

Original Research Article**Free Radical Scavenging Activity of *Jatropha curcas* Leaves,****Phytochemical And Antibacterial Analysis of its Butanol Fraction.****ABSTRACT**

The use of different plant parts for the prevention and cure of ailments, infections and diseases as an alternative to the use of orthodox medicine is gaining more attention. Moreso, resistance to orthodox drugs has been confirmed in various literatures. The leaves of *Jatropha curcas*, an ever-green perennial plant was used in this study. They were washed, air-dried and pulverized for active extraction of the composition of the plant in butanol. Fresh leaves of the plant were tested for its DPPH scavenging activity (indicator of antioxidant), while the butanol leaf extract was accessed for the presence or absence of phytochemicals and was also tested against some gram positive and gram negative bacteria. The result of the free radical scavenging activity of the leaves was higher to that of ascorbic acid (a standard antioxidant). The qualitative analysis showed the presence of alkaloids, tannins, saponin, flavonoids, steroid and cardiac glycoside. The quantitative analysis on the other hand showed the presence of alkaloids in an appreciable amount, followed by saponin while others had relatively low values. The average values of the antibacterial (inhibitory) activity as compared with standard antibiotics used had no significant difference except for *pseudomonas sp.* which was very low to the antibiotics used. It

20 could be concluded that *Jatropha curcas* is of great medicinal potential which could be
21 explored further.

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

23 1. INTRODUCTION

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

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

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

33 Synthetic drugs have become expensive and not easily accessible by the less privileged.
34 Moreover, resistance to most synthetic drugs is a serious health concern in the world
35 today. In addition, people have returned to traditional medicine and natural plants are
36 now used as drugs for various ailments based on their folkloric uses. Plant derived-
37 bioactive compounds have received considerable attention due to their therapeutic
38 potential as antimicrobial, anti-inflammatory properties and antioxidant activities [4].

39 *Jatropha curcas* leaves has been used as cure for various ailments like; skin infection,
40 diarrhea, cancer e.t.c.

41 Therefore, this research is targeted at evaluating the free radical scavenging activities of
42 *Jatropha curcas* leaves, phytochemicals present in its butanol fraction as well as the
43 antibacterial activities of its butanol fraction in order to ascertain some of its claims in
44 traditional healing.

45 **1.1 Free radicals and DPPH scavenging activities**

46 Free radicals are fundamental to any biochemical process and represent an
47 essential part of aerobic life and metabolism [5]. Reactive oxygen (ROS) and
48 reactive nitrogen species (RNS) are products of normal cellular metabolism. The
49 most common ROS include superoxide anion Peroxy nitrite anion [6]. These reactive
50 species play an important role in pathogenesis, cardiovascular diseases, and rheumatoid
51 ulcerative colitis. It is possible to reduce the risk of chronic diseases and prevent
52 diseases progression by either enhancing the body's natural antioxidant defenses or
53 by supplementing with dietary antioxidants [7]. Antioxidant offer resistance against
54 oxidative stress by scavenging the free radicals inhibiting the lipid peroxidation
55 and by many other mechanisms and thus prevent the diseases progression.
56 DPPH is a stable nitrogen centered free radical commonly used for testing
57 radical scavenging activity of the compound or plant extracts. When stable DPPH
58 radical accept an electron from the antioxidant compound the violet color of the
59 DPPH reduce to yellow color or red. Diphenylpicrylhydrazyl radical which was
60 measured colorimetrically. Substance which are able to perform this reaction can be
61 considered as antioxidant and therefore a radical scavenger [8].

63 **1.2 Antibacterial**

64 Antibacterial also known as antibiotics is a type of antimicrobial drug used in
65 the treatment and prevention of bacterial infection. They may kill or inhibit the
66 growth of bacterial. They are not effective against viruses such as common cold
67 or influenza. Antibiotics may be given as a preventive measure (prophylactic) and
68 this is usually limited to at risk populations such as those with a weakened
69 immune system (particularly in HIV cases to prevent pneumonia) [9].

70 **2.0 MATERIALS AND METHODS**

71 **2.1 Experimental material**

72 Some of the experimental apparatus used for this work included;

73 Beakers, test tubes, petri dishes, conical flasks, boiling tubes, Bunsen burner, measuring
74 cylinder

75 Experimental material included; *J. curcas* leaves,

76 Reagents included;

77 HCl, H₂SO₄, Ferric chloride solution, Potassium iodide, Mayer's reagent, Butanol,
78 Ethanol, Ethyl acetate, Dilute ammonia, Potassium ferrocyanide, Acetic acid, Olive oil,
79 MacConkey agar, Nutrient agar.

