Production of cytotoxic compounds in dedifferentiated cells of *Jatropha curcas* L. (Euphorbiaceae)

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This study addresses the *in vitro* culture as an alternative to obtain compounds with cytotoxic activity from the medicinal plant *Jatropha curcas* (Euphorbiaceae). We determined the presence of cytotoxic compounds in both whole plants and dedifferentiated cells. We evaluated the effect of auxin, cytokinins and light on callus induction in cotyledon explants. We found that the most effective combination to induce callus was the auxin 2, 4-D (5 mM) with the cytokinin 6-BAP (2.5 mM), on Murashige-Skoog medium in darkness. We compared the callogenic potential among accessions from different geographic origins, finding that ARR-251107-MFG7 is most prone to form callus. The roots of *J. curcas* grown in field produced a compound chromatographically similar to the cytotoxic diterpene jatrophone. The profile of compounds extracted from the dedifferentiated cells was similar to that of the whole plant, including a relatively abundant stilbene-like compound. This study contributes to the future establishment of protocols to produce anti-cancer compounds from *J. curcas* cultivated in vitro.

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2 **(Euphorbiaceae)**

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20   **Abstract**

21   This study addresses the *in vitro* culture as an alternative to obtain compounds with cytotoxic

22   activity from the medicinal plant *Jatropha curcas* (Euphorbiaceae). We determined the presence

23   of cytotoxic compounds in both whole plants and dedifferentiated cells. We evaluated the effect

24   of auxin, cytokinins and light on callus induction in cotyledon explants. We found that the most

25   effective combination to induce callus was the auxin 2, 4-D (5 mM) with the cytokinin 6-BAP

26   (2.5 mM), on Murashige-Skoog medium in darkness. We compared the callogenic potential

27   among accessions from different geographic origins, finding that ARR-251107-MFG7 is most

28   prone to form callus. The roots of *J. curcas* grown in field produced a compound

29   chromatographically similar to the cytotoxic diterpene jatrophone. The profile of compounds

30   extracted from the dedifferentiated cells was similar to that of the whole plant, including a

31   relatively abundant stilbene-like compound. This study contributes to the future establishment of

32   protocols to produce anti-cancer compounds from *J. curcas* cultivated in vitro.

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41   **Introduction**

42   The search for less toxic and more potent anti-carcinogenic drugs is based on the fact that current

43   drugs are scarcely selective and highly toxic to normal cells (Mohan et al. 2012). The most

44   promising sources of such molecules are plants, especially those used for herbal medicine

45   (Alonso-Castro et al. 2011). In this sense, *Jatropha curcas*, a plant native to Mesoamerica and

46   recently rediscovered due to its potential as a source of raw material for biofuels (Salvador-

47   Figueroa et al. 2014), it has been used in traditional medicine by various peoples of Asia, Africa

48   and Latin America (Kumar & Sharma 2008). A variety of compounds with different biological

49   activities have been isolated from species belonging to the genus *Jatropha* (Devappa et al. 2010).

50   Among them are: jatrophol, a molecule with rodenticide activity (Jing 2005), the curcusones *a*, *b*

51   and *c*, and jatropholone *A*, with antineoplastic properties (Muangman et al. 2005;

52   Naengchomnong et al. 1986, 1994; Theoduloz et al. 2009), hydroxyl-jatrophone and other

53   diterpenes with potential antimetastatic activity (Devappa et al. 2011). There is evidence

54   suggesting that methanol extracts of the leaves have anti-metastatic and anti-proliferative activity

55   (Balaji et al. 2009). That compounds have been isolated from different plant parts in several

56   species of *Jatropha*, although the concentration is usually low (Goulart et al. 1993). This

57   situation is not unique to this genus, as in other plants bioactive molecules are also found in low

58   concentrations.

59   Given the above, the *in vitro* culture of dedifferentiated plant cells is an alternative for increasing

60   the concentration of the compounds of interest (Roberts 2007). In this regard, Fett- Neto et al.

61   (1994) obtained 100 times more taxoid in *Taxus cuspidata* callus than in the field plant.

62   However, *in vitro* culture does not always improve the concentration of the metabolite of interest

63   (Pletsch & Charlwood 1997), given the difficulties to obtain friable callus, the genetic variations

64   throughout the culture and the formation of cell aggregates (Chattopadhyay et al. 2002).

