

2 **Free Radical Scavenging Activity of *Jatropha curcas* Leaves,**

3 **Phytochemical and Antibacterial Analysis of its Butanol crude extract.**

4 **ABSTRACT**

5 The use of different plant parts for the prevention and cure of ailments, infections and  
6 diseases as an alternative to the use of orthodox medicine is gaining more attention.  
7 Moreso, resistance to orthodox drugs has been confirmed in various literature. The free  
8 radical scavenging activities of *Jatropha curcas* (*Euphorbiaceae*) leaves, phytochemicals  
9 present in its butanol crude extract as well as the antibacterial activities of its butanol crude  
10 extract were accessed. The leaves of *Jatropha curcas* were washed, air-dried and pulverized  
11 for active extraction of the composition of the plant in butanol. Fresh leaves of the plant  
12 were tested for its DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity (indicator of  
13 antioxidant), while the butanol crude leaf extract was accessed for the presence or absence  
14 of phytochemicals and was also tested against some gram positive and gram negative  
15 bacteria all according to standard procedures. All analysis was carried out at the Chemistry  
16 Laboratory of the Federal College of Animal Health and Production Technology, Moor  
17 Plantation, Ibadan for four weeks. The result of the free radical scavenging activity of the  
18 leaves (74.73 mg/kg) was higher than that of ascorbic acid (31.01 mg/kg) (a standard  
19 antioxidant). The qualitative analysis showed the presence of alkaloids, tannins, saponin,  
20 flavonoids, steroid, phenols phlobatanins and cardiac glycoside. The quantitative analysis

21 on the other hand showed the presence of alkaloids in an appreciable amount (0.567%),  
22 followed by saponin (0.316%) while others had relatively low values (below 0.19%). The  
23 average values of the antibacterial (inhibitory) activity as compared with standard  
24 antibiotics used had no significant difference except for *pseudomonas sp.* which was very  
25 low to the antibiotics used. It could be concluded that *Jatropha curcas* is of great medicinal  
26 potential.

27 Keywords; *Jatropha curcas*, Antibacterial, Scavenging activity, Phytochemicals.

## 28 1. INTRODUCTION

29 Ethnomedicine refers to the study of traditional medicinal practice which is concerned  
30 with the cultural interpretation of health diseases and illness and also addresses the  
31 health care seeking process and healing practices [1].

32 Ethnomedicinal plants have been used since ancient time for human healthcare and still  
33 remain the most widely used medication system in developing and least developed  
34 nations [2].

35 The reliance of people on ethnomedicine has been for reasons of cost-effectiveness,  
36 acceptability, biomedical benefits and accessibility. However, there has been a  
37 continuous growth of demand for herbal medicine globally [3].

38 Synthetic drugs have become expensive and not easily accessible by the less privileged.  
39 Moreover, resistance to most synthetic drugs is a serious health concern in the world  
40 today. In addition, people have returned to traditional medicine and natural plants are  
41 now used as drugs for various ailments based on their folkloric uses. Plant derived-

42 bioactive compounds have received considerable attention due to their therapeutic  
43 potential as antimicrobial, anti-inflammatory properties and antioxidant activities [4].

44 *Jatropha curcas* (*J. curcas*) is a specie of flowering plant in the spurge family –  
45 Euphorbiaceae [5].

46 *Jatropha curcas* leaves have been used as cure for various ailments like; skin infection,  
47 diarrhea and cancer e.t.c. [6, 7, 8]. It has also been explored for alopecia, anasorea,  
48 ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhea, eczema, fever,  
49 rashes, sores, ache, and rheumatism [9].

50 Therefore, this research is targeted at evaluating the free radical scavenging activities of  
51 *Jatropha curcas* leaves, phytochemicals present in its butanol crude extract as well as the  
52 antibacterial activities of its butanol crude extract in order to ascertain some of its  
53 claims in traditional healing.

