanol. The mixture was vortexed and incubated in a dark chamber for 30 minutes after which the absorbance of each sample and the standard was measured at 517 nm against a DPPH control containing only 1 ml of methanol (in place of the extract). The percentage scavenging activity was calculated using the expression.

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.5 Cytotoxicity Bioassay

The modified method of Solis et al., (1992) was employed in the cytotoxicity study of *J. curcas* and *P. daemia* leaf extracts. Brine Shrimps (*Artamia salina*) were hatched using shrimp eggs in a plastic vessel (500 ml), filled with sterile artificial sea water (prepared using NaCl Salt (38g/L) and adjusted to pH 8.5 using 40% NaOH) under constant aeration for 48h. After hatching, active *nauplii* free from egg shells were harvested from brighter portion of the hatching chamber and used for the assay. Ten *nauplii* were drawn through a glass capillary and placed in each vial containing 5 ml of brine solution. A portion (50 ml) of different concentrations of crude ethanol extracts (1000, 500, 250, 125, 62.25 µg/ml) solution in DMSO – artificial sea water was added into each well (vial bottles) containing 10 newly hatched brine shrimps and then incubated at room temperature for 24h. All samples were repeated in two wells to make the overall tested organisms of 20 for each. The living shrimps were counted under a hard magnifying lens. Same procedure was followed using potassium dichromate as the reference toxicant (standard).

Plot of % lethality versus log concentration, substituted y = 50 in the resulted linear equation to obtain the X value. The antilog of X was the LC_{50} (concentration of 50% lethality) value (Ballantyne et al., 1995)

2.6 Experimental Animals

The animal selected for this study was albino rat (*Rattus norvergicus*); all were female with average weight of 150 – 152.47g. The animals were obtained from Ahmadu Bellow University, Zaria and were housed under standardized environmental conditions for a period of 7 days before the experiment, for the purpose of acclimatization. Animals were fed with commercial feeds bought from “Top feeds” in Anyigba, Nigeria. The animal experiment was carried out in the Department of Biochemistry, Kogi State University, Anyigba, according to the University’s ethics on animal handling.

2.7 Preparation of Plant Extracts

Cold extraction method was used in the extraction of the bioactive extracts. Portions (200g) of the powdered samples of both plants were weighed into two separate conical flasks. 600 ml of pure methanol (99%) was added into individual conical flasks and sealed tightly with foil paper. The samples were left for 72 hours, after which they were filtered under vacuum pressure, and the filtrates were concentrated using rotary evaporator.

2.8 Ointment Formulation

An alcohol-free extract of the leaf gel was used for the ointment formulation (Yagi et al., 1998). A 50% (W/W) of extract ointment of *Jatropha curcas* and *Pergularia daemia* was formulated using 2.5 g of the leaf extracts and 2.5 g of soft white paraffin base (Cooper, 1987).

2.9 Wound Creation

The animals were anaesthetized with 0.3 ml of Lindocane adrexyl (local anesthetic) to prevent any movement of the animals for at least two hours.

The hairs were removed by shaving the dorsal back of the rats and methylated spirit was applied as antiseptic to the shaved region. A millimeter rule was used to measure 7 X 7 mm^2 on the skin and an excision wound was made by removing a thick piece of the measured region of the skin.

2.10 Animal Grouping and Treatment

The animals were divided into four (4) groups of four (4) rats per group.

Group 1: Control (Wound treated with paraffin base).
Group 2: Standard (Wound treated with povidone iodine).
Group 3: (Wound treated with *Jatropha curcas* extract ointment)
Group 4: (Wound treated with *Pergularia daemia* extract ointment).

Measurement of wound closure, wound surface microbial load, and wound surface protein were taken on day 4, 8, 12 and 16 post wound creation. The wound area was traced and measured parametrically and the values recorded.

