

1    **Antioxidant, anti-enzymatic, antimicrobial and cytotoxic properties of *Euphorbia tirucalli* L.**

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9    Running title: Bioactivity of *Euphorbia tirucalli*.

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12 **Antioxidant, anti-enzymatic, antimicrobial and cytotoxic properties of *Euphorbia tirucalli* L.**

13 **Abstract**

14 Medicinal properties of *Euphorbia tirucalli* L. have been investigated in vitro. Water extract from  
15 the plant latex was freeze dried and tested against 3 digestive enzymes and showed IC50 as  
16  $39.8 \pm 0.22$ ,  $79.43 \pm 0.38$  and  $316.22 \pm 0.3$  for lipase, alpha- glucosidase and alpha- amylase,  
17 respectively. These results were incomparable with drugs such as acarbose and orlistat.  
18 Antioxidant property showed that the IC50 values of *E. tirucalli* and Trolox were  $79.43 \pm 0.69$  and  
19  $3.1 \pm 0.92$  respectively. Also, the plant extract exhibited a range of antimicrobial activity against  
20 gram-positive and gram-negative bacteria and pathogenic yeast and fungi. Cytotoxicity of the plant  
21 extract tested on Caco-2 cells was determined using MTT method. The effect was linear and almost  
22 0.5 mg/ml extract has inhibited 50% of cells relative to the control. *Euphorbia tirucalli* showed  
23 promising activities and is potential source of active ingredient with functional properties.

24 Key words: *Euphorbia tirucalli* L, bioactive properties, cytotoxicity, Caco-2.

25 **Introduction**

26 Phylogenetic chemicals play an essential value in drugs investigations. Many of drug candidates and  
27 clinically approved therapeutic agents have been isolated from plant products [1]. In fact, several  
28 isolated molecules from herbal sources utilized in medicine, have the most potent therapeutic  
29 activity among chemically produced medicines such as vincristine, taxol, atropine, morphine,  
30 pilocarpine and many others [2]. The potent physiological and therapeutic effects of plant materials  
31 usually resulted from the effects of plant secondary metabolic compounds.

32 Diabetes mellitus, obesity, and overweight are dangerous metabolic disorders and considered the  
33 major reasons for mortality and morbidity for thousands of people in many of the developing  
34 countries. However, different investigations demonstrated that obesity and overweight are

35 associated with an increased risk of diabetes. In fact, both of the obesity and overweight elevate  
36 the costs of immense health care because they are significantly associated with diabetes, arthritis,  
37 asthma, high level of blood cholesterol, high blood pressure and poor quality of life [3].  
38 Malignant tumor is commonly known as cancer which is a large group of diseases that may affect  
39 every part of the human body with a rapid formation of abnormal cells and usually distributed to  
40 other organs. However, the death from cancer usually occurred from the metastases process and  
41 considered a second leading cause of death world widely. In 2018 cancer was responsible for about  
42 9.6 million deaths with 70% of deaths were from middle- and low -income regions [4-6].  
43 Evidently, the anti-microbial resistance is a global medical and public health concern as it is not  
44 limited to bacterial pathogens but also distributed to the parasites, fungi and viruses. Moreover,  
45 the most therapeutically effective and safe drugs have already been developed, and newer effective  
46 drugs often have more toxicity and other drawbacks, including higher cost [7].  
47 The imbalance between antioxidants and free radicals in human body called oxidative stress. While  
48 oxidation is a normal internal biochemical process in living cells which results in production of  
49 free radicals causing long chains of chemical reactions with other compounds in the body, other  
50 external such as alcohol, high sugar, fat diet, pollution, radiation, smoking and pesticides are some  
51 of the risk factors of oxidative stress [8]. However, antioxidants compounds can donate an electron  
52 to free radicals sparing the reactions with vital molecules in the cell such as DNA or lipids of cell  
53 membrane. This can cause these free radicals to be more stable. In fact, oxidative stress can  
54 contribute to the development of many diseases including heart disease, inflammation, glaucoma,  
55 diabetes, chronic obstructive pulmonary disease, atherosclerosis and cancer [9].  
56 *Euphorbia tirucalli* L. is a perennial laticiferous, evergreen shrubby plant belongs to  
57 Euphorbiaceae family. It grows wildly in tropical and subtropical regions or cultivated for

58 decorative purposes as ornamental plant. The *E. tirucalli* branches have a pencil shape from which  
59 obtained its vernacular name, the pencil-tree plant. However, in all its parts, *E. tirucalli* contains  
60 white milky latex that contains triterpenoids as cyclotirucanenol [10, 11], diterpene tirucallicine  
61 [12], euphol, amyrin, cycloeuphordenol, tirucallol, lanosterol, glut-5-en-3-b-ol, cycloartenol  
62 euphorginol [13] and steroids [14].

63 In addition, *E. tirucalli* aqueous extract contains various types of phenolic acids and flavonoids  
64 including gallic acid, chlorogenic acid, caffeic acid, kaempferol, quercetin and rutin [15].

65 In various folk medicines, *E. tirucalli* white latex used for the treatment of warts, epilepsy, sexual  
66 impotence, snake bites, hemorrhoids, toothache, corn, rheumatism, acne [16], cancer [17] and  
67 antimicrobial agent [18, 19].

68 Some in vitro studies were found in the literature regarding *E. tirucalli* biological and  
69 pharmacological properties which revealed that the plant has antiviral, larvicidal, molluscicide and  
70 anti-arthritic activities [20-23]. Moreover, *E. tirucalli* showed cytotoxicity, genotoxicity and made  
71 some changes in antioxidant gene expression in human leukocytes [15].

72 On the other hand, African *E. tirucalli* latex revealed a co-carcinogenic property and specific  
73 cellular immunity reduction associated to the infection from Epstein-Barr virus [24].

74 The current investigation aims to evaluate the inhibitory effects of *E. tirucalli* freeze dried juice  
75 against  $\alpha$ -amylase,  $\alpha$ -glucosidase, lipase, microbial growth also aims to assess its antioxidant and  
76 cytotoxic properties.