#### 54 **1.1 Free radicals and DPPH scavenging activities**

55 Free radicals are fundamental to any biochemical process and represent an  
56 essential part of aerobic life and metabolism [10]. Reactive oxygen (ROS) and  
57 reactive nitrogen species (RNS) are products of normal cellular metabolism. The  
58 most common ROS include superoxide anion Peroxy nitrite anion [11]. These reactive  
59 species play an important role in pathogenesis, cardiovascular diseases, and  
60 rheumatism. It is possible to reduce the risk of chronic diseases and prevent  
61 diseases progression by either enhancing the body's natural antioxidant defenses or  
62 by supplementing with dietary antioxidants [12]. Antioxidant offer resistance

63 against oxidative stress by scavenging the free radicals inhibiting lipid  
64 peroxidation and by many other mechanisms and thus prevent disease progression.

65 DPPH is a stable nitrogen centered free radical commonly used for testing  
66 radical scavenging activity of the compound or plant extracts. When stable DPPH  
67 radical accept an electron from the antioxidant compound the violet color of the  
68 DPPH reduce to yellow color or red. Diphenylpicrylhydrazyl radical which was  
69 measured colorimetrically. Substance which are able to perform this reaction can be  
70 considered as antioxidant and therefore a radical scavenger [13].

71

## 72 **1.2 Antibacterial**

73 Antibacterial also known as antibiotics is a type of antimicrobial drug used in  
74 the treatment and prevention of bacterial infection. They may kill or inhibit the  
75 growth of **bacteria**. They are not effective against viruses such as common cold  
76 or influenza. Antibiotics may be given as a preventive measure (prophylactic) and  
77 this is usually limited to at risk populations such as those with a weakened  
78 immune system (particularly in HIV cases to prevent pneumonia) [14].

## 79 **2.0 MATERIALS AND METHODS**

### 80 **2.1 Experimental material**

81 Some of the experimental apparatus used for this work included; Petri dishes, 25 ml  
82 conical flasks, boiling tubes, Bunsen burner, 5 ml, 10 ml and 20 ml measuring  
83 cylinders. Experimental material included; *J. curcas* leaves, while the reagents utilized

84 included; Butanol, Ethanol, Ethyl acetate, Chloroform used were all BDH general  
85 purpose chemicals and distilled prior to use. 2,2-diphenyl- 1-picrylhydrazyl (DPPH),  
86 Ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO). HCl, Conc.  
87 H<sub>2</sub>SO<sub>4</sub>, Potassium iodide, Mayer's reagent, Dilute ammonia, Potassium ferrocyanide,  
88 Acetic acid, Olive oil, Ferric chloride solution MacConkey agar, Nutrient agar were all  
89 BDH general purpose chemicals. Some of the Instruments used in this research  
90 included; Mettler analytical balance H80 (UK), Water Bath (Gallenkamp), UV-Visible  
91 spectrophotometer (Unico1200 & Perkin Elmer lambda 25 models) .

## 92 2.2 Experimental procedure

93 **Sample collection:** Fresh plant sample (leaves) was collected around the quarters of  
94 the Federal college of Animal Health and Production Technology, Moor Plantation,  
95 Ibadan (around September, 2016). They were then taken to the Botany unit of the Institute  
96 of Agricultural Research and Training, Moor Plantation, Ibadan for proper identification.  
97 1kg of fresh leaves were harvested, the fresh leaves were used for the DPPH scavenging  
98 activities while the rest were air-dried to preserve some phytochemical constituent which  
99 are thermolabile and could be denatured by heat and also to prevent physiological  
100 change of the leaves.

101 The air-dried samples were pulverized, using an electric blender **in order to** reduce  
102 them to fine particles for effective extraction of the bioactive compounds.

103 Exactly, 100g of the **dried pulverized** leaves were weighed into a 1000 ml beaker and  
104 **excess** butanol was **added** until the samples were fully immersed, the extraction was  
105 carried out by decanting the butanol (supernatant) every 24 hours **and were filtered**

106 through a muslin. This was replaced with fresh volumes of butanol and was repeated  
107 for 10days.

108 The samples were concentrated using rotary evaporator until all the butanol had  
109 evaporated and the extract was obtained.

