

## Original Research Article

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

### Phytochemical and Antibacterial Analysis of its Butanol crude extract.

#### ABSTRACT

**Comment [SMHAPD1]:** Wrong format!  
It should contain aims, study design, place and duration of study, methodology, results and conclusion.

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* (*Euphorbiaceae*), an ever-green perennial plant were 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 (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity (indicator of antioxidant), while the butanol crude 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 (74.73 mg/kg) was higher than that of ascorbic acid (31.01 mg/kg)(a standard antioxidant). The qualitative analysis showed the presence of alkaloids, tannins, saponin, flavonoids, steroid, phenols phlobatanins and cardiac glycoside. The quantitative analysis on the other hand showed the presence of alkaloids in an appreciable amount (0.567%), followed by saponin (0.316%) while others had relatively low values (below 0.19%). The average values of the antibacterial (inhibitory) activity as compared with standard antibiotics used had no significant difference except for *pseudomonas sp.*

21 which was very low to the antibiotics used. It could be concluded that *Jatropha curcas* is  
22 of great medicinal potential.

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

## 24 1. INTRODUCTION

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

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

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

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

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

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

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

#### 50 **1.1 Free radicals and DPPH scavenging activities**

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

61 DPPH is a stable nitrogen centered free radical commonly used for testing  
62 radical scavenging activity of the compound or plant extracts. When stable DPPH  
63 radical accept an electron from the antioxidant compound the violet color of the

64 DPPH reduce to yellow color or red. Diphenylpicrylhydrazyl radical which was  
65 measured colorimetrically. Substance which are able to perform this reaction can be  
66 considered as antioxidant and therefore a radical scavenger [13].

67

## 68 1.2 Antibacterial

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

## 75 2.0 MATERIALS AND METHODS

### 76 2.1 Experimental material

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

78 Petri dishes, 25 ml conical flasks, boiling tubes, Bunsen burner, 5 ml, 10 ml and 20 ml  
79 measuring cylinders.

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

81 Reagents included;

82 Butanol, Ethanol, Ethyl acetate, Chloroform used were all BDH general purpose chemicals  
83 and distilled prior to use. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid were

**Comment [SMHAPD2]:** Incorrect format.  
This section must be written in paragraph form.

84 obtained from Sigma Chemical Co. (St. Louis, MO). HCl, Conc. H<sub>2</sub>SO<sub>4</sub>, Potassium iodide,  
85 Mayer's reagent, Dilute ammonia, Potassium ferrocyanide, Acetic acid, Olive oil, Ferric  
86 chloride solution MacConkey agar, Nutrient agar were all BDH general purpose chemicals

87 Instruments included;

88 Mettler analytical balance H80 (UK), Water Bath (Gallenkamp), UV-Visible  
89 spectrophotometer (Unico1200 & Perkin Elmer lambda 25 models).

## 90 2.2 Experimental procedure

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

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

101 Exactly, 100g of the dried pulverized leaves were weighed into a 1000 ml beaker and  
102 excess butanol was added until the samples were fully immersed, the extraction was  
103 carried out by decanting the butanol (supernatant) every 24 hours and were filtered  
104 through a muslin. This was replaced with fresh volumes of butanol and was repeated  
105 for 10days.

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

108

### 109 2.3. DPPH free radical scavenging Activity

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

112 100g of fresh sample was weighed into a beaker, 100ml of ethanol was added and  
113 shaken vigorously for 2min, it was stirred with a magnetic stirrer for 15min, it was  
114 allowed to stand for 2hours for proper extraction, then centrifuged at 2500rpm for  
115 10min. The supernatant was poured into another beaker and concentrated by  
116 evaporating in a water bath at 80<sup>0</sup>C. The concentrated extract was kept for further assay.

117 1mM DPPH was prepared in ethanol (394.32mg DPPH dissolved in 1litre of  
118 ethanol. It was dissolved with 10mg of the concentrated sample extract in 10ml of  
119 ethanol (1mg/ml) 1.5ml of the extract was pipette into a test tube. 1.5ml DPPH  
120 solution prepared was added into the test tube. The spectrophotometer was zeroed with  
121 ethanol as blank. Thereafter the absorbance\optical density of the control (DPPH  
122 solution) and that of the sample was read at 517nm.