78 **Material and methods**

79 **Instrument for anticancer test**

80 Microplate reader [Unilab, 6000, Mandaluyong, USA], CO<sub>2</sub> incubator [Esco, 2012-74317, Changi,  
81 Singapore], inverted microscope [MRC, IX73, Hong Kong, China], UV-Visible  
82 Spectrophotometer [Jenway 7315, Staffordshire, UK], vortex [Heidolph Company, 090626691,  
83 Schwabach, Germany], ultrasonic cleaner [MRC Laboratory Equipment, 1108142200049, Essex,  
84 UK], autoclave [MRC Laboratory Equipment, A13182, Essex, UK], Water bath [Lab  
85 Tech, 2011051806, S. Korea], Stir-Mixer [Tuttnaver, 300303159, USA], Cooled incubator  
86 [Gallenkamp, SG92/01/244, Loc, United Kingdom], Micropipette [MRC Laboratory Equipment,  
87 MPC-1000, Essex, UK], Multichannel Micropipette [MRC Laboratory Equipment, MPC-8-50,  
88 Essex, UK].

89 **Instruments for antioxidant, lipase, amylase and glycosidase inhibition tests**

90 UV-Visible Spectrophotometer [Jenway 7135, England], filter papers [Whitman no.1, USA],  
91 shaker device [Memmert shaking incubator, Germany], rotatory evaporator [Heidolph vv2000  
92 Heidolph OB2000, Germany], grinder [Moulinex model, Uno, China], balance [Red wag, AS  
93 220/c/2, Poland] and freeze dryer [Mill rock technology BT85, China].

94 **Instruments for antimicrobial test**

95 Sonicator [ultrasonic cleaner, MRC laboratory equipment, 1108142200049, Essex, UK],  
96 Autoclave [MRC laboratory equipment, A13182, Essex, UK], Water bath [Iso 9001 certified, Lab  
97 tech, 2011051806, Korea], Incubator [EN500, Nuve, A08 No.789244, Turkey], Stir-Mixer

98 [vortex] [Tuttnaver co., 300303159, USA], Cooled incubator [Gallenkamp, SG92/01/244, Loc,  
99 UK], Micropipette [MRC, MPC-1000, UK], Multichannel Micropipette [MRC, MPC-8-50, UK].

100 **Chemicals and reagents**

101 BBL Mueller Hinton II broth, cation adjusted [Becton Dickinson, USA], Difco Sabouraud agar,  
102 modified [Becton Dickinson, USA], Acarbose, pNPG,  $\alpha$ -glucosidase [Baker's Yeast alpha  
103 glucosidase],  $\alpha$ -amylase, DNSA and potassium phosphate from Sigma-Aldrich, USA. Methanol,  
104 NaOH, n-hexane, and acetone were purchased from Lobachemie [India], [DPPH] 2,2-Diphenyl-  
105 1-picrylhydrazyl was obtained from Sigma-Aldrich [Germany] and DMSO [Dimethyl sulfoxide]  
106 was obtained from Riedeldehan [Germany]. In addition, Trolox [(s)-(-)-6 hydroxy-2, 5, 7, 8-  
107 tetramethylchroman-2-carboxylic acid] was obtained from Sigma-Aldrich [Denmark]. Alpha-  
108 amylase [Sigma, Mumbai, India], DNSA [3, 5-dinitrosalicylic acid], Acarbose [Sigma, St. Louis,  
109 USA], p-nitrophenyl butyrate, Orlistat, tris-HCl buffer and Porcine pancreatic lipase type II were  
110 purchased from Sigma [USA].

111 **Plant material**

112 The pencil shape branches of *E. tirucalli* were collected from Tulkarm region of Palestine in 2018.  
113 The taxonomical characterization was established at An-Najah National University in Natural  
114 Products laboratory, Department of Pharmacy and the herbarium was stored under the voucher  
115 specimen code Pharm-PCT-1002.

116 The collected branches were washed with sterile water and the cleaned branches were grated and  
117 pressed using mechanical Juicer Extractor Machine [Aicok Juicer, China]. Plant juice was  
118 sterilized utilizing Millipore Sigma membrane filtration device [Germany]. The produced liquid

119 was dried using a freeze-drier apparatus and then kept in an air-tight brown jars at 4°C for further  
120 use.

## 121 Free radical scavenging assay

122 Methanolic stock solution [1mg/ml] was set for *E. tirucalli* dried juice and for vitamin E analogue  
123 [Trolox] which used as a control with a potent antioxidant activity. Then, various concentrations  
124 from the previous stock solution were prepared. Plant working solution [1 ml] was mixed with 1ml  
125 freshly prepared DPPH [0.002 g/ml] methanolic solution and 1ml methanol was then added to the  
126 previous mixture. The blank solution contained DPPH and methanol only in a ratio of 1:1. The  
127 solutions were incubated at room temperature [25°C] in a dark place for 30 min. Then, their optical  
128 densities were measured by the UV/Vis spectrophotometer [Jenway 7135, England] at 517 nm.

129 Antioxidant activity was calculated per the following equation (1):

130

$$DPPH \text{ inhibition\%} = \frac{AB - As}{Ab} \times 100 \dots \dots \dots (1)$$

132  $A_b$  is the recorded absorbance of the blank solution

133  $A_s$  is the recorded absorbance of the sample solution or control.