110

### 111 2.3. DPPH free radical scavenging Activity

112 The DPPH scavenging activity of the leaves was carried out according to the methods as  
113 described by [15, 16]

114 100g of fresh sample was weighed into a beaker, 100ml of ethanol was added and  
115 shaken vigorously for 2min, it was stirred with a magnetic stirrer for 15min, it was  
116 allowed to stand for 2hours for proper extraction, then centrifuged at 2500rpm for  
117 10min. The supernatant was poured into another beaker and concentrated by  
118 evaporating in a water bath at 80<sup>0</sup>C. The concentrated extract was kept for further assay.

119 1mM DPPH was prepared in ethanol (394.32mg DPPH dissolved in 1litre of  
120 ethanol. It was dissolved with 10mg of the concentrated sample extract in 10ml of  
121 ethanol (1mg/ml) 1.5ml of the extract was pipette into a test tube. 1.5ml DPPH  
122 solution prepared was added into the test tube. The spectrophotometer was zeroed with  
123 ethanol as blank. Thereafter the absorbance\optical density of the control (DPPH  
124 solution) and that of the sample was read at 517nm.

125 DPPH Scavanged % =  $\frac{\text{absorbance of control} - \text{absorbance of test sample} \times 100}{\text{absorbance of control} + \text{sample weight}}$  ....(Equation 1)

126 This procedure was also carried out for Vitamin C (ascorbic acid) a standard antioxidant.

## 127 **2.4 Qualitative phytochemical Analysis**

128 The crude extract was subjected to qualitative and quantitative phytochemical tests for  
129 tannins, alkaloids, saponins, flavonoids, steroids and cardiac glycosides. Qualitative test  
130 was carried out on the extract in the Federal College of Animal Health and Production  
131 Technology using standard procedures as described by [17, 18, 19, 20].

### 132 **2.4.1 Test for Alkaloids:**

133 0.5g of the plant sample was dissolved in 5ml dilute HCl in a steam bath and filtered.  
134 1ml of the above filtrate was treated with few drops of Mayer's reagent. A creamy  
135 white precipitate was observed which indicated the presence of alkaloids.

### 136 **2.4.2 Test for Tannin**

137 Exactly 0.5g of each plant extract was stirred with about 20 ml of distilled water  
138 and then filtered. Few drops of 0.1% ferric chloride solution were added to the  
139 filtrate. A dirty green precipitate was observed, indicative of the presence of tannin.

### 140 **2.4.3 Test for saponin**

141 2 gram of each portion was boiled with 20ml of distilled water filtered. To the  
142 filtrate about 3ml of distilled water was further added and shake vigorously for  
143 about 5min frothing which persisted on warming was observed. This was a positive  
144 test for saponin.

### 145 **2.4.4 Test for flavonoids**

146 A portion of the powdered plant sample was heated with 10ml ethyl acetate over a  
147 steam bath for 3min. it was filtered and 4ml of the filtrate was shaken with 1ml of  
148 dilute ammonia .A yellow coloration indicating the presence of flavonoids was  
149 observed

#### 150 **2.4.5 Test for steroids**

151 About 0.2g of the plant extract, 2ml of acetic acid was added and the solution  
152 was cooled well, Conc. H<sub>2</sub>SO<sub>4</sub> was added to the solution. A violet to blue color  
153 indicates the presence of a steroidal ring.

154

### 155 **2.5 Quantitative phytochemical analysis**

#### 156 **2.5.1 Test for Alkaloids**

157 2g of the extracted sample was weighed into a 100ml beaker and 20ml of 80%  
158 absolute alcohol added to give a smooth paste. The mixture was transferred to a  
159 250ml flask and more alcohol added to make up to 100ml and 1g magnesium  
160 oxide added. The mixture was digested in boiling water for 1.5 hours under a  
161 reflux air condenser with occasional shaking. The mixture was filtered while hot  
162 through a small bucher funnel. The residue was returned to the flask and  
163 redistilled for 30minutes with 50ml hot water to replace the alcohol lost. When all  
164 the alcohol had been removed, 3 drops of 10% HCl was added. The whole solution  
165 was later transferred into a 250ml volumetric flask 5ml of zinc acetate solution



166 and 5ml of Potassium Ferro cyanide solution was added thoroughly mixed to give  
167 homogenous solution.