The actual value was converted into percentage value taking the size of the wound at the time of wound creation as 100%. The surface of the wounds were swabbed using swab sticks (dipped in sterile water) for analysis of microbial load, and the granulation tissue were removed (by scrapping wound surface) for the analysis of total protein.

2.11 Wound Surface Protein Determination

The wound surface protein was determined following the method described by A.O.A.C. (1980).

Sample (0.01 g) was weighed out into a Kjeldahl flask and 5g of anhydrous sodium sulphate was added. This was followed up with the addition of 1g of copper sulphate and 1 tablet of Kjeldahl catalyst (each tablet contains 1g of Na_2So_4+0.05 selenium). 10 ml of concentrated tetraoxo sulphate (vi) acid and 5 glass beads (to prevent bumping during heating) were introduced into the mixture. The mixture was transferred into a fume cupboard where it was heated with occasional shaking till the solution assumed a green color (temperature of digester was above 420^0C for about 30 minutes). The mixture was then cooled. Black particles at the mouth and neck of the flask was washed down with distilled water and reheated gently until the green colour disappeared. After cooling, the digest was transferred with several washings into a 250 ml volumetric flask and up to mark with distilled water.

This was distilled using the Markham distillation apparatus. A 100 ml conical flask containing 5 ml boric acid was placed under the condenser such that the condenser tip was under the liquid. 5 ml of the digest was pipette into the condenser via the small funnel aperture, and washed down with distilled water which was followed by 5 ml of 40% NaOH solution. The distillation apparatus was steamed through for about 5 – 7 minutes to collect enough

ammonium sulphate. The solution in the receiving flask was filtrated using 0.01N HCl and the nitrogen content was calculated.

2.12 Wound Surface Microbial Load Count

The method used for the estimation of wound surface microbial load was the swab method as described by Aneda (2005). All glass wares used were thoroughly washed, rinsed with distilled water, dried and sterilized at 120^0C for 30 minutes. A portion (4.2g) of nutrient agar was dissolved in 150 ml of distilled water and was autoclaved at 121^0C for 15 minutes to remove all contaminants that may influence the growth of interfering microorganisms. It was then left to cool to room temperature after which it was poured into large Petri dishes and left to solidify, sterile swab was carefully unwrapped, dipped into sterile water, gently pressed and was used to swab the surface of the wounded rat skin. The swab stick was streaked gently on the surface of the molten agar in a zigzag manner to ensure an even distribution on the plates (Petri dishes). The culture plate was incubated at 37^0C for 24 hours and the microbial load counts were done. This was done for the entire sample and for each experimental rat.

2.13 Statistical Analysis

All data were expressed as mean ± S.E.M and Graph Pad Instat – [Dataset 1. ISD] was applied to determine the significance of the difference at $P<0.05$.

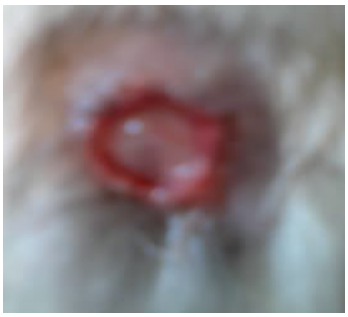
3. RESULTS AND DISCUSSION

The result of the effects of *J. curcas* and *P. daemia* extract ointment on the wound healing is presented on table 1. The extract ointments exhibited significant wound healing properties on the experimental animal's wound when compared with the standard (povidone iodine and the control (paraffin base) (Figure 1-5).

Table 1: Effect of *J. curcas* and *P. daemia* leaf extract ointment on wound healing

Groups	Percentage Wound Closure			
	Day 4	Day 8	Day 12	Day 16
Control	17.35 ± 3.06^{x}	12.04 ± 0.51^{ai}	58.67 ± 3.76^{17}	69.90 ± 1.93^{xa}
Povidone iodine treated	26.53 ± 4.99^{xa}	45.92 ± 2.70^{y}	72.45 ± 1.77^{g}	82.14 ± 0.51^{xb}
J. Curcas treated	31.63 ± 2.70^{xb}	57.14 ± 2.04^{y}	80.61 ± 1.77^{h}	91.33 ± 0.51^{xc}
P. daemia treated	37.24 ± 3.05^{xc}	69.39 ± 3.91^{aii}	91.84 ± 0.42^{hi}	97.19 ± 0.99^{xd}

Results are expressed as mean ± SEM, n = 4 per group. Values along the column with different superscripts are considered significant (P<0.05).