## 134 Porcine pancreatic lipase inhibitory assay

135 A porcine pancreatic lipase inhibition assay was carried out to assess the activity *E. tirucalli*.  
136 Orlistat, a commercially available anti-obesity, and an anti-lipase therapeutic agent was used as a  
137 reference control. The porcine pancreatic lipase inhibitory method was performed according to the

138 protocol of Jaradat *et al.*[2019] [25]. With some modifications. A 500  $\mu$ g/mL stock solution from  
139 each plant extract was dissolved in 10% dimethyl sulfoxide [DMSO], from which five different  
140 dilutions were prepared [50, 100, 200, 300 and 400  $\mu$ g/ml]. Then, a 1 mg/ml stock solution of  
141 porcine pancreatic lipase was freshly prepared before use, which was dispersed in Tris-HCl buffer.  
142 The substrate used was *p*-nitrophenyl butyrate [PNPB] [Sigma-Aldrich, Germany], prepared by  
143 dissolving 20.9 mg in 2 mL of acetonitrile. In addition, for each working test tube, 0.1 ml of  
144 porcine pancreatic lipase [1 mg/ml] was mixed with 0.2 mL of the plant extract from each diluted  
145 solution series for each plant extract. The resulting mixture then completed to 1 ml by adding Tri-  
146 HCl solution and incubated at 37°C for 15 minutes. After this incubation period, 0.1 ml of *p*-  
147 nitrophenyl butyrate solution was added to each test tube. The mixture was then incubated for  
148 30 minutes at 37°C. Pancreatic lipase activity determined by measuring the hydrolysis of PNPB  
149 into *p*-nitrophenolate ions at 410 nm using a UV-vis spectrophotometer; the same procedure was  
150 repeated for Orlistat [Sigma-Aldrich, Germany]. The inhibitory percentage of the anti-lipase  
151 activity was calculated using the following equation (2):

$$152 \quad \text{Lipase inhibition \%} = \frac{AB - Ats}{AB} \times 100 \quad \dots \dots \dots \quad (2)$$

153 where AB is the recorded absorbance of the blank solution and Ats is the recorded absorbance of  
154 the tested sample solution.

### 155 ***In vitro* $\alpha$ -amylase inhibitory activity**

156 The  $\alpha$ -amylase inhibitory activity of *E. tirucalli* dried juice was carried out according to the  
157 standard method of [26] with minor modification. Briefly, each extract fraction was dissolved in  
158 few milliliters of 10% DMSO and then further dissolved in a buffer [[Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> [0.02  
159 M], NaCl [0.006 M] at pH 6.9] to give concentrations of 1000  $\mu$ g/ml. The following dilutions were

160 prepared [10, 50, 70, 100, 500 µg/ml]. A volume of 0.2 ml of porcine pancreatic  $\alpha$ -amylase enzyme  
161 solution with concentration of [2 units/ml] was mixed with 0.2 ml of the plant juice then incubated  
162 for 10 min at 30°C. Thereafter, 0.2 ml a freshly prepared starch solution [1%] was added and the  
163 mixture was incubated for at least 3 min. The reaction was stopped by the addition of 0.2 ml  
164 Dinitrosalicylic acid [DNSA] then the mixture was diluted with 5 ml of distilled water and heated  
165 for 10 min in a water bath at 90°C. The mixture was left to cool down to room temperature, then  
166 the absorbance was taken at 540 nm. A blank was prepared following the same procedure replacing  
167 the plant fraction with 0.2ml of previous buffer.

168 Acarbose was used as positive control following the same procedure. The  $\alpha$ -amylase inhibitory  
169 activity was calculated using the following equation:

170 
$$\text{alpha - amylase inhibition \%} = \frac{A_b - A_s}{A_b} \times 100 \dots\dots\dots (3).$$

171 where:

172  $A_b$ : is the absorbance of blank

173  $A_s$ : is the absorbance of tested sample or control.

174  **$\alpha$ -glucosidase inhibitory activity**

175 The  $\alpha$ -glucosidase inhibitory activity of the dried *E. tirucalli* juice was carried out in accordance  
176 to the standard biochemical method with minor modification [27]. In each eppendorf tube a  
177 reaction mixture containing 50 µl phosphate buffer [100 mM, pH = 6. 8], 10 µl alpha-glucosidase  
178 [1 U/ml], and 20 µl of varying concentrations of the plant juice [100, 200, 300, 400 and 500 mg/ml]  
179 which was incubated at 37°C for 15 min. Then pre-incubated 20 µl of [5 mM] *p*-NPG was added

180 as a substrate of the reaction and again incubated at 37°C for further 20 min. The reaction was  
181 terminated by adding 50 µl Na<sub>2</sub>CO<sub>3</sub> [0.1M]. The absorbance of the released *p*-nitrophenol was  
182 measured by a UV/Vis spectrophotometer at 405 nm. Acarbose, with similar concentrations to *E.*  
183 *tirucalli* dried juice, was used as a positive control.

184 Inhibition percentage was calculated using the following equation:

185 
$$\text{alpha-glucosidase inhibition \%} = \frac{A_b - A_s}{A_b} \times 100 \dots\dots\dots (3).$$

186

187 Where:

188 A<sub>b</sub>: is the absorbance of blank

189 A<sub>s</sub>: is the absorbance of tested sample or control.

## 190 **Cell proliferation assay for Caco-2 cells**

191 Colorectal adenocarcinoma cells [Caco-2, ATCC® HTB-37™] were propagated in RPMI-1640  
192 media, followed by the addition of 10% fetal bovine serum, also 1% penicillin-streptomycin  
193 antibiotics, and 1% l-glutamine. Caco-2 cells were grown in a moist atmosphere which contain 5%  
194 CO<sub>2</sub> at 37°C. Cells were implanted at 2.6 x 10<sup>4</sup> cells/well in a 96-well plate. After 24h, the cells  
195 were aggregated, and media were changed then cells were incubated with 5, 2.5, 1.25, 0.625,  
196 0.3125 mg/ml for 24 h. Cell viability was defined by Cell Titer 96® Aqueous One Solution Cell  
197 Proliferation [MTS] Assay according to the manufacturer's instructions [Promega Corporation,  
198 Madison, WI]. At the end of treatment, 20 µl of MTS solution/100 µl of media was added to every  
199 single well and were incubated for 2 h at 37°C. Finally, absorbance was measured at 490 nm.