65   Therefore, the objectives of this study were a) to establish a procedure for obtaining friable and

66   fast growing calluses, and b) to evaluate the production of cytotoxic compounds in *J. curcas*

67   dedifferentiated cells.

68

69   **Materials and methods**

70   *Biological Materials*

71   Five accessions of *J. curcas* (Table 3) representing the regions of Chiapas (Mexico) were used,

72   from the Institute of Biosciences (IBC, initials in Spanish) *Jatropha* Germplasm Bank of the

73   Autonomous University of Chiapas (Mexico) located in the municipality of Tapachula, Chiapas

74   (14.4976 N, 92.4774 W, 58 m a.s.l., annual average temperature 30.7 ℃, annual average

75   humidity 80 %, average rainfall of 2632.9 mm and andosol-type soil.) For *in vitro* culture 50

76   seeds of each of the accessions were collected. For the whole plant phytochemical analysis,

77   samples of leaf, stem and root of the accession MAP-011107-G8 were used. In a parallel study

78   (not yet published), that accession was the most toxic among many Mesoamerican *J. curcas*

79   accessions analyzed. Those samples were washed with tap water, dried at 60 °C for 48 h and

80   ground to particle size of 500 μm.

81   *Induction of dedifferentiated tissue*

82   Cotyledons of different *J. curcas* accessions were used as explants for induction of

83   dedifferentiated tissue. In the first phase, the seeds of the accession MAP-011107-G8 were sown

84   on MS medium (Murashige & Skoog 1962), after disinfection with sodium hypochlorite at 5%,

85   following the procedure described by Soomro & Memon (2007), and kept in 2 d darkness and 2d

86   in light. After that period, the seeds were cut transversely, the embryo was removed and

87   cotyledons were sown on a MS medium supplemented with different hormone combinations, and

88   under different lighting conditions. For this phase, we used a full-randomized design with 32

89   treatments including a control without hormones, with three replications. Explants were

90   maintained for 20 d, at the end of which the dry weight of callus generated was quantified. Based

91   on the treatment that induced the highest amount of callus, the optimization process was

92   conducted based on the concentration of hormones, using a 6^2 factorial design, where the factors

93   were the hormones (2, 4-D and BAP) at six levels each, with four replications. In these

94   treatments the dry weight of callus was determined after 30 d of culture. Lastly, cotyledons of all

95   accessions were placed under the best conditions to induce callus, comparing among accessions.

96   *Determination of jatrophone content in field plants*

97   Three grams (± 0.1 g) of particles of different plant parts were subjected to extraction in

98   triplicate using refluxing (60 °C, 20 cycles) with 80 mL hexane in Soxhlet equipment. The

99   hexane was evaporated in a rotary evaporator to 50 °C and the yield (w/w) was calculated. The

100   separation and identification of jatrophone was performed by thin layer chromatography using

101   silica gel 60 plates of 5 x 20 cm (Sigma-Aldrich®, Fluka, Germany) washed with MeOH

102   (Hycel®, Mexico, purity 99.8%) activated at 50 °C for 5 min. For this, the residue obtained as

103   previously described was dissolved in hexane to achieve concentrations of 0.1 g/mL. An aliquot

104   (15 µL) of each of the extracts and of a mixture of jatrophone (10 mM) with jatropholone *a* and *b*

105   (4 mM based on jatropholone *a* dissolved in Hexane: Ethyl Acetate 7:3, kindly provided by Dr.

106   G. Schmeda-Hirschmann of the University of Talca, Chile), were placed individually on the

107   chromoplate lanes. The chromatogram was developed at 28 °C as a mobile phase a mixture of

108   Hexane: Ethyl Acetate 7:3. The compounds were revealed with sulphuric anisaldehyde using the

109   procedure reported by Pertino et al. (2007). Under the above conditions the Rf values for

132 then the residue with ethanol 96 °GL (Goulart *et al.*, 1993). Extracts were analyzed by GC-MS,

133 as described previously.

134 *Data analysis*

135 The concentrations of jatrophone and jatropholone *a* and *b* were obtained based on the areas

136 under the curve of the samples peaks in relation to those of the standards. The abundance of an

137 abundant stilbene-like compound (**1**) in callus was estimated using the peak's area of the

138 chromatogram. Data from all assays were subjected to analysis of variance (ANOVA) and

139 comparison of means (Tukey $\alpha \leq 0.05$).