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

Comment [SMHAPD3]: each equation is sequentially numbered

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

### 125 2.4 Qualitative phytochemical Analysis

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

#### 130 **2.4.1 Test for Alkaloids:**

131 0.5g of the plant sample was dissolved in 5ml dilute HCl in a steam bath and filtered.  
132 1ml of the above filtrate was treated with few drops of Mayer's reagent. A creamy  
133 white precipitate was observed which indicated the presence of alkaloids.

#### 134 **2.4.2 Test for Tannin**

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

#### 138 **2.4.3 Test for saponin**

139 2 gram of each portion was boiled with 20ml of distilled water filtered. To the  
140 filtrate about 3ml of distilled water was further added and shake vigorously for  
141 about 5min frothing which persisted on warming was observed. This was a positive  
142 test for saponin.

#### 143 **2.4.4 Test for flavonoids**

144 A portion of the powdered plant sample was heated with 10ml ethyl acetate over a  
145 steam bath for 3min. it was filtered and 4ml of the filtrate was shaken with 1ml of

146 dilute ammonia .A yellow coloration indicating the presence of flavonoids was  
147 observed

#### 148 **2.4.5 Test for steroids**

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

152

### 153 **2.5 Quantitative phytochemical analysis**

#### 154 **2.5.1 Test for Alkaloids**

155 2g of the extracted sample was weighed into a 100ml beaker and 20ml of 80%  
156 absolute alcohol added to give a smooth paste. The mixture was transferred to a  
157 250ml flask and more alcohol added to make up to 100ml and 1g magnesium  
158 oxide added. The mixture was digested in boiling water for 1.5 hours under a  
159 reflux air condenser with occasional shaking. The mixture was filtered while hot  
160 through a small bucher funnel. The residue was returned to the flask and  
161 redistilled for 30minutes with 50ml hot water to replace the alcohol lost. When all  
162 the alcohol had been removed, 3 drops of 10% HCl was added. The whole solution  
163 was later transferred into a 250ml volumetric flask 5ml of zinc acetate solution  
164 and 5ml of Potassium Ferro cyanide solution was added thoroughly mixed to give  
165 homogenous solution.



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

174 
$$\% \text{ total alkaloid} = \%N \times 3.26$$

**Comment [SMHAPD4]:** each equation is sequentially numbered

175

### 176 2.5.2 Test for flavonoids

177 Exactly 0.50g of the extracted sample was weighed into a 100ml beaker and 80ml  
178 of 95% ethanol added and stirred with a glass rod to prevent lumping. The  
179 mixture was filtered through a whatman NO 1 filter paper into a 100ml volumetric  
180 flask and made up to mark with ethanol. 1ml of the extract was pipette into  
181 500ml volumetric flask , four drops of conc. HCl added via a dropping pipette  
182 after which 0.5g of magnesium turning was added to develop a magenta red  
183 coloration . Standard solution were read on digital labomed 200 spectrophotometer  
184 at a wavelength of 520nm . the percentage flavonoids was calculated using the  
185 formula

186 
$$\% \text{ flavonoid} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{weight of sample} \times 10000}$$

**Comment [SMHAPD5]:** each equation is sequentially numbered

187 **2.5.3 Test for tannin**

188 Exactly 0.20g extracted sample was measured into a 500ml of beaker , 20ml of  
189 50% methanol was added and covered with paraffin and placed in water bath at  
190 77-80°C for 1 hour . It was shaken thoroughly to ensure uniform mixing. The  
191 extract was quantitavely filtered using a double layered whatman NO. 41 filter  
192 paper into a 100ml volumetric flask, 20ml water added, 2.5ml folin-denis reagent  
193 and 10ml of 17% Na<sub>2</sub>CO<sub>3</sub> were added and mixed properly . the mixture was made  
194 up to mark with water mixed well and and allow to stand for 20mins, the bluish-  
195 green color which developed at the end of range 0-10ppm were treated similarly  
196 as 1ml sample above .