## 200 **Antimicrobial method**

201 **Microbial strains**

202 Reference microbial strains were obtained from American Type Culture Collection [ATCC].  
203 Bacterial strains were *Staphylococcus aureus* [ATCC 25923], *Shigella sonne* [ATCC 25931],  
204 *Pseudomonas aeruginosa* [ATCC 27853], *Escherichia coli* [ATCC 25922] and *Enterococcus*  
205 *faecium* [ATCC 700221]. While, the fungal strains were *Candida albicans* [ATCC 90028] and  
206 *Epidermophyton floccosum* [ATCC52066]. However, to carry out the antimicrobial activity, the  
207 *E. tirucalli* latex was dissolved in water to prepare 50 mg/ml solution.

208 **Micro plate broth dilution method**

209 This method was carried out according to [28] for determination of minimal concentration  
210 inhibition [MIC] for both bacteria and yeast reference strains. Plant extract was examined in  
211 duplicate in each run. Using multichannel micropipette, 100  $\mu$ l Mueller-Hinton II Broth [Becton  
212 Dickinson, France] was pipetted into each well of a 96 - well plate. Then in the first row a 100  $\mu$ l  
213 from plant extract was placed and mixed thoroughly, followed by transferring a 100  $\mu$ l to next raw.  
214 This was repeated to the 10<sup>th</sup> row then the 11<sup>th</sup> row has got 100  $\mu$ l plant extract representing the  
215 negative control for bacterial growth. The 12<sup>th</sup> row in the plate was left as positive control for  
216 microbial growth containing no plant extract. After this serial dilution, the examined plant extract  
217 concentrations were [ $\mu$ g/ $\mu$ l]; 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390 etc.  
218 Bacterial suspension from fresh culture was prepared and turbidity was made equivalent to that of  
219 0.5 McFarland standard so that the concentration of bacterial isolate was about  $1.5 \times 10^8$  CFU/ml.  
220 Then the bacterial suspension was diluted 1:3 in sterile broth. Bacterial suspension [1 $\mu$ l] was mixed  
221 thoroughly with solutions in rows 1-10 and 12 while leaving raw 11 with no bacterial suspension.  
222 The plates were incubated at 35 °C for 18 hours.

223 For yeast, a yeast suspension was prepared with turbidity equivalent to 0.5 McFarland standard.  
224 The yeast suspension was diluted 1:1000 in sterile broth. A 100 $\mu$ l was transferred to a 96-well  
225 plate as described above. The plate was incubated at 35°C for 48 hours.

## 226 **Agar diffusion well-variant method**

227 This method was made according to [29, 30]. Bacterial suspension with turbidity equivalent to 0.5  
228 McFarland standard was prepared from fresh culture. A sterile cotton swab was used to distribute  
229 the bacterial inoculum uniformly on surface of Mueller-Hinton II agar. In each inoculated plate  
230 using sterile glass cylinder, 5 of 6-mm diameter wells were made with 2.5-cm gaps. In each well,  
231 80  $\mu$ l plant extract were placed. The plates were then incubated at 37 °C for 18 hours. The diameters  
232 of inhibition zones were measured. Each plant extract test was made in duplicate and the average  
233 of diameter was calculated. Ciprofloxacin antibiotic [50 mg/ml] was used as positive control.

## 234 **Agar dilution method**

### 235 **Preparation of Sabouraud dextrose agar [SDA]**

236 Twelve tubes after autoclaving were containing 1 ml SDA. They were divided into 2 groups and  
237 numbered with E1-E6. Tubes, then, were placed in the water bath at 45 °C to remain in a liquid  
238 state. In addition, tubes containing microfiltered plant extracts were also placed in the same water  
239 bath.

240 One ml of the extract was transferred to E1 then 1 ml serially transferred to tubes E2-E5  
241 respectively. All tubes were positioned in slant to solidify. Tube E6 was left as a control. The same  
242 steps were repeated on the second set of tubes.

## 243 **Yeast Enumeration**

244 Yeast cultures were grown on potato dextrose broth and transferred every 72 h to assure purity. A  
245 freshly prepared yeast culture grown on potato dextrose agar was flooded with 10 ml of 0.05%

246 Tween 20 in normal saline, then 2ml of the yeast suspension was pipetted twice by a micropipette  
247 and transferred to a tube containing 5 ml normal saline. The yeast suspension was prepared to  
248 achieve a turbidity equivalent to 0.5 McFarland.  
249 Twenty- $\mu$ l of suspension of fungus was aseptically transferred to each tube that previously  
250 numbered from E1-E6 of each group except for tubes numbered E5.  
251 The tubes were left for one day then incubated at 25°C for further 14 days.

## 252 **Results**

### 253 **Digestive enzymes inhibitory activity**

254 The inhibitory activities against  $\alpha$ -amylase,  $\alpha$ -glucosidase and lipase enzymes were conducted  
255 according to standard biochemical tests. The results showed that the dried juice of *E. tirucalli* plant  
256 has a strong  $\alpha$ -glucosidase and lipase enzymes inhibitory activity with IC<sub>50</sub> values of 79.43 $\pm$ 0.38  
257 and 39.8 $\pm$ 0.22, respectively as shown in Table 1 and Fig. 2. While the inhibitory activity against  
258  $\alpha$ -amylase was weak in comparison with Acarbose drug.

### 259 **Antioxidant activity**

260 The DPPH method was utilized to assess the antioxidant potential of the dried juice of *E. tirucalli*  
261 plant and Trolox. Table 4 and Fig. 4 depicted that the plant has mild antioxidant potentials in  
262 comparison to Trolox.

### 263 **Agar diffusion well- variant method**

264 The juice of *E. tirucalli* plant was milky and even after dilution all wells remained unclear, so  
265 micro broth dilution method was not suitable for evaluation of antimicrobial activities.