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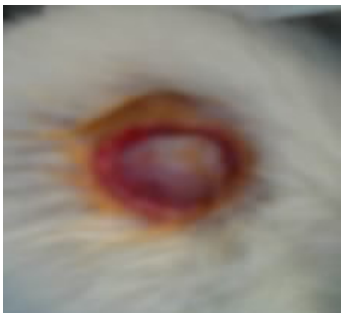

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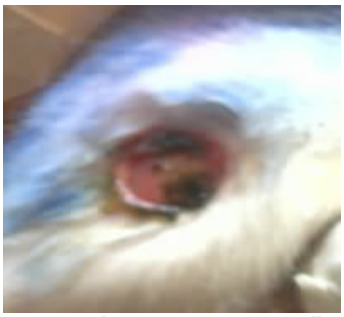
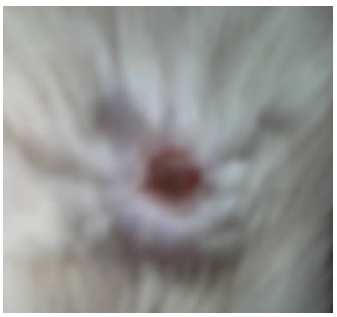


Test 1 (*J. curcas* treated)

Test 2 (*P. daemia* treated)

Fig. 1. Excision wound model on Day 0



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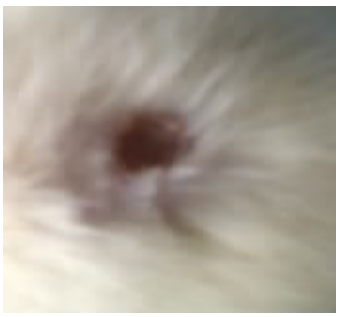

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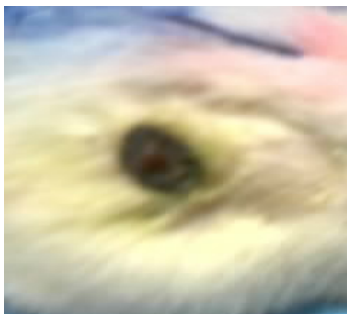

Test 1 (*J. curcas* treated)

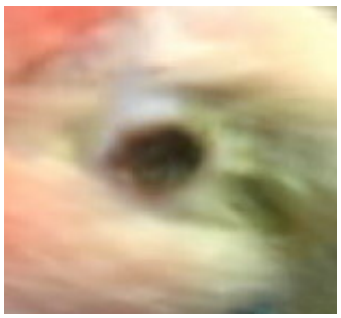

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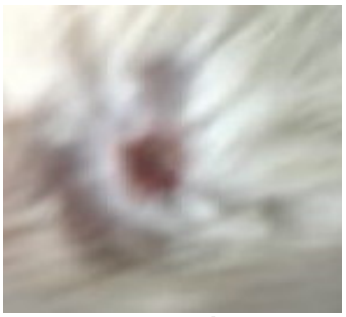
Fig. 2. Excision wound model on Day 4



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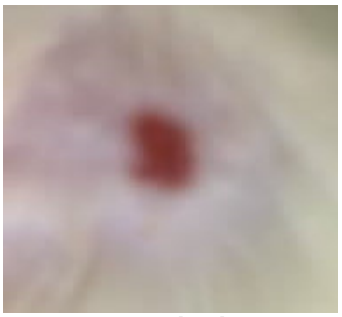