168 The flask was allowed to stand for a few minutes filtered through a dry filter  
169 paper and 10ml of the filtrate was transferred into a separatory funnel and  
170 alkaloids present were extracted vigorously by shaking with five succession  
171 portions of chloroform. The residue obtained was dissolved in 10ml hot distilled  
172 water and transferred into a kjeldalh tube with the addition of 0.20g sucrose and  
173 10ml Conc. H<sub>2</sub>SO<sub>4</sub> and 0.02g selenium for digestion to colorless solution to  
174 determine %N by kjeldahl distillation method. % N got was converted to % total  
175 alkaloid by multiplying with a factor 3.26 i.e

176 
$$\% \text{ total alkaloid} = \%N + 3.26 \dots\dots\dots(\text{Equation 2})$$

177

### 178 **2.5.2 Test for flavonoids**

179 Exactly 0.50g of the extracted sample was weighed into a 100ml beaker and 80ml  
180 of 95% ethanol added and stirred with a glass rod to prevent lumping. The  
181 mixture was filtered through a whatman NO 1 filter paper into a 100ml volumetric  
182 flask and made up to mark with ethanol. 1ml of the extract was pipette into  
183 500ml volumetric flask , four drops of conc. HCl added via a dropping pipette  
184 after which 0.5g of magnesium turning was added to develop a magenta red  
185 coloration . Standard solution were read on digital labomed 200 spectrophotometer

186 at a wavelength of 520nm . the percentage flavonoids was calculated using the  
187 formul

$$188 \quad \% \text{ flavonoid} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{weight of sample} \times 10000} \dots(\text{Equation 3})$$

189

190

### 191 **2.5.3 Test for tannin**

192 Exactly 0.20g extracted sample was measured into a 500ml of beaker , 20ml of  
193 50% methanol was added and covered with paraffin and placed in water bath at  
194 77-80°C for 1 hour . It was shaken thoroughly to ensure uniform mixing. The  
195 extract was quantitatively filtered using a double layered whatman NO. 41 filter  
196 paper into a 100ml volumetric flask, 20ml water added, 2.5ml folin-denis reagent  
197 and 10ml of 17% Na<sub>2</sub>CO<sub>3</sub> were added and mixed properly . the mixture was made  
198 up to mark with water mixed well and and allow to stand for 20mins, the bluish-  
199 green color which developed at the end of range 0-10ppm were treated similarly  
200 as 1ml sample above .

201 The absorbance of the tannic acid standard solutions as well as samples were read  
202 after color development on a spectrophotometer at a wavelength of 70mm.  
203 %tannin was calculated using the formula:

$$204 \quad \% \text{ tannin} = \frac{\text{absorbance of sample} + \text{average gradient factor} + \text{dilution factor}}{\text{Weight sample} \times 10000} \dots(\text{Equation 4})$$

205 **2.5.4 Test for saponin**

206 1g of sample was weighed into a 250ml beaker and 10ml of butyl alcohol was  
207 added. The mixture was shaken on a UDY shaker for 5hrs to ensure uniform  
208 mixing. Thereafter the mixture was filtered through a whatman NO.1 filter paper  
209 into a 100ml beaker and 20ml of 40% saturated solution of magnesium  
210 carbonate was added. The mixture obtained with saturated  $MgCO_3$  was again  
211 filtered through a whatman NO. 1 filter paper to obtain a clear colorless solution  
212 was pipette into a 500ml volumetric flask and 2ml of 5%  $FeCl_3$  solution was  
213 added and made up to mark with distilled water . It was allowed to stand for 30  
214 min for blood red color to development in a Jenway V6300 spectrophotometer at  
215 a wavelength of 380nm.

$$\% \text{ saponin} = \frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000} \dots\dots\dots(\text{Equation 5})$$

217 **2.5.5. Test for steroid**

218 0.5g of sample extract was weighed into a 100ml beaker 20ml of chloroform-  
219 methanol (2:1) mixture was added to dissolve the extract upon shaking for 30mins  
220 on a shaker . The whole mixture was filtered through a whatman NO. 1 filter paper  
221 into another dry clean 100ml conical flask /beaker.

