

1 **ORIGINAL RESEARCH ARTICLE**

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2 **Anti-hemorrhoid Evaluation of Selected Medicinal plants used in**

3 **Bali North-East Nigeria for the Treatment of Hemorrhoids (Pile)**

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4 **ABSTRACT**

5 *A medicinal plant is any plant in which one or more of its organs contain substance that can*  
6 *be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. In*  
7 *this research, five (5) selected plants were screened for antihemorrhoid activities in mice.*  
8 *The extracts were obtained from whole plant or part of plants such as root, stem, leaves and*  
9 *seeds and include the following plants: Khaya senegalensis, Euphorbia hirta, Parkia*  
10 *biglobosa, Newbouldia leavis and Prosopis africana. Hemorrhoid (pile) was induced in*  
11 *group of five mice of five animals per group using Jatropa oil(Jatropa curcas,*  
12 *Euphorbiaceae)(I.P) and using Pilex granule as the control drug. Group I received 10 mg/kg*  
13 *Pilex granule, and 100, 200, 400, and 600 mg/kg b.w of A. leiocarpus, N. leavis, P. africana,*  
14 *and KEP for groups II, III, IV and V respectively. A. leiocarpus and KEP(mixture of K.*  
15 *senegalensis, E. hirta and P. africana) showed the highest antihemorrhoidal activities in*  
16 *mice than the other plants and compared with the standard drug Pilex granule. However, all*  
17 *the plant extracts showed significant rectoanal coefficient at potent levels. The study showed*  
18 *that the extracts of the plants investigated possessed antihemorrhoid activities with*  
19 *A.leiocarpus and KEP demonstrating the highest activity in mice.*

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20 **Keywords:** Anti-hemorrhoid, medicinal plants, mice, pilex granule, Jatropa oil.

21 **Introduction**

22 Hemorrhoid represents the dilation of varicose of the vessel of the superior inferior rectal  
23 plexuses of veins. It has ve been a common and painful human disease for decades. The exact  
24 prevalence in most developing countries has not been determined in spite of the growing  
25 problems associated with this ailment especially among the local populace in North-east  
26 Nigeria States, which are majorly due to dietary habits. This disease is frequently  
27 encountered in developing countries [1], Various dilations of the internal hemorrhoids; as  
28 well as physiological dilation which exist at infancy, is presumed to have develop into

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29 varicosities under the influence of wide range of factors. The predisposing factors include  
30 hereditary, age, anal sex, and prolong labor at pregnancy. The precipitating factors comprises  
31 cathartic abuse, diarrhoea, enemata, constipation, infection and spasms or a stony dietary  
32 intake which puts extra pressure on the intestinal mucosa linings which may subsequently  
33 result in rupture of the mucosa linings of the small intestine [2].

34 There are two types of piles, internal piles and external piles. Internal piles expand inside,  
35 along the anal. The common symptoms of internal piles are the painless blood loss. The  
36 internal piles are totally prolapsed. External piles extend close to the anus. The colour of  
37 external piles is same as the skin. The outside piles forms thrombus. The outside piles are  
38 painful. When the external pile ruptures it bleeds. The blood loss is more disturbing, and it is  
39 typical concern for considering a therapeutic measure by a medical practitioner. Prolapsed on  
40 the other hand, is an oral dysfunction effect of piles [3].

41 This research was aimed at evaluating some selected medicinal plants used in North-east  
42 Nigeria for the treatment of hemorrhoids with a view to ascertain this claim in traditional  
43 medicine, thereby identifying which of the plant has the best anti-hemorrhoids property in  
44 mice models.

## 45 MATERIALS AND METHODS

### 46 Plant Collection and Identification

47 The plants species (*Parkia biglobosa*, *Prosopis africana*, *Euphorbia hirta*, *Khayasenegalensis*,  
48 *Newbouldia leavis* and *Anogeissus leiocarpus*) were collected from Bali and Takum forests in  
49 2014, and were identified by Mr. Cletus A. Ukwubile of Science Laboratory Technology  
50 Department, Federal Polytechnic Bali, where vouchernumbers were deposited for the plants.