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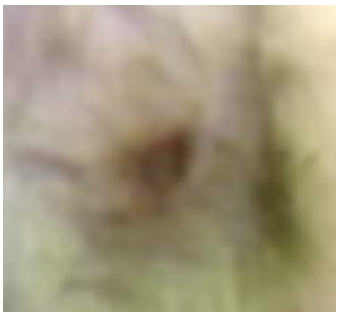
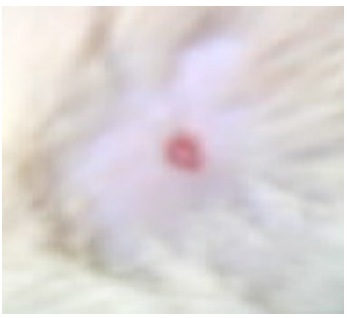


Test 1 (*J. curcas* treated)

Test 2 (*P. daemia* treated)

Fig. 3. Excision wound model on Day 8



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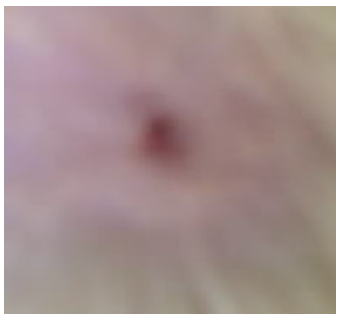

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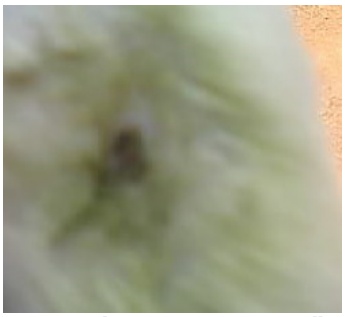

Test 1 (*J. curcas* treated)

Test 2 (*P. daemia* treated)

Fig. 4. Excision wound model on Day 12

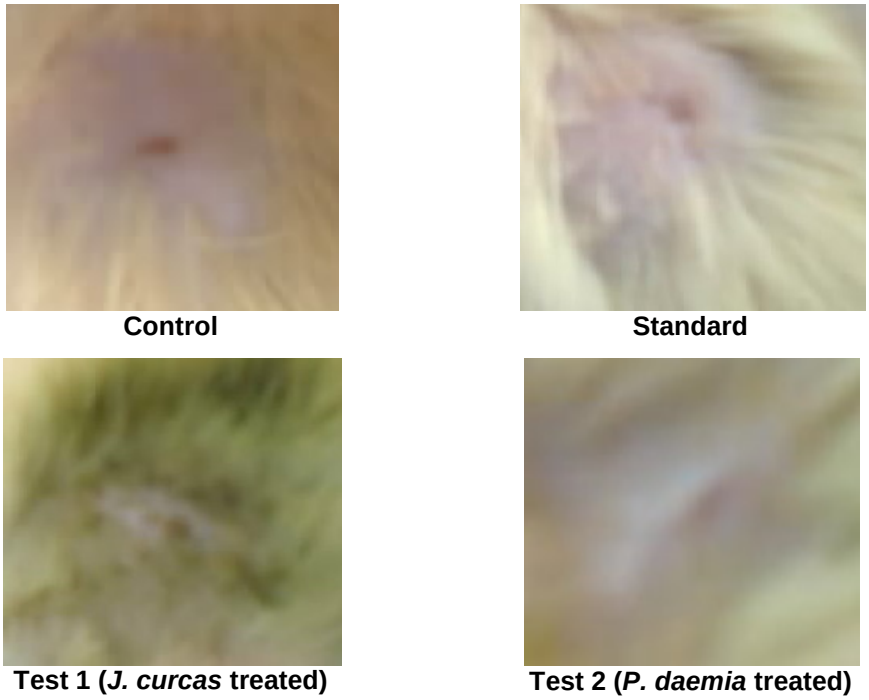

Fig. 5. Excision wound model on Day 16

Table 2 shows the effects of the leaf extracts ointment of *J. curcas* and *P. daemia* on the synthesis of wound surface protein. There was a progressive synthesis of protein (Collagen) in all groups after the wound creation. Animals in group 4 had the highest concentration of protein deposition followed by animals in group 3.