222 The resultant residue was repeatedly treated with chloroform- methanol mixture  
223 until it was free of steroids. 1ml of the filtrate was pipette into a 300ml test tube  
224 and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a  
225 homogenous mixture. The mixture was later placed in a water bath set at 37°C-

226 40°C for 90mins. It was cooled to room temperature and 10ml of petroluem ether  
227 was added followed by the addition of 5ml of chard reagent to residue in dry  
228 bottle and absorbance taken at a wavelength of 620nm on spectronic 20D  
229 spectrophotometer. Stand steroids of concentration of 0.4mg/l were prepared from  
230 100mg/ml stock steroid was calculated using equation 6:

231 
$$\frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000} \dots\dots(\text{Equation 6})$$

232

### 233 2.5.6 Test for glycosides

234 10ml of extract was pipetted into a 250ml conical flask. 50ml chloroform was added  
235 and shaken on a vortex mixer for 1hour,the mixture was filtered into 100ml conical  
236 flask and pyridine, 2ml of 2% sodium nitroprusside were added and shaken thoroughly  
237 for 10 minutes. 3ml of 20% NaOH was later added to give a brownish yellow color. The  
238 absorbance of the sample and the standards were read on a spectronic 20D digital  
239 spectrophotometer at a wavelength of 510nm. Percentage glycoside was calculated using  
240 equation 7:

$$\frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000} \dots\dots(\text{Equation 7})$$

### 242 2.6 Antibacterial susceptibility test

243 Agar well diffusion method was used in the assessment of the antibacterial activity of the  
244 extract as described by [16]. Tested bacteria were Gram positive (*Staphylococcus sp*,  
245 *Bacillus cereus* and *Clostridium sp.*). Gram negative (*Escherichia coli*, *Proteus sp* and  
246 *Pseudomonas sp.*). Media used were Nutrient agar and MacConkey agar

### 247 **2.6.1 Preparation of culture media**

248 MacConkey agar of 24.00g and 14.00g of nutrient agar were weighed into two  
249 different conical flasks (500 ml each), covered with aluminum foil and shaken  
250 thoroughly and then left to disperse. They were then sterilized at 121°C for 15mins in  
251 autoclave. They were then poured into petri dishes and left to solidify. After which  
252 it was incubated for 24hrs at 37°C.

253

### 254 **2.6.2 Isolation of organism**

255 The inoculating loops were sterilized and were used to pick the samples. They were  
256 then used to streak the surface of each labeled petri-dishes containing the prepared  
257 media. The streaked media were then kept for the bacterial susceptibility test.

### 258 **2.6.3 Bacterial susceptibility test**

259 24hrs old standardized culture of bacteria was subcultured into distilled water and  
260 1ml of the broth was used to flood the surface of the media prepared and  
261 allowed to dry. A sterile cork borer of 1cm was used to make holes and 1ml of  
262 the extract was dropped into each hole of the labeled dishes. Antibiotics were also  
263 placed on the surface of the media containing the cultured bacterial which were then  
264 incubated at 37°C for 24hrs, the minimum zone of inhibition of the extract and  
265 antibiotics were compared and records were taken.

266

### 267 3 RESULTS AND DISCUSSIONS

268

269 TABLE 1: DPPH free radical scavenging activity of fresh *Jatropha curcas* leaves

270

271 Parameter	Fresh leaves(mg/kg)	Ascorbic acid (mg/kg)
272 Antioxidant	74.73	31.01

273

274

275

276 Table 1 reveals the DPPH scavenging activity (indicator of the antioxidant) in fresh  
277 *Jatropha curcas* leaves in which the value was relatively high compare to the  
278 antioxidant ability of ascorbic acid a standard antioxidant which proves that the leaves  
279 have the ability of scavenging free radicals and the oxidation process in the body. These  
280 free radicals are responsible for some diseases and therefore *Jatropha curcas* has the  
281 ability to cure disease since it has the ability to inhibit the oxidation processes in  
282 human body that are responsible for these diseases.