266 There wasn't any inhibition zone around wells.  
267 showed inhibition zones, which were 4, 5.5, 5.5, 5.5 and 5.8 cm for *S. aureus*, *Shigella sonnie*,  
268 *Pseudomonas aeruginosa*, *E. coli* and *E. faecium*, respectively.

269 **Agar dilution method**

270 Anti-mold effects of the plant extract are shown on the Table 6. There was no growth of the fungus  
271 in the tubes numbered from E1-E5, but fungus growth was observed in the tube E6. These results  
272 revealed that the plant dried juice inhibited the growth of *Epidermophyton floccosum*.

273

274 **Cytotoxic effect of extracts derived from *Euphorbia tirucalli***

275 As shown in Fig. 5 treatment of Caco-2 cells with 5, 2.5, 1.25, 0.625, 0.3125 mg/ml of *Euphorbia*  
276 *tirucalli* dried juice induced cytotoxicity significantly [ $p \leq 0.001$ ] by approximately 86%, 88%,  
277 80%, 37% and 13% respectively.

278 **Discussion**

279 Plants are natural source of many bioactive compounds. Some plants exhibit a wide range of  
280 bioactivities due to production of secondary metabolites they produce. Such metabolites are  
281 usually produced to protect the plants from the harsh environment and natural enemies. In this  
282 context *E. tirucalli*, though an ever-green plant, has been rarely fed by herbivores. Moreover,  
283 reports showed that it has few pests and diseases due to its poisonous latex. In this study we  
284 examined the bioactivities of the latex of *E. tirucalli*. The chemical composition of the latex was  
285 reported in many researches. Its complexity may explain the wide varieties of the functions it has.  
286 The extract activity against digestive enzymes was a half and a third of that of the controls for  
287 glucosidase and lipase respectively while it was weaker with amylase this may suggest that the  
288 extract compete with the substrates on the active sites, however, in case of amylase which reacts  
289 with larger molecule such as starch with many possible sites of reaction this will make competition  
290 process less likely. Moreover, the variety of the extract constituents may result in different  
291 affinities to react with different proteins [enzymes]. This is in agreement with many researches  
292 which found that plant extracts have variable activities against digestive enzymes due to levels of

293 polyphenols, flavonoids, terpenoids etc [31,32]. On the other hand, the current study of the  
294 antimicrobial activity of the crude alcoholic extracts of *E. tirucalli* against some types of pathogens  
295 has shown that it exhibited a low MIC against *S. aureus* and other bacteria under investigation.  
296 This has a great significance in the healthcare delivery system, so it could be used as an alternative  
297 to orthodox antibiotics in treatment of some infections caused by these microbes, especially as  
298 they usually developing resistance to the known antibiotics.

299 Antimicrobial activity can be explained due to the fact that *E. tirucalli* is high in tannins. Tannins  
300 are known to be surfactants and may inactivate microbial adhesions and also complex with  
301 membrane polysaccharides. Many plant genetic sources have been analyzed for their active  
302 components have antimicrobial activities. The antimicrobial activity exhibited by *E. tirucalli* may  
303 be attributed to the various active constituent's present, which also because of their individual or  
304 combined action, shows antimicrobial activity [33, 34]. Moreover, *E. tirucalli* showed complete  
305 inhibition of the yeast cells used in this experiment.

306 Antioxidant ability of bioactive compounds is very crucial in fighting against tumors as it was  
307 found that free radicals are significant players in the etiology of cancer. *E. tirucalli*'s extract was  
308 found to have mild ability in reacting with DPPH compared with control. This is may be due to  
309 that the extract was mechanically prepared and was mixed with methanol, meaning that not all  
310 active constituents were presented in the extract. Polyphenols could play an important role in this  
311 context and their effects have been studied *in vivo* and *in vitro*. Many polyphenols, such as  
312 anthocyanin, proanthocyanins, flavonoid, resveratrol, tannins, epigallocatechin-3-gallate, and  
313 gallic acid, have been tested; all of them showed promising effects however, their mechanisms of  
314 action were variable [35]. *E. tirucalli*'s extract showed potential toxicity against Caco-2 cell line.

315 A lot of pharmacological activities of *E. tirucalli* have been documented by many studies as  
316 molluscicidal activity, antimicrobial activity, antiherpetic and anti-mutagenic activity. The latex  
317 also shows anti-carcinogenic activities. In the northeast region of Brazil, the extract of *E. tirucalli*  
318 is used; as an antibacterial agent; a laxative effect; to treat intestinal parasites; to treat cough,  
319 asthma, rheumatism, cancer, sarcoma, and epithelioma skin tumors [36].

320 **Conclusion**

321 Taken together, our results suggest that the dried latex of *E. tirucalli* induces cytotoxicity to human  
322 cancer cells. However, the exact mechanism of action of the plant on the examined cells needs to  
323 be further explored. Moreover, the dried juice of *E. tirucalli* plant has a strong  $\alpha$ -glucosidase and  
324 lipase enzymes inhibitory activity. While the inhibitory activities against  $\alpha$ -amylase was weak in  
325 comparison with Acarbose drug. In addition, the *E. tirucalli* plant dried juice showed mild  
326 antioxidant effect. All these facts suggest the benefits of the *E. tirucalli* plant use in the  
327 pharmaceutical and functional foods industries due to its potential health benefits.