197 The absorbance of the tannic acid standard solutions as well as samples were read  
198 after color development on a spectrophotometer at a wavelength of 70mm.  
199 %tannin was calculated using the formula:

200 % tannin = 
$$\frac{\text{absorbance of sample} + \text{average gradient factor} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

**Comment [SMHAPD6]:** each equation is sequentially numbered

201 **2.5.4 Test for saponin**

202 1g of sample was weighed into a 250ml beaker and 10ml of butyl alcohol was  
203 added. The mixture was shaken on a UDY shaker for 5hrs to ensure uniform  
204 mixing. Thereafter the mixture was filtered through a whatman NO.1 filter paper  
205 into a 100ml beaker and 20ml of 40% saturated solution of magnesium  
206 carbonate was added. The mixture obtained with saturated MgCO<sub>3</sub> was again  
207 filtered through a whatman NO. 1 filter paper to obtain a clear colorless solution

208 was pipette into a 500ml volumetric flask and 2ml of 5% FeCl<sub>3</sub> solution was  
209 added and made up to mark with distilled water .It was allowed to stand for 30  
210 min for blood red color to development in a Jenway V6300 spectrophotometer at  
211 a wavelength of 380nm.

212 
$$\% \text{ saponin} = \frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

Comment [SMHAPD7]: each equation is sequentially numbered

### 213 2.5.5. Test for steroid

214 0.5g of sample extract was weighed into a 100ml beaker 20ml of chloroform-  
215 methanol (2:1) mixture was added to dissolve the extract upon shaking for 30mins  
216 on a shaker . The whole mixture was filtered through a whatman NO. 1 filter paper  
217 into another dry clean 100ml conical flask /beaker.

218 The resultant residue was repeatedly treated with chloroform- methanol mixture  
219 until it was free of steroids. 1ml of the filtrate was pipette into a 300ml test tube  
220 and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a  
221 homogenous mixture. The mixture was later placed in a water bath set at 37°C-  
222 40°C for 90mins. It was cooled to room temperature and 10ml of petroluem ether  
223 was added followed by the addition of 5ml of chard reagent to residue in dry  
224 bottle and absorbance taken at a wavelength of 620nm on spectronic 20D  
225 spectrophotometer. Stand steroids of concentration of 0.4mg/l were prepared from  
226 100mg/ml stock steroid was calculated using the formular:

Comment [SMHAPD8]: Typo error??

227 
$$\frac{\text{absorbance of sample} + \text{average gradient} + \text{dilution factor}}{\text{Weight sample} \times 10000}$$

Comment [SMHAPD9]: each equation is sequentially numbered

228 **2.5.6 Test for glycosides**

229 10ml of extract was pipetted into a 250ml conical flask. 50ml chloroform was added  
230 and shaken on a vortex mixer for 1hour,the mixture was filtered into 100ml conical  
231 flask and pyridine, 2ml of 2% sodium nitroprusside were added and shaken thoroughly  
232 for 10 minutes. 3ml of 20% NaOH was later added to give a brownish yellow color. The  
233 absorbance of the sample and the standards were read on a spectronic 20D digital  
234 spectrophotometer at a wavelength of 510nm. Percentage glycoside was calculated using  
235 the formula:

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

Comment [SMHAPD10]: each equation is sequentially numbered

237 **2.6 Antibacterial susceptibility test**

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

242 **2.6.1 Preparation of culture media**

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

248 **2.6.2 Isolation of organism**

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

252 **2.6.3 Bacterial susceptibility test**

253 24hrs old standardized culture of **bacteria** was subcultured into distilled water and  
254 1ml of the broth was used to flood the surface of the media prepared and  
255 allowed to dry. A sterile cork borer of 1cm was used to make holes and 1ml of  
256 the extract was dropped into each hole of the labeled dishes. Antibiotics were also  
257 placed on the surface of the media containing the cultured bacterial which were then  
258 incubated at 37<sup>o</sup>C for 24hrs , the minimum zone of inhibition of the extract and  
259 antibiotics were compared and records were taken.