Table 2. Effects of *J. curcas* and *P. daemia* leaf extract ointment on wound surface protein

Groups	Percentage Protein			
	Day 4	Day 8	Day 12	Day 16
Control	5.80 ± 0.55^{z}	12.75 ± 0.30^{h}	14.63 ± 0.24^{l}	17.19 ± 0.12^{xa}
Povidone iodine treated	11.67 ± 0.27^{s}	14.76 ± 0.46^{h}	19.10 ± 0.29^{k}	17.19 ± 0.12^{xa}
J. curcas treated	12.90 ± 0.10^{s}	17.97 ± 0.53^{p}	21.06 ± 0.39^{k}	30.68 ± 0.21^{aa}
P. daemia treated	19.57 ± 1.0^{f}	23.76 ± 0.86^{q}	29.90 ± 0.92^{m}	39.76 ± 1.41^{xd}

Results are expressed as mean ± SEM, n = 4 per group. Values along the same column with different superscripts are considered significant (P<0.05).

Table 3 shows the antimicrobial effect of the extracts on wound surface microbial load. Generally, the microbial load on the wound surfaces of all groups decreased with the wound closure. Povidone iodine treated group (2) had the lowest microbial count. *P. daemia* leaf extract ointment had a better antimicrobial effect than *J. curcas* when compared to the reference standard (Povidone iodine).

Table 3. Antimicrobial Effects of *J. curcas* and *P. daemia* leaf extract ointment on wound surface microbial load

Groups	Microbial Count (CFU/ml) X 10^2			
	Day 4	Day 8	Day 12	Day 16
Control	34.25 ± 1.66^{x}	31.49 ± 1.55^{xl}	28.50 ± 0.99^{xxl}	26.94 ± 0.77^{xa}
Povidone iodine treated	3.35 ± 0.64^{xa}	0.59 ± 0.06^{xb}	0.30 ± 0.02^{xd}	0.21 ± 0.05^{xf}
J. curcas treated	28.75 ± 0.29^{xb}	26.43 ± 0.42^{xc}	23.33 ± 0.36^{xd}	20.30 ± 0.39^{xg}
P. daemia treated	21.95 ± 0.67^{xc}	21.45 ± 0.51^{xd}	9.27 ± 0.34^{xe}	15.88 ± 0.62^{xh}

Results are expressed as mean ± SEM, n = 4 per group. Values along the same column with different superscripts are considered significant (P<0.05).

The estimated quantity of the phytoconstituents of *J. curcas* and *P. daemia* are as presented in Table 4. *J. curcas* contains higher phenol, and alkaloid content. An appreciable amount of Saponins were not detected in *P. daemia* leaves. *P. daemia* is richer in Tannins than *J. curcas*.

Table 4. Quantitative estimation of the phytoconstituents of *J. curcas* and *P. daemia* leaves

Phytochemical	*Jatropha curcas*	*Pergularia daemia*
Flavonoid (Mg/g)	0.662±0.022	0.492±0.023
Tannins (%)	0.301±0.010	0.507±0.018
Phenol (Mg/g)	0.290±0.040	0.190±0.039
Glycosides (Mg/g)	0.015±0.017	0.024±0.005
Saponins (%)	0.207±0.009	-
Alkaloids (%)	6.000±0.031	3.000±0.026

The free radical scavenging activity of *J. curcas* and *P. daemia* is presented in Table 5. *J. curcas* has higher free radical scavenging activity than *P. daemia* when compared with the reference standard (Quercetin).