283 TABLE 2: Qualitative and Quantitative analysis of butanol crude extract of *Jatropha*  
284 *curcas* leaves.

285 Parameters	Qualitative	Quantitative (%)	Inference
286 Alkaloids	+	0.5670	present
287 Phlobatannins	+	0.0005	present
288 Tannins	+	0.0018	present
289 Saponin	+	0.3160	present
290 Flavonoids	+	0.0024	present
291 Steroid	+	0.0037	present
292 Cardiac glycosides	+	0.1880	present
293 Phenol	+	0.0760	present

294

295 The qualitative and quantitative phytochemical analysis of the butanol extract of the leaves  
 296 of *J.curcas* (Table 2), showed the presence of most phytochemicals like alkaloid, tannin,  
 297 saponin, flavonoid, glycoside and phenol. This corroborates the findings of [22].

298 The medicinal value of this plant lies in its phytochemical constituents since each  
 299 phytochemical is said to have a definite physiological and pharmacological action on the  
 300 human body. Different classes of phytochemicals have been found to possess wide range of  
 301 activities which helps in prevention and protection against diseases. The presence of  
 302 alkaloid in the butanol extract could make the plant active against malaria, asthma and  
 303 support its use as an analgesic.

304 Phytochemicals such as flavonoids, phenol are effective as antioxidant while saponin and  
 305 glycosides are effective as anti-inflammatory and antibiotics respectively [12].

306

307 TABLE 3: Average value of the antibacterial activity of butanol extract of *Jatropha curcas*  
 308 leaves.

309 Isolate	310 Zone of inhibition (mm)	
	311 Antibiotics	312 Butanol extract
313 <b>Gram +ve</b>		
314 <i>Staphylococcus sp.</i>	32(gen), 29(ofl)	30
315 <i>Bacillus cereus</i>	22(gen), 20(ofl)	20
316 <i>Clostridium sp.</i>	15 (gen), 20(ofl)	15
317 <b>Gram –ve</b>		
318 <i>Escherichia coli</i>	28(gen), 25(ofl)	41
319 <i>Proteus sp.</i>	20(ch), 20(ch)	17
320 <i>Pseudomonas sp</i>	40(cpx), 35(pef,ofl)	22

321 **KEY**

- 322 Cpx-ciprofloxacin
- 323 Pef-pefloxacin
- 324 Ofi-ofloxacin
- 325 Ch-chloraphenicol
- Gen –gentamycin

326

327 Antibacterial activity of the extract (Table 3) revealed that the minimum zones of inhibition  
328 (mm) values were not significantly different when compared with the antibiotics used  
329 which shows that the leaves could be used as antibacterial agents with the exception  
330 of *pseudomonas sp.* in which the its inhibitory zone value was relatively low to that  
331 of the antibiotics used .

332 [23] reported that presence of biologically active principle (as confirmed in table 2) may  
333 aid the antimicrobial activities of *J. curcas* as these secondary metabolite exerts  
334 antimicrobial activity through different mechanisms.

335 This indicates that the plant extract is effective against most of the bacteria used in this  
336 study. Therefore, the plant extracts have medicinal properties which support its use in the  
337 treatment of various forms of infections.

#### 338 4. CONCLUSION

339 It can therefore be concluded from these research that *Jatropha curcas* leaves has  
340 bioactive compounds (phytochemicals) as well as a high free radical scavenging activity  
341 (indicator of antioxidant activity). This is also evident in its ability to inhibit the growth of  
342 some bacteria. Therefore, *Jatropha curcas* has great medicinal potential and this could  
343 justify the use of its leaves in folklore therapy.

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346



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