51 | Attempt has been made to expel scyballous masses from the rectum by traditional  
52 | medical practitioners using different plant species using any of or the following preparations:  
53 | concoction, decoction and maceration.

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#### 54 | **Preparation and Extraction of Plant Materials**

55 | The plants were air-dried for two weeks, ~~and then, plant parts were~~ grounded into powder and  
56 | weighed and then stored for onward use. One of the plants (*Newbouldia leavis*) was extracted  
57 | with water following the method of its preparation in traditional medicine while others were  
58 | extracted with absolute ethanol 99.1% (v/v), and were soaked for 24 h using cold maceration  
59 | technique. The extracts were concentrated to dryness under reduced pressure in rotary  
60 | evaporator and dried in a desiccators. Percentage yields for the extracts were as follows:  
61 | *Parkia biglobosa* (5.1%), *Prosopis africana* (5.6%), *Euphorbia hirta* (4.2%), *Khaya*  
62 | *senegalensis* (6.2%), *Newbouldia leavis*(8.2%) and *Anogeissus leiocarpus*(4.5%).

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#### 63 | **Grouping of Swiss albino rats**

64 | The animals were grouped into five (5) groups of 5 according to each plant extracts.

#### 65 | **Experimental Animals**

66 | Inbred male and female Swiss albino mice (18-29 g in weight) that were housed in standard  
67 | conditions of temperature ( $22 \pm 3^{\circ}\text{C}$ ), relative humidity ( $55 \pm 5\%$ ), and light (12h light-dark  
68 | cycle) before and during the study were ~~included-used~~ in this experiment. They were fed with  
69 | standard pellet diet (obtained from animal house of Department of Pharmacology and  
70 | Clinical Therapeutics, Ahmadu Bello University Zaria) and water *ad libitum*. All the  
71 | experimental protocols were approved by the Institutional Animal Ethic Committee (IAEC)  
72 | of the Ahmadu Bello University Zaria and Health Research Extension Act of 1985(Public  
73 | Law November 20, page 99-158) USA. The animals received humane care as per the

74 | guidelines prescribed by Committee for the Purpose of Control and Supervision of  
75 | Experiments on Animals (CPCSEA), Federal Ministry of Environment and Forestry, Nigeria.

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## 76 | **Experimental Protocols**

77 | Two sets of experiments were carried out. The first set was used to improve an existing  
78 | experimental model of hemorrhoids mentioned by [3], and to validate the same by using pilex  
79 | granules (PG), *Newbouldia leavis* extract (NE) which was the plant used widely by traditional  
80 | healers in North-east Nigeria to treat piles, and a combination of both extracts. The protocol  
81 | was designed to quantify the extent of plasma exudation and to determine the levels of  
82 | inflammatory cytokines such as TNF- $\alpha$  and IL-6, which are associated with hemorrhoids [3].

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83 | In the second set, the effect of PG, AL, and a combination of some plant extracts were  
84 | further evaluated by determining the rectoanal coefficient (RAC), severity score, and the  
85 | histopathological evaluation [4].

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## 86 | **Evaluation of Anti-hemorrhoid property against *Jatropha* oil-induced hemorrhoid in** 87 | **mice**

88 | Mice of both sexes (20-29g) were randomized based on their body weights and were divided  
89 | into 5 groups (G-1 to G-5), with each group consisting of 5 animals (n=5). G-1 animals  
90 | received PG (Pilex granule) (10 mg/kg) and served as positive control; G-2 animals received  
91 | AL (100 mg/kg), while G-3, G-4, and G-5 animals received NL, PA and KEP (200, 400, and  
92 | 600 mg/kg b.w; i.p, respectively). Hemorrhoids were induced in all the groups, except normal  
93 | control group, by applying *Jatropha* oil preparation in five days. A day after induction, all the  
94 | animals were subjected to respective treatment as assigned to the group once daily for five  
95 | days. On the fifth day, 1 h after the treatment, all the animals were euthanized by  
96 | exsanguination under deep isoflurane anaesthesia and rectoanal tissues (20mm in length) were

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97 isolated. They were evaluated for their severity score, weighed, and fixed in 10% formalin  
98 solution for histological examination.