260

261 **3 RESULTS AND DISCUSSIONS**

262

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

264

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

268

269

270 Table 1 reveals the DPPH scavenging activity (indicator of the antioxidant) in fresh  
271 *Jatropha curcas* leaves in which the value was relatively high compare to the  
272 antioxidant ability of ascorbic acid a standard antioxidant which proves that the leaves

273 have the ability of scavenging free radicals and the oxidation process in the body. These  
274 free radicals are responsible for some diseases and therefore *Jatropha curcas* has the  
275 ability to cure disease since it has the ability to inhibit the oxidation processes in  
276 human body that are responsible for these diseases.

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

279	Parameters	Qualitative	Quantitative (%)	Inference
280	Alkaloids	+	0.5670	present
281	Phlobatannins	+	0.0005	present
282	Tannins	+	0.0018	present
283	Saponin	+	0.3160	present
284	Flavonoids	+	0.0024	present
285	Steroid	+	0.0037	present
286	Cardiac glycosides	+	0.1880	present
287	Phenol	+	0.0760	present

288

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

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

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

300

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

303 Isolate	303 Zone of inhibition (mm)	
	304 Antibiotics	304 Butanol extract
305 <b>Gram +ve</b>		
306 <i>Staphylococcus sp.</i>	32(gen), 29(ofl)	30
307 <i>Bacillus cereus</i>	22(gen), 20(ofl)	20
308 <i>Clostridium sp.</i>	15 (gen), 20(ofl)	15
309 <b>Gram -ve</b>		
310 <i>Escherichia coli</i>	28(gen), 25(ofl)	41
311 <i>Proteus sp.</i>	20(ch), 20(ch)	17
312 <i>Pseudomonas sp</i>	40(cpx), 35(pef,ofl)	22

313 **KEY**

- 315 Cpx-ciprofloxacin
- 316 Pef-pefloxacin
- 317 Ofi-ofloxacin
- 318 Ch-chloraphenicol
- 319 Gen –gentamycin

320

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

326 [23] reported that presence of biologically active principle (as confirmed in table 2) may  
 327 aid the antimicrobial activities of *J. curcas* as these secondary metabolite exerts  
 328 antimicrobial activity through different mechanisms.

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

**Comment [SMHAPD11]:** Grammatically error.  
Manuscript should be written in concise English  
Use have .....

#### 332 4. CONCLUSION

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

338

339

340

#### 341 REFERENCES

342 1. Krippner S. Models of ethnomedicinal healing. Paper presented at the ethnomedicine  
343 conferences, Munich ,Germany. 2003, April 26-27 and October 11-12.

344 2. Abebe D: The role of ethnomedicinal plants in health care coverag of ethopia, the  
345 possible benefits of integration. *J. ethnobiol. & ethnomed.* 2001; 9:32.

**Comment [SMHAPD12]:** Wrong format –page  
number  
Hilly M, Adams ML, Nelson SC. A study of digit  
fusion in the mouse embryo. *Clin Exp Allergy.*  
2002;32(4):489-98.

346 3. Srivastava R. Studying the information needs of medicinal plant stakeholders in Europe.  
347 U.K. *J. appl. & environ. Microb.* 2000,(2):42-45

**Comment [SMHAPD13]:** Wrong format –  
comma  
Hilly M, Adams ML, Nelson SC. A study of digit  
fusion in the mouse embryo. *Clin Exp Allergy.*  
2002;32(4):489-98.



348 4. Rathee P, Hema C, Sushila R and Dharmender R. Mechanism of action of flavonoids as  
349 anti-inflammatory. *J.of nutr.* 2009, 2:(98).

**Comment [SMHAPD14]:** Wrong format – journal name

350 5. Janick J. and Robert E. Encyclopedia of fruits and nuts, 2008. Cabi publisher, U.K.

Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. *Clin Exp Allergy.* 2002;32(4):489-98.