140

141 **Results**

142 *Effect of auxins and cytokinins on the induction of callus*

143 All hormone treatments induced callus formation in cotyledons of *J. curcas*, accession MAP-011

144 107-G8. In all cases, the callogenesis started on the edge where the cutting was made and then

145 covered the rest of the explant. Table 1 shows the callus induction by the different phytohormone

146 treatments and lighting conditions. It was observed that exogenous phytohormones under any

147 lighting condition did not influence the formation of callus, as they were statistically similar to

148 the control ($p \leq 0.05$). The auxin 2,4- dichlorophenoxyacetic acid (2,4-D) presented lower values

149 of callus formation, but its callogenic effect was potentiated by combining it with the cytokinin

150 6-benzylaminopurine (BAP), either under light or darkness. Besides this, it was observed that

151 calluses from treatments with light showed signs of differentiation, producing chlorophyll and

152 compacting themselves, while those cultivated in darkness remained undifferentiated and were

153 friable (Figure 1).

154   To obtain the appropriate callus induction, different concentrations of phytohormones 2,4-D and

155   BAP were evaluated under darkness. In the overall analysis of the treatments, the combinations

156   of 2,4-D and BAP at 7.5 + 2 μM and 5 + 2.5 μM produced the largest amount of callus, were

157   statistically different from the other treatments, but equal among them (Table 2). Statistically

158   significant differences were found among the amounts of cell callus formed in the five

159   accessions evaluated (Table 3). The ARR-251107-MFG7 accession was superior in its capacity

160   of callogenesis.

161   *Cytotoxic compounds in mother plants.*

162   The yield of the hexane extracts depended on the type of tissue of the *J. curcas* studied. The

163   highest extract concentration was found in the roots (50 ± 2.7 mg/g dry weight), which had 1.6 -

164   1.8 times more extract than in the leaves (30 ± 2.8 mg/g dry weight) and bark (27.1 ± 3.9 mg/g

165   dry weight). Separation by thin layer chromatography of the components in all the hexane

166   extracts showed a compound with Rf similar to jatropholone (0.814), while for jatrophone (Rf =

167   0.772) only one spot with similar Rf (0.768) was found in the root extract (Figure 2). Separation

168   and analysis by gas chromatography-mass spectrometry of jatrophone positive fractions revealed

169   that the root hexane extract contains, in addition to other compounds, jatrophone and

170   jatropholones *a* and *b* (Table 4). Figure 3 shows the chromatogram and mass spectrum of the

171   fraction in which jatrophone was identified.

172   *Production of compounds in dedifferentiated cells*

173   Although 19 compounds (alkanes, fatty acids, among others) were found under the assayed

174   conditions of gas chromatography- mass spectrometry, no jatrophone or jatropholones were

175   detected in dedifferentiated cells. A major stilbene-like compound (**1**) was found, which was

176   present in all extracts with retention time of 27.21 min (Figure S1) and whose fragmentogram

177   showed the following ions and relative abundances: [**280** (**8**), 279 (12), 273 (14), 167 (53), 166

178   (14), 165 (19), 163 (62), 150 (52), 149 (32), 148 (40), 147 (32), **145** (**100**), 112 (13), 110 (13),

179   101 (7), 83(11), 70 (40), 68 (24), 57 (19), 53 (19)].

180   There were no differences in the amount of (**1**) produced by calluses cultivated in the light or

181   darkness. It was found that this metabolite is dependent on genotype as the accession ARR-

182   1251107-MFG7 had the greatest peak's area of this substance in comparison with the remainder.

183   Compared to the roots of field plants, calluses had 26 times more (**1**).

184

185   **Discussion**

186   Although a variety of compounds with cytotoxic activity isolated from *J. curcas* has been

187   reported (Misra & Misra 2010; Ravindranath et al. 2004; Van den Berg et al. 1995), our study

188   focused on three compounds: jatrophone, jatropholones *a* and *b*, which were present, especially

189   in the roots. In particular, jatrophone is a diterpene present in the genus *Jatropha,* which had not

190   been previously reported in *J. curcas.* Unlike the findings by Goulart et al. (1993), who detected

191   jatrophone in *J. elliptica* using hexane as eluent in column chromatography, in this study it was

192   detected in fractions where the elution solvent polarity was increased (Hexane: Ethyl Acetate

193   6:1).