Table 6 shows the inhibitory effects of the extracts on brine – shrimps (*Artemia salina*). Both extracts showed mild inhibition on the brine shrimps when compared with the reference standard used (Potassium dichromate). The higher the LC_{50} value, the lower the toxicity. *J. curcas* is less toxic than *P. daemia*.

Table 5. Comparative DPPH Radical scavenging activity of the plant extracts

Sample	Concentration (µg/ml)	Log Concentration	Scavenging activity (%)	IC_{50} (µg/ml)
J. curcas	1000	3.000	85.37	90.83[a]
	500	2.699	75.30	
	250	2.398	64.35	
	125	2.097	52.25	
	62.5	1.796	46.26	
	31.25	1.495	34.62	
P. daemia	1000	3.000	68.43	214.16[b]
	500	2.699	62.52	
	250	2.398	48.61	
	125	2.097	41.82	
	62.5	1.796	36.86	
	31.25	1.495	28.27	
Quercetin	1000	3.000	92.77	50.71[c]
(Standard)	500	2.699	84.14	
	250	2.398	72.15	
	125	2.097	63.52	
	62.5	1.796	51.57	
	31.25	1.495	44.01	

(a) Linear equation: y = 33.505 X – 15.610; (b) Linear equation: y = 27.012 X – 12.958; (c) Linear equation: y = 33.236X – 6.671

Table 6. Cytotoxic effect of the plant extracts on *Artemia salina*

Sample	Concentration (µg/ml)	Log Concentration	Percentage Lethality	IC_{50} (µg/ml)
J. curcas	1000	3.000	60	586.79[a]
	500	2.699	50	
	250	2.398	30	
	125	2.097	20	
	62.5	1.796	10	
P. daemia	1000	3.000	70	344.26[b]
	500	2.699	60	
	250	2.398	40	
	125	2.097	30	
	62.5	1.796	20	
Potassium	1000	1000	3.000	50.71[c]
Dichromate	500	500	2.699	
(standard)	250	250	2.398	
	125	125	2.097	
	62.5	62.5	1.796	

(a) Linear equation: y = 43.189 X – 69.568; (b) Linear equation: y = 43.19 X – 59.568; (c) Linear equation: y = 39.867X – 21.601

The comparative study on the wound healing potentials of the two plants revealed that in all cases, there was a progressive decrease in wound area with time, indicating efficacy of the formulations in healing the induced wounds. In table 1, the leaf gels of *J. curcas* and *P.*

daemia exhibited significant (P<0.05) healing properties on the experimental animals when compared with the standard (Povidone iodine) and the control (Paraffin base). However, *P. daemia* – treated rats had a higher healing rate, higher percentage of wound contraction, a very minimal scar formation when compared to the healing properties exhibited by *J. curcas* treated rats (Figure 1-5).

Wound healing or repair is a natural process of regenerating dermal and epidermal tissue, and may be categorized into three phases, Viz, inflammation, proliferation and remodeling phase. In the inflammation phase, various growth factors such as tumor necrosis factor (TNF), interleukins (IL) are released to initiate the proliferation phase. The later is characterized by angiogenesis, collagen deposition, granular tissue formation, epithelization and wound contraction (Chang et al., 2004). In the last phase, the levels of collagen production and degradation equalize, after which disorganized fibres are rearranged thus increasing the tensile strength of the wound (Smith, 1985).

The capacity of wound to heal depends, in part, on its depth, as well as on the overall health and nutritional status of the individual. Following injury, inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated. It is well known that stages in healing, namely, coagulation, inflammation, microphasia, fibroblasts formation, and collagenation, are intimately interlinked (Nayak et al., 2005). The process of wound contraction and epithelization is separate and independent. The activity of fibroblast is responsible for wound contraction and involves movement of entire dermis. It is known that stabilization of lysosomal membranes, inhibition of cellular migration and inhibition of fibroblast contraction are responsible for their anti-healing effects (Nayak et al., 2007). Thus intervention in any one of these phases by drugs would eventually lead to either promotion or depression of collagenation, wound contraction and epithelization (Bigoniya and Rana, 2007).