99 The RAC was calculated using the formula:

100

$$101 \text{ Rectoanal coefficient} = \frac{\text{Weight of rectoanal tissue (mg)}}{\text{Body weight (g)}}$$

102

103 Histological observation of the rectoanal tissue was carried out in order to determine the  
104 appearance of inflammatory cells, congestion, haemorrhage, vasodilatation, and medium to  
105 high degrees of necrosis [5].

106

## 107 RESULTS AND DISCUSSION

108 The study showed the oil from *Jatropha* seeds was toxic at 1000 mg/kg body weight in the  
109 mice (table 1).

110 **Table 1: LD<sub>50</sub> Determination of *Jatropha* oil from *Jatropha curcas***

Dosage (mg/kg)	Animal died / Animal survived
10	0/5
100	0/5
1000*	1/4
1600	ND

111 LD<sub>50</sub> = 1118 mg/kg b.w, ND (not determine), \* toxic dose

112 Hemorrhoid induction was on the increase as the dosage was increase, leading to weight loss  
113 in mice (table 2) [6]. The fact that oil from the seeds of *Jatropha* caused acute inflammation in  
114 the rectum of the mice as well as mortality witnessed at the dose 1000 mg/kg b.w suggest  
115 that at higher dose, the extract is toxic, making it a potential carcinogen in cancer research  
116 [7]. In this case, loss of weight in the animals after induction is a symptom of hemorrhoids due

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117 to loss of blood and severe inflammation in the rectoanal region of the mice, coupled with  
118 anal itching as seen in the animal groups.

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119 **Table 2: Effect of Jatropha oil on the body weights of mice before and after induction**

Test groups	Weight Before (g)	Weight After (g)
Group I Control 20 mg/kg	29.6	25.2
Group II 50 mg/kg	28.6	24.1
Group III 100 mg/kg	25.2	20.1
Group IV 150 mg/kg	28.0	22.2
Group V 200 mg/kg	26.6	20.2

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121 Previous research had proved that hemorrhoids are pathological condition, which is  
122 characterized by severe vasodilation at the rectoanal region, which leads to inflammation of  
123 the surrounding tissues, thus further leading to secondary complications such as  
124 extravasations of fluid into interstitial space mainly due to increased vascular permeability  
125 and migration of large quantity of inflammatory white blood cells (granulocytes and  
126 monocytes)[8].

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127 In the present study, Jatropha oil from seeds of *Jatropha curcas* (*Euphorbiaceae*) has been  
128 used as an inducer or proinflammatory agent to induce experimental hemorrhoids. In this study, it is  
129 possible that Jatropha oil causes inflammation due to the release of soluble factors involving  
130 inflammatory lipid metabolites. These factors, alone or in combination with other factors,  
131 regulate the activation of resident cells (Fibroblasts, endothelial cells, macrophages, and mast  
132 cells) and newly recruited inflammatory cells (Monocytes, lymphocytes, neutrophils, and

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133 eosinophils) leading to systemic response to inflammation [8-9], This mechanism explained  
134 here was not different from that of the present study (Figure 1d).

135 The normal control group showed normal cell architecture of the rectoanal region [10-  
136 11](Figure 1a). However, intraperitoneal administration of plant extracts of AL, NL, PA, and  
137 KEP showed remarkable vasoconstriction of the rectum (table 3). The greatest healing of the  
138 rectum were shown by AL (*Anogeissus leiocarpus*) and KEP (*K. senegalensis*, *Euphorbia*  
139 *hirta*, *Parkia biglobosa*) mixed in 40:40:40 mg/kg ratios, which was evident in the reduction of  
140 tissue weights after treatment, and these also confirmed the healing of the mucosa linings of  
141 the small intestine of mice by the plant extracts (Figure 1 a-c). Plant extracts has been used  
142 for healing wounds, correct disorders internally, and promotes immune responses [11]. The  
143 extracts from these must have exerted the same effects on the experimental animals in dose-  
144 dependent fashion. These results were comparable with that of the standard control drug  
145 (Pilex) at  $p \leq 0.05$  (one-way ANOVA). All the extracts produced better rectoanal coefficient  
146 values than the first line drug Pilex (table 3).