80 Instruments included

81 Weighing balance, UV - spectrophotometer

82 **2.2 Experimental procedure**

83 **Sample collection:** The plant sample was collected around the quarters of the
84 Federal college of Animal Health and production technology. The plant samples
85 was air-dried to preserve some phytochemical constituent which are thermolabile
86 and could be denatured by heat and also to prevent physiological change of the
87 leaves.

88 The air-dried samples were pulverized, using an electric blender in other to
89 reduce them to fine particles for effective extraction of the bioactive compounds.

90 Exactly, 100g of the leaves were weighed into a beaker and butanol was poured
91 into it until the samples were fully immersed, the extraction was carried out by
92 decanting the butanol (supernatant) every 24 hours. This was replaced with fresh
93 volumes of butanol and was repeated for 10days.

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

96

97 **2.3. DPPH free radical scavenging Activity**

98 100g of fresh sample was weighed into a beaker, 100ml of ethanol was added and
99 shaken vigorously for 2min, it was stirred with a magnetic stirrer for 15min, it
100 was allowed to stand for 2hours for proper extraction, then centrifuged at 2500rpm
101 for 10min. The supernatant was poured into another beaker and concentrated by

102 evaporating in a water bath at 80°C. The concentrated extract was kept for further
103 assay.

104 1mM DPPH was prepared in ethanol (394.32mg DPPH dissolved in 1litre of
105 ethanol. It was dissolved with 10mg of the concentrated sample extract in 10ml of
106 ethanol (1mg/ml) 1.5ml of the extract was pipette into a test tube. 1.5ml DPPH
107 solution prepared was added into the test tube. The spectrophotometer was zeroed with
108 ethanol as blank. Thereafter the absorbance\optical density of the control (DPPH
109 solution) and that of the sample was read at 517nm.

110
$$\text{DPPH Scavanged \%} = \frac{\text{absorbance of control} - \text{absorbance of test sample} \times 100}{\text{absorbance of control} + \text{sample weight}}$$

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

112 **2.4 Qualitative phytochemical Analysis**

113 **2.4.1 Test for Alkaloids:**

114 0.5g of the plant sample was dissolved in 5ml dilute HCl in a steam bath and filtered.
115 1ml of the above filterate was treated with few drops of Mayer's reagent. A creamy
116 white precipitate was observed which indicated the presence of alkaloids.

117 **2.4.2 Test for Tannin**

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

121 **2.4.3 Test for saponin**

122 2 gram of each portion was boiled with 20ml of distilled water filtered. To the
123 filtrate about 3ml of distilled water was further added and shake vigorously for
124 about 5min frothing which persisted on warming was observed. This was a positive
125 test for saponin.

126 **2.4.4 Test for flavonoids**

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

131 **2.4.5 Test for steroids**

132 About 0.2g of the plant extract, 2ml of acetic acid was added and the solution
133 was cooled well, Conc. H_2SO_4 was added to the solution. A violet to blue color
134 indicates the presence of a steroidal ring.

135

136 **2.5 Quantitative phytochemical analysis**

137 **2.5.1 Test for Alkaloids**

138 2g of the extracted sample was weighed into a 100ml beaker and 20ml of 80%
139 absolute alcohol added to give a smooth paste. The mixture was transferred to a
140 250ml flask and more alcohol added to make up to 100ml and 1g magnesium

141 oxide added. The mixture was digested in boiling water for 1.5 hours under a
142 reflux air condenser with occasional shaking. The mixture was filtered while hot
143 through a small bucher funnel. The residue was returned to the flask and
144 redistilled for 30minutes with 50ml water to replace the alcohol will be
145 evaporated adding hot water to replace the alcohol lost. When all the alcohol had
146 been removed, 3 drops of 10% HCl was added. The whole solution was later
147 transferred into a 250ml volumetric flask 5ml of zinc acetate solution and 5ml of
148 Potassium Ferro cyanide solution was added thoroughly mixed to give
149 homogenous solution.