328 **Conflict of interest**

329 The authors declare that they have no conflict of interest with this research or its outcomes.

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433

435 **Table 1. The  $\alpha$ -glucosidase inhibitory activity and IC<sub>50</sub> values of *E. tirucalli* and Acarbose.**

436

Conc.	Acarbose	<i>E. tirucalli</i>
0	0±0	0±0
100	65.8±0.42	57.76±0.47
200	67.75±0.35	58.1±0.95
300	73.2±0.42	65.7±0.23
400	85.35±0.35	65.7±0.23
500	92.22±0.11	66.8±0
$\alpha$ -glucosidase inhibitory activity IC <sub>50</sub> [μg/ml], ±SD	37.1 ±0.33	79.43±0.38

437

439 **Table 2. Anti-lipase inhibitory activity and IC<sub>50</sub> values of *E. tirucalli* and Orlistat.**

440

Conc.	Orlistat	<i>E. tirucalli</i>
0	0±0	0±0
50	91.1±0.8	53.4±0.46
100	93.1±0.4	57.8±0.47
200	94.3±0.4	66.8±0
300	97.4±0.1	66.8±0
400	97.5±0	94.4±0.17
Anti-lipase activity IC <sub>50</sub> [μg/ml], ±SD	12.56±0.35	39.8±0.22

441

443 **Table 3. The  $\alpha$ -amylase inhibitory activity and IC<sub>50</sub> values of *E. tirucalli* and Acarbose**  
444

Conc.	Acarbose	<i>E. tirucalli</i>
0	0±0	0±0
10	53.1±0.22	31.4±0.67
50	55.0±0.13	34.5±0.34
70	66.1±0.04	53.1±0.22
100	66.1±0	55.0±0.13
500	72.3±0.38	55.0±0.13
$\alpha$ -amylase inhibitory activity IC <sub>50</sub> [μg/ml], ±SD	28.84±0.15	316.22±0.3

445

447 **Table 4. DPPH free radical scavenging property and IC<sub>50</sub> values of *E. tirucalli* and Trolox.**

448

Conc.	Trolox	<i>E. tirucalli</i>
0	0±0	0±0
2	53.4±3.46	26.5±0.51
5	61.5±0.34	28.2±0.25
10	91.4±0.34	31.3±0.52
20	97.3±0.34	34.4±0.26
50	97.3±0.34	34.4±0.26
100	97.3±0.69	54.4±2.31
Antioxidant activity IC <sub>50</sub> [μg/ml], ±SD	3.1 ±0.92	79.43±0.69

449

451 **Table 5. Inhibition zone of Ciprofloxacin [positive control]**

452

Bacterial strain	Inhibition zone [cm]
<i>S. aureus</i>	4
<i>Shigella sonnie</i>	5.5
<i>Pseudomonas aeruginosa</i>	5.5
<i>Escherichia coli</i>	5.5
<i>Enterococcus faecium</i>	5.8

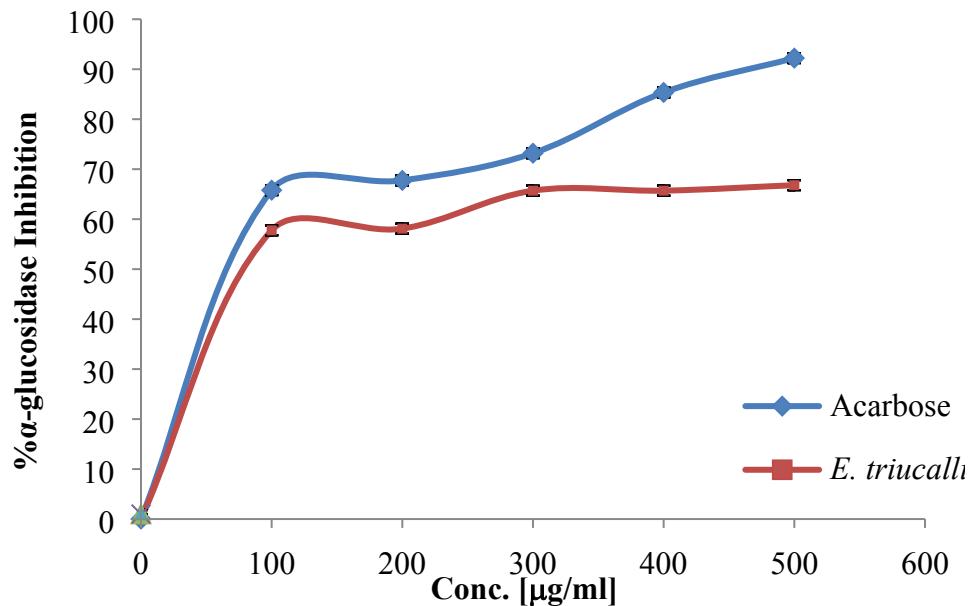
453

455 **Table 6. The growth of the fungus on the slant agar.**

456

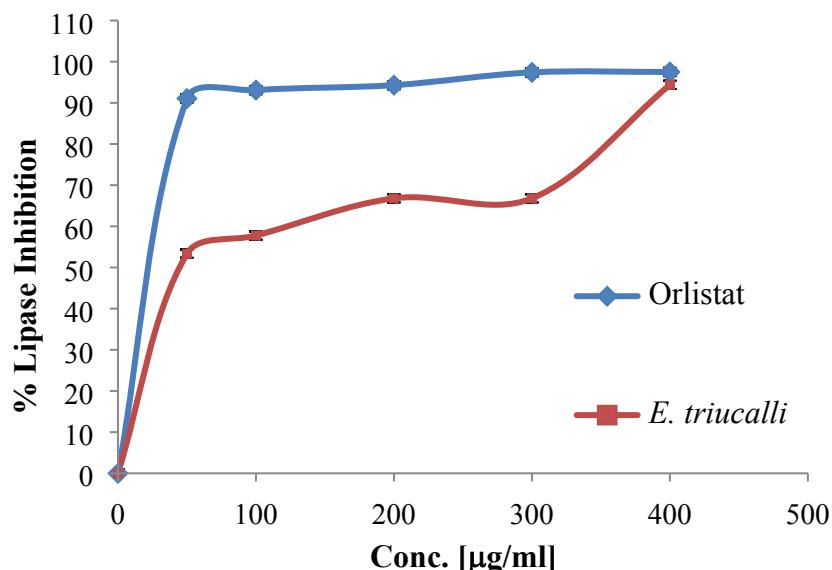
Tube number	Growth result
E1	No fungus growth
E2	No fungus growth
E3	No fungus growth
E4	No fungus growth
E5	No fungus growth
E6	Fungus growth