351 6. Duke JA, Bogenschutz-Godwin MJ, Duceellier J and Duke PA: Handbook of  
352 medicinal herbs. 2<sup>nd</sup> edition, 2002. Boca press.Pg 32

**Comment [SMHAPD15]:** Wrong format – remove and

Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. *Clin Exp Allergy.* 2002;32(4):489-98.

353 7. Agbogidi OM and Ekeke EA. *Jatropha curcas* linn; An important but neglected plant  
354 species in Nigeria. *J. of boil. & chem. Res.* 2011, 28-1:52:62

**Comment [SMHAPD16]:** Wrong format – remove and

Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. *Clin Exp Allergy.* 2002;32(4):489-98.

355 8. Aliyu BS. Some ethnomedicinal plants of the savannah regions of West Africa:  
356 description and phytochemicals. *J. of boil. Sci.* 2006, 5:314-321.

357 9. Osemene KP, Ilori MO and Elujoba AA. Examining the nature of herbal research  
358 and development in Nigeria. *Intl J of bio, pharm& allied sci.* 2013, 1(2):133-142.

**Comment [SMHAPD17]:** Wrong format – journal name

Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. *Clin Exp Allergy.* 2002;32(4):489-98.

359 10. Tiwari A. Imbalance in antioxidant defense and human diseases, *J. of mult.*  
360 *approach of natural antioxd therpy.* 2001, 81:1179-1187.

361 11. Sudha G, Sangeethapriya M, Indhushee R and Vadiuukkaras S. *In vitro* free radical  
362 scavenging activity of raw pepino fruit. *Int. J. of pharm.* 2011; (7) :137-140.

363 12. Stanner SA, Hugher J, Kelly CN and Butlris JA. Review of the epidemiological  
364 evidence for the antioxidant hypothesis. *J. pub hlth nutr.* 2000, 7: 401-422.

**Comment [SMHAPD18]:** Wrong format – capital letter of P

Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. *Clin Exp Allergy.* 2002;32(4):489-98.

365 13. Dehpour AA, Ebrahimzadel MA, Nabari SF, Fazelin M and Eslami B. *In vitro*  
366 antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana*  
367 growing in Iran. *Pharmgsy mag.*, 2009, 4:123-127.

- 368 14. Aminov RI. A brief history of the antibiotic era. Lessons and challenges for the future.  
369 *J. of frontiers in microb.* 2010, 1:134.
- 370 15. Koleva II, Van-Beck TA, Linssen JP, De-Groot A. and Evstaliva LN. Screening of plant  
371 for antioxidant activity. A comparative study on three testing methods. 2002. *Phytochem.*  
372 *Anal.* 13: 8-17.
- 373 16. Oloyede KG and Farombi OE. Antioxidant properties of *Crinum ornatum* bulb extract.  
374 2010. *World J. Chem.*, 5: 32-36.
- 375 17. Edeoga HO, Okwu DE and Mbaebre BO. Phytochemical constituents of some Nigeria  
376 plants. *Afr J. of Biotech.* 2005; 447:685-688.
- 377 18. Sofowara A. Medicinal plants and traditional medicine in Africa. Spectrum books  
378 limited, Ibadan, Nigeria, 1993; pp 289
- 379 19. Trease GE and Evans WC. Pharmacognosy, 14<sup>th</sup> edition, WB Saunders Company  
380 Limited. U.K, 1996: pp. 1098-1115.
- 381 20. Harborne JP. Phytochemical methods, London. Chapman and hall, limited. 1973: pp.49-  
382 188.
- 383 21. Stokes EJ. *Clinical Bacteriology*, 1968. 3rd Edition pp179.
- 384
- 385 22. Thomas T, Sah NK and Sharma PB. Therapeutic biology of *Jatropha curcas*: A mini  
386 review. *Curr Pharm Biotechnol*; 2008, 9(4):24-315.
- 387 23. Igbinsosa OO, Igbinsosa EO and Aiyegoro OA. Antimicrobial activity and phytochemical  
388 screening of stem bark extracts from *J. curcas* (linn). *Afr. J. of pharm. & pharmcgy* 2009,  
389 3:58-62.