150 The flask was allowed to stand for a few minutes filtered through a dry filter
151 paper and 10ml of the filtrate was transferred into a separatory funnel and
152 alkaloids present were extracted vigorously by shaking with five succession
153 portions of chloroform. The residue obtained was dissolved in 10ml hot distilled
154 water and transferred into a kjeldalh tube with the addition of 0.20g sucrose and
155 10ml Conc. H_2SO_4 and 0.02g selenium for digestion to colorless solution to
156 determine %N by kjeldahl distillation method. % N got was converted to % total
157 alkaloid by multiplying with a factor 3.26 i.e

$$158 \quad \% \text{ total alkaloid} = \%N + 3.26$$

$$159 \quad \% \text{ alkaloids} = \% N + 3.26.$$

160

161

162 2.5.2 Test for flavonoids

163 Exactly 0.50g of the extracted sample was weighed into a 100ml beaker and 80ml
164 of 95% ethanol added and stirred with a glass rod to prevent lumping. The
165 mixture was filtered through a whatman NO 1 filter paper into a 100ml volumetric
166 flask and made up to mark with ethanol. 1ml of the extract was pipette into
167 500ml volumetric flask , four drops of conc. HCl added via a dropping pipette
168 after which 0.5g of magnesium turning was added to develop a magenta red
169 coloration . Standard solution were read on digitallabomed 200 spectrophotometer
170 at a wavelength of 520nm . the percentage flavonoids was calculated using the
171 formula

$$172 \quad \% \text{ flavonoid} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{weight of sample} \times 10000}$$

173 2.5.3 Test for tannin

174 Exactly 0.20g extracted sample was measured into a 500ml of beaker , 20ml of
175 50% methanol was added and covered with paraffin and placed in water bath at
176 77-80°C for 1 hour . It was shaken thoroughly to ensure uniform mixing. The
177 extract was quantitavely filtered using a double layered whatman NO. 41 filter
178 paper into a 100ml volumetric flask, 20ml water added, 2.5ml folin-denis reagent
179 and 10ml of 17% Na_2CO_3 were added and mixed properly . the mixture was made
180 up to mark with water mixed well and and allow to stand for 20mins, the bluish-
181 green color which developed at the end of range 0-10ppm were treated similarly
182 as 1ml sample above .

183 The absorbance of the tannic acid standard solutions as well as samples were read
 184 after color development on a spectrophotometer at a wavelength of 70nm.
 185 %tannin was calculated using the formula:

$$186 \quad \% \text{ tannin} = \frac{\text{absorbance of sample} + \text{average gradient factor} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

187 2.5.4 Test for saponin

188 1g of sample was weighed into a 250ml beaker and 10ml of butyl alcohol was
 189 added. The mixture was shaken on a UDY shaker for 5hrs to ensure uniform
 190 mixing. Thereafter the mixture was filtered through a whatman NO.1 filter paper
 191 into a 100ml beaker and 20ml of 40% saturated solution of magnesium
 192 carbonate was added. The mixture obtained with saturated MgCO_3 was again
 193 filtered through a whatman NO. 1 filter paper to obtain a clear colorless solution
 194 was pipette into a 500ml volumetric flask and 2ml of 5% FeCl_3 solution was
 195 added and made up to mark with distilled water. It was allowed to stand for 30
 196 min for blood red color to development in a Jenway V6300 spectrophotometer at
 197 a wavelength of 380nm.

$$198 \quad \% \text{ saponin} = \frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

199 2.5.5. Test for steroid

200 0.5g of sample extract was weighed into a 100ml beaker 20ml of chloroform-
 201 methanol (2:1) mixture was added to dissolve the extract upon shaking for 30mins

202 on a shaker . The whole mixture was filtered through a whatman NO. 1 filter paper
203 into another dry clean 100ml conical flask /beaker.

204 The resultant residue was reportedly treated with chloroform- methanol mixture
205 until it is free of steroids. 1ml of the filtrate was pipette into a 300ml test tube
206 and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a
207 homogenous mixture. The mixture was later placed in a water bath set at 37°C-
208 40°C for 90mins. It was cooled to room temperature and 10ml of petroluem ether
209 was added followed by the addition of 5ml of charad reagent to residue in dry
210 bottle and absorbance taken at a wavelength of 620nm on spectronic 20D
211 spectrophotometer. Stand steroids of concentration of 0.4mg/l were prepared from
212 100mg/ml stock steroid was calculated using the formular:

$$213 \quad \frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

214 **2.5.6 Test for glycosides**

215 10ml of extract was pipetted into a 250ml conical flask. 50ml chloroform was added
216 and shaken on a vortex mixer for 1hour, the mixture was filtered into 100ml conical
217 flask and pyridine, 2ml of 2% sodium nitroprusside were added and shaken thoroughly
218 for 10 minutes. 3ml of 20% NaOH was later added to give a brownish yellow color. The
219 absorbance of the sample and the standards were read on a spectronic 20D digital
220 spectrophotometer at a wavelength of 510nm. Percentage glycoside was calculated using
221 the formula:

$$222 \quad \frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

223 **2.6 Antibacterial susceptibility test**

224 **2.6.1 Preparation of culture media**

225 MacConkey agar of 24.00g and 14.00g of nutrient agar were weighed into two
226 different with aluminum foil and shaken thoroughly and then left to disperse. They
227 were then sterilized at 121^oC for 15mins in autoclave. They were then poured into
228 petri dishes and left to solidify. After which it was incubated for 24hrs at 37^oC.

229 **2.6.2 Isolation of organism**

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

233 **2.6.3 Bacterial susceptibility test**

234 24hrs old standardized culture of bacterial was subcultured into distilled water and
235 1ml of the broth was used to flood the surface of the media prepared and
236 allowed to dry. A sterile cork borer of 1cm was used to make holes and 1ml of
237 the extract was dropped into each hole of the labeled dishes. Antibiotics were also
238 placed on the surface of the media containing the cultured bacterial which were then
239 incubated at 37^oC for 24hrs, the minimum zone of inhibition of the extract and
240 antibiotics were compared and records were taken.

241

242

243 **3 RESULTS AND DISCUSSIONS**

244

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

246

247 Parameter	247 Fresh leaves(mg/kg)	247 Ascorbic acid (mg/kg)
248 Antioxidant	248 74.73	248 31.01

249

250

251

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

259 TABLE 2: Qualitative and Quantitative analysis of butanol fraction of *Jatropha curcas*
 260 leaves.

261 Parameters	261 Qualitative	261 Quantitative (%)	261 Inference
262 Alkaloids	262 +	262 0.5670	262 present
263 Phlobatannins	263 -	263 0.0005	263 absent
264 Tannins	264 +	264 0.0018	264 present
265 Saponin	265 +	265 0.3160	265 present
266 Flavonoids	266 +	266 0.0024	266 present
267 Steroid	267 +	267 0.0037	267 present
268 Cardiac glycosides	268 +	268 0.1880	268 present
269 Phenol	269 +	269 0.0760	269 present

270

271 The qualitative and quantitative phytochemical analysis of the butanol extract of the leaves
 272 of *J.curcas* (Table 2), showed the presence of most phytochemicals like alkaloid, tannin,
 273 saponin, flavonoid, glycoside and phenol. This corroborates the findings of Thomas *et al*
 274 [10].

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

281 Phytochemicals such as flavonoids, phenol are effective as antioxidant while saponin and
 282 glycosides are effective as anti-inflammatory and antibiotics respectively [11].

283
 284 TABLE 3: Average value of the antibacterial activity of butanol extract of *Jatropha curcas*
 285 leaves.

286 287 Isolate	Zone of inhibition (mm)	
	Antibiotics	Butanol extract
288 Gram +ve		
289 <i>Staphylococcus sp.</i>	32(gen), 29(ofl)	30
290 <i>Bacillus cereus</i>	22(gen), 20(ofl)	20
291 <i>Clostridium sp.</i>	15 (gen), 20(ofl)	15
292 Gram -ve		
293 <i>Escherichia coli</i>	28(gen), 25(ofl)	41
294 <i>Proteus sp.</i>	20(ch), 20(ch)	17
295 <i>Pseudomonas sp</i>	40(cpx), 35(pef,ofl)	22

297 **KEY**
 298 Cpx-ciprofloxacin
 299 Pef-pefloxacin
 300 Ofi-ofloxacin

301 Ch-chloraphenicol
302 Gen –gentamycin
303

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

309 Igbinsa *et al.*,2009 opined that presence of biologically active principle (as confirmed in
310 table 2) may aid the antimicrobial activities of *J. curcas* as these secondary metabolite
311 exerts antimicrobial activity through different mechanisms.

312 This indicates that the plant extract is effective against most of the bacteria used in this
313 study. Therefore, the plant extracts has medicinal properties which supports its use in the
314 treatment of various forms of infections.

315 **4. CONCLUSION**

316 It can therefore be concluded from these research that *Jatropha curcas* leaves has
317 bioactive compounds (phytochemicals) as well as a high free radical scavenging activity
318 (indicator of antioxidant activity). Therefore, *Jatropha curcas* has great medicinal potential
319 and this could justify the use of its leaves in folklore therapy.

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