457

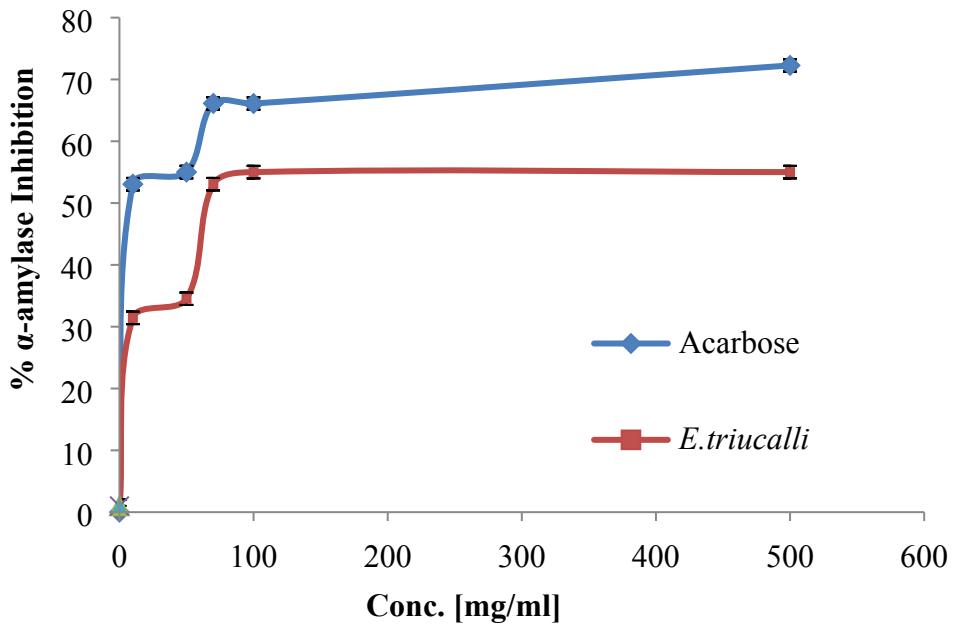


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**Figure 1. The dried juice of *E. tirucalli* plant and Acarbose antidiabetic drug  $\alpha$ -glucosidase inhibitory activities**



464  
465 **Figure 2. The dried juice of *E. tirucalli* plant and Acarbose antidiabetic drug lipase inhibitory**  
466 **activities**



468  
469 **Figure 3. The dried juice of *E. tirucalli* plant and Acarbose antidiabetic drug  $\alpha$ -amylase**  
470 **inhibitory activities**

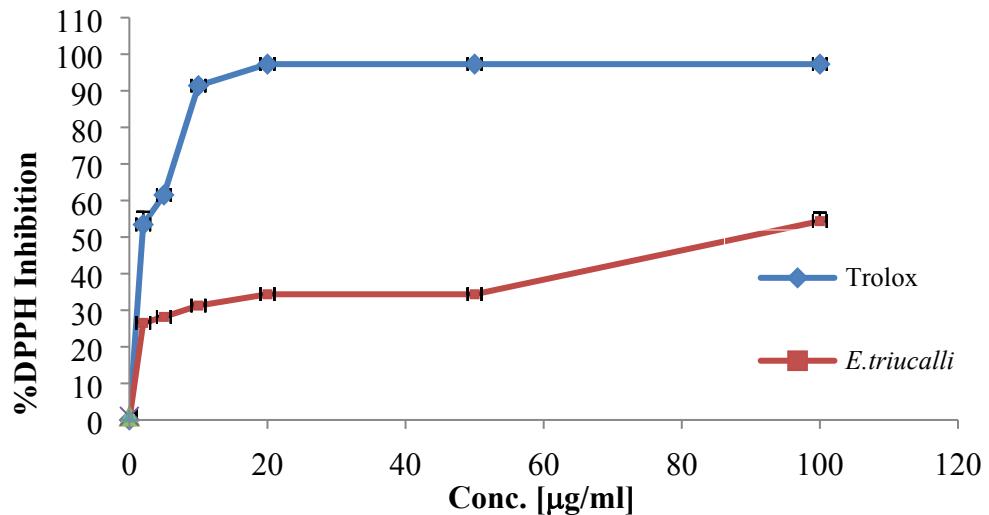
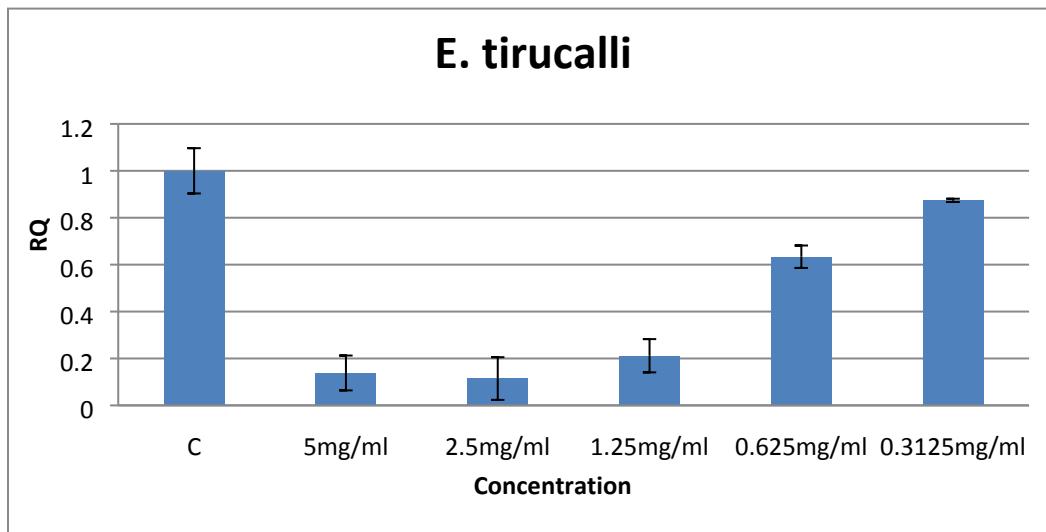


Figure 4. DPPH free radical scavenging property of *E. tirucalli* and Trolox.