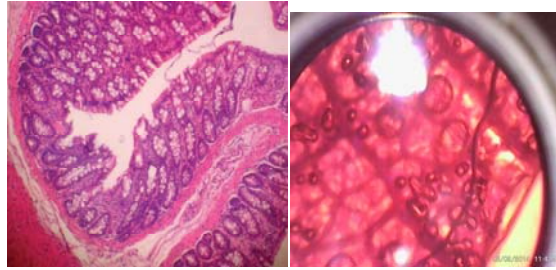
147 **Table 3: Effect of extracts on rectum after drug administration (i.p)**

Extract dose (mg/kg b.w) ( n=5)	Rectoanal coefficients (g)	Inference
Group I Control Pilex (10 mg)	$0.6 \pm 0.22^*$	Moderate healing
Group II AL 100	$0.1 \pm 0.20^{TM}$	*Strong healing
Group III NL 200	$0.4 \pm 0.18$	Moderate healing
Group IV PA 400	$0.3 \pm 0.15$	Moderate healing
Group V KEP 600	$0.2 \pm 0.10^{TM}$	*Strong healing

148 AL (*Anogeissus leiocarpus*), NL (*Newbouldia leavis*), PA (*Prosopis africana*), KEP  
149 (*Khayasenegalensis*, *Euphorbia hirta*, *Parkia biglobosa*), Results are means  $\pm$  SEM. The

150 lower the values, the more efficacy the drug, <sup>TM</sup> More efficacy, \* Statistical significant at  $p \leq$   
151 **0.05 (One-way ANOVA).**

152



153

154 **a.b.**



155

156 **c.d.**

157 **Figure 1:** Medicinal plants effects on rectoanal tissue in mice in *Jatropha* oil-induced hemorrhoids; a;  
158 hemorrhoid induced in recto anal tissue **40x**, b-c **40x**; healing of tissue after drug administration  
159 with *Anogeissus leiocarpus*, d; mouse developed pile after five days of induction with *Jatropha* oil,

160 **indicates higher degrees of necrosis.** **medium necrosis.**

## 161 CONCLUSION

162 Medicinal plants are a source of many biological ingredients which cannot be ignored. The  
163 study therefore showed that extracts of *Anogeissus leiocarpus*, *Khaya senegalensis*,  
164 *Euphorbia hirta*, *Parkia biglobosa* and *Newbouldia leavis* possess antihemorrhoid properties



165 in mice. But from the study, extracts of *Anogeissus leiocarpus* and *Khayasenegalensis*,  
166 *Euphorbia hirta*, *Parkia biglobosa*) presented the strongest antihemorrhoid activity in mice  
167 than the rest of the plants. These plants can be used as medication for the treatment of  
168 hemorrhoids (pile) in traditional medicine, and justified their acclaimed use for treating of  
169 piles in North-east Nigeria.

170 The plants thus, represent sure sources towards the development of conventional medicine for  
171 the treatment of piles other than surgery, which normally is very expensive with high risk.  
172 However, the precise molecular mechanism behind the antihemorrhoidal activities of these  
173 plant extracts and the compounds responsible for the observed activity, need to be explored in  
174 future studies.

#### 175 ACKNOWLEDGMENT

176 The authors are grateful to Mr. Ibrahim of the Department of Pharmacology and Clinical  
177 Therapeutics, Ahmadu Bello University Zaria, for his help in this work, and also the  
178 traditional medical practitioners in Bali, Taraba State and beyond, who helped us during the  
179 ethnomedicinal survey of these plants especially Mama Daniya Bali (Age 102 years).

#### 180 CONFLICT OF INTERESTS

181 We declare no competing interests.

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