The percentage protein on the wound surfaces of animals treated with *J. curcas* and *P. daemia* increased progressively as these was an enhanced synthesis of collagen which aided the healing process (Table 2) collagen, a major protein of the extra cellular matrix, which is synthesized by fibroblast cells, ultimately contributes to wound strength and wound healing. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomical structure and function. Although, if too much collagen is deposited in the wound site, normal anatomical structure is lost, function is compromised and fibrosis occurs. Conversely, if an insufficient amount of collagen is deposited, the wound is weak and healing is delayed. Rats in groups 3 and 4 had higher protein deposition on the wound surface compared to those in groups 1 and 2 which may have fostered the healing process. *Pergularia daemia* – treated rats, having the highest protein deposition, exhibited the best healing property.

The leaves of these plants are rich in tannins, alkaloids, phenol, flavonoids and glycosides. *J. curcas* had an appreciable amount of Saponins; however, Saponins were not detected in the leaf extract of *P. daemia* (Table 4). These phytochemicals have been reported to possess diverse medicinal properties like anti – inflammation, antioxidant, hypoglycemic, anti-tumor, hepatoprotective and antiviral effects as well as exhibiting physiological activities (Sofowora, 1993).

Flavonoids and phenolic compounds are contributory to antioxidant properties of medical plants. Tannins hasten the healing of injuries, especially from burns and they stop the bleeding of cuts. This is contributory to the observed wound healing in the experimental rats.

The antimicrobial effects of these plants are presented in Table 3. The microbial load on the wound surface decreased with days of treatment and wound contraction. The observed antimicrobial effects are due to the presence of some of the phytochemicals such alkaloids, tannins, and flavonoids identified in the leaves of both plants. Saponins demonstrate antimicrobial properties particularly against fungi, and some bacteria and protozoa (Vinoth et al., 2009) *Pergularia daemia* showed a more potent antimicrobial activity than *J. curcas* in this experiment.

The antioxidant activities of the two plants are presented in table 5. *J. curcas* possessed more radical scavenging potential than *P. daemia*. Both plants possessed concentration dependent radical scavenging activities. Flavonoid and phenolic compounds detected in extracts can explain their free radical scavenging activities.

Reactive oxygen species (ROS) play a vital role in wound healing and can trigger various beneficial oxygen free radicals. ROS also play an important role in the failure of ischemic wound healing while antioxidants improve healing in ischemic skin wounds (Senel et al., 1997). Elevated lipid peroxide levels have also been demonstrated in certain inflammatory skin lesions such as wound and dermatitis (Niwa et al., 1987). Therefore, if a compound has antioxidant potential, it can be a good therapeutic agent for enhancing the wound healing process (Udupa et al., 1995).

The cytotoxic potential of the two plants are presented in table 6. Both plants exhibited mild toxic effect on *Artemia salina*. Crude extracts having LC_{50} values less than 250 µg/ml is considered significantly active and having the potentials for further investigation (Rieser et al., 1996). However, *J. curcas* and *P. daemia* having LC_{50} values of 586.79 and 344.26 µg/ml, respectively are considered less cytotoxic and this could be correlated with their continual traditional utilization in the management of diverse diseases.

4. CONCLUSION

The aim of wound care is promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient. The present study showed that formulating *J. curcas* and *P. daemia* extracts into ointment is effective in wound repair and encourages the harnessing of the extracts in the formulation of commercial dermatological ointments. In comparism, the ointment prepared with *P. daemia* leaf extract had better wound healing characteristics compared to *J. curcas*, suggesting *P. daemia* is a better candidate for wound healing. This finding thus, strongly supports the verbal claim and justifies the use of these plants in folkloric medicine for wound healing. Further work is therefore necessary to isolate and identify the active principle responsible for the biological activities observed in this investigation.

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