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475



476

477 **Figure 5. The cytotoxicity of *E. tirucalli* on the Caco-2 cells.. Results were depicted as relative**  
478 **quantities [RQ] compared to the control [with only media; C]. \*P<0.001. Error bars**  
479 **represent SD.**

## Figure caption

**Figure 1.** The dried juice of *E. tirucalli* plant and Acarbose antidiabetic drug  $\alpha$ -glucosidase inhibitory activities.

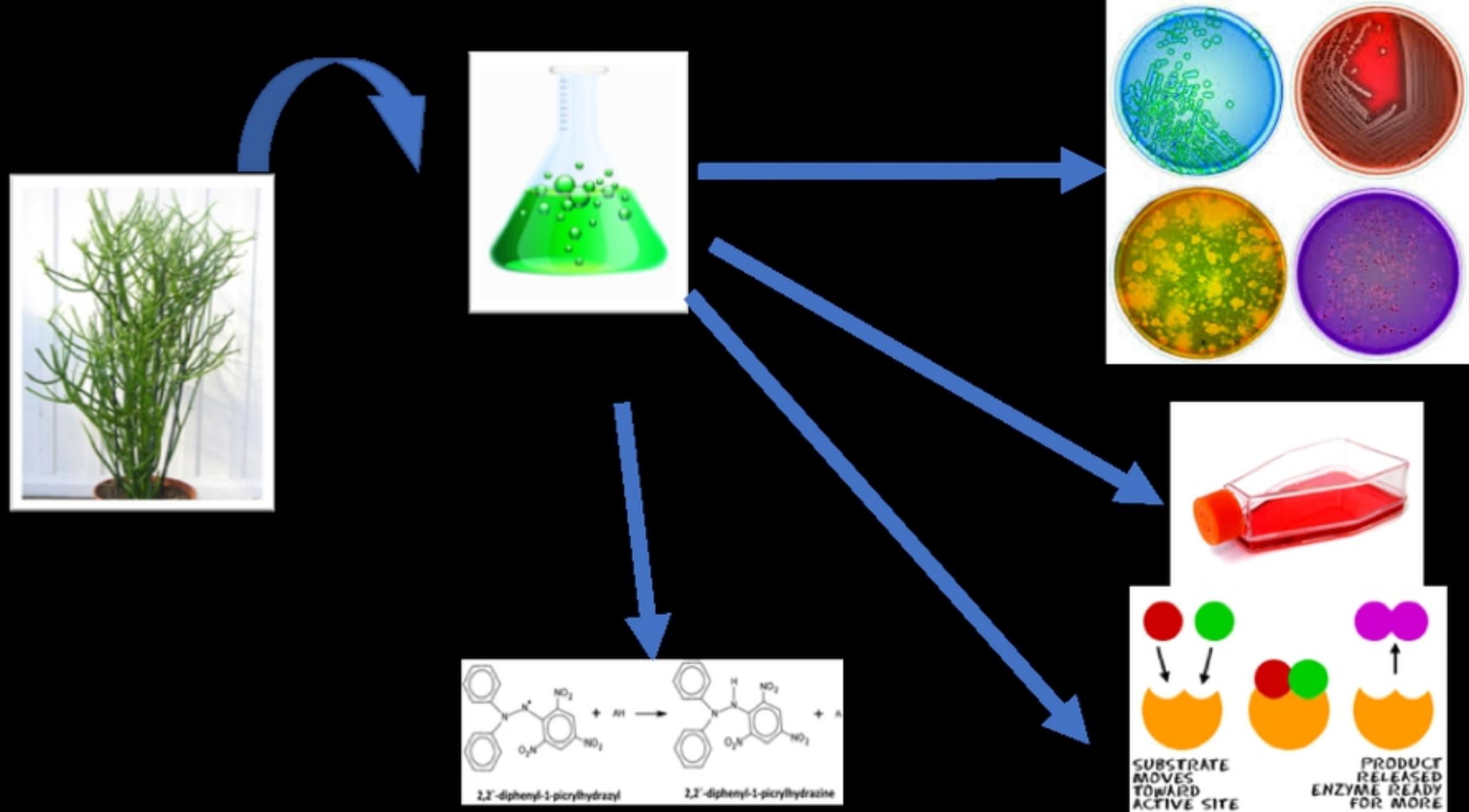
**Figure 2.** The dried juice of *E. tirucalli* plant and Acarbose antidiabetic drug lipase inhibitory activities. A 500  $\mu$ g/mL stock solution from each plant extract was dissolved in 10% dimethyl sulfoxide (DMSO), The substrate used was *p*-nitrophenyl butyrate (PNPB) and 0.1 ml of porcine pancreatic lipase (1 mg/ml) was mixed

**Figure 3.** The dried juice of *E. tirucalli* plant and Acarbose antidiabetic drug  $\alpha$ -amylase inhibitory activities

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**Figure 4.** DPPH free radical scavenging property of *E. tirucalli* and Trolox. Methanolic stock solution (1mg/ml) was set for *E. tirucalli* dried juice. Plant working solution (1 ml) was mixed with 1ml freshly prepared DPPH (0.002 g/ml) methanolic solution. The solutions were incubated at room temperature (25°C) in a dark place for 30 min. Then, their optical densities were measured by the UV/Vis spectrophotometer at 517 nm.

**Figure 5.** The effect of *E. tirucalli* on the cytotoxicity of Caco-2 cells. Caco-2 cells were treated with 5, 2.5, 1.25, 0.625, 0.3125 mg/ml of *Euphorbia tirucalli* crude extract for 24 h. Cytotoxicity was determined by MTT assay. Results were depicted as relative quantities (RQ) compared to the control (with only media; C). \* $P<0.001$ . Error bars represent SD.



Other