194   Further studies of the extracts are required to corroborate the presence of jatrophone and discard

195   the possibility that we are dealing with other terpenoid compounds with similar chemical

196   structure. However, comparison of the mass spectrum of the compound detected [**312** (**14**), 297

197   (4), **284** (**29**), 216 (36), 202 (33), **187** (**34**), 175 (45), 173 (83), **159** (**100**), 147 (30), 145 (70), 133

198 (30), 130 (45), 119 (30), 107 (35), 105 (56), 91 (32), **79 (40)**, 77 (50), 69 (35)] with that of the

199 jatrophol, a terpenoid with the same molecular weight (Naengchomnong et al. 1994), showed

200 that they have different ionization patterns [312 (100) 297 (11), 281 (43), 253 (34), 240 (37), 225

201 (35)]. Conversely, we emphasize that the compound identified as jatrophone has a ionization

202 pattern very similar to that of the standard [**312 (15)**, **297 (6)**, 241 (67), 189 (66), 187 (34), 175

203 (31), 173 (43), 160 (75), **159 (100)**, 147 (41), 145 (38), 115 (39), 91 (43), 81 (51), 79 (43), 53

204 (67)] and to that reported by Pertino et al. (2007) for jatrophone.

205 In this work a maximum of 2038 µg/g of jatrophone was found, which represents 22.6 times the

206 maximum reported for *J. gossypiifolia* (90 µg/g, Kupchan et al. 1976) and 1.4 times than in *J.*

207 *isabelli* (1450 µg/g, dos Santos and Goulart 1999), but 4.9 times less than in *J. elliptica* (10 000

208 µg/g, Goulart et al. 1993). The *J. curcas* accession MAP-011107-G8 has a high amount of

209 jatrophone, although it is possible that the yields are affected by the extraction method. Although

210 these results are promising with respect to other species, it must be remembered that in all cases

211 the extraction is performed at the root, which involves waiting for the development of the latter

212 and the complete sacrifice of the plant.

213 In regard to the study of induction for dedifferentiation, it was found that when the exogenous

214 phytohormones auxin and cytokinin were at low concentrations did not influence the formation

215 of cell callus. The formation of callus in the control treatment was remarkable (Table 1). The

216 results of this study show that explants of *J. curcas* tend spontaneously to *in vitro* callogenesis,

217 denoting a high endogenous hormone load or high responsiveness of the plant tissues to healing,

218 since callogenesis invariably started at the cutting sites of the explant. In this sense, Sujatha et al.

219 (2005) mention that *J. curcas* leaf explants develop callus from the cutting margins. In other

220 species, *in vitro* spontaneous callogenesis has been observed, which has been linked either to

221   high hormone concentrations or to its *de novo* synthesis. In order to determine the cause of

222   spontaneous callogenesis in *J. curcas,* studies are needed regarding the dynamics of hormone

223   concentration in intact tissues and *in vitro* cultured.

224   Although auxin 2,4-D presented the lowest values of callus formation, its effect was potentiated

225   when combined with cytokinin BAP. For this reason wider ranges of concentration of both

226   hormones were explored and found that at higher concentrations (greater than 2.5 µM in the case

227   of auxins and above 2 µM in the case of cytokinins) there is an increased production of callus.

228   Similar results were obtained by Kaewpoo & Te- Chato (2009), who evaluated different growth

229   regulators in epicotyl and hypocotyl explants of *J. curcas* finding friable, soft and slightly yellow

230   callus in all treatments with 2,4-D (6.78 µM), while compact callus or shoots were obtained with

231   the other auxins evaluated (IBA or NAA). In the present study friable callus induction was

232   observed when using NAA although in lesser amounts, which is consistent with results obtained

233   by Shrivastava & Banerjee (2008) who at assessing concentrations from 0.2 µM to 5.37 µM of

234   NAA obtained the highest amount of callus at the highest concentration.

235   In the study for optimization of concentrations, it was observed that the largest amount of callus

236   is formed at concentrations of 5 µM and 7.5 µM of 2, 4-D, which are statistically equal (Table

237   2). However, we suggest the use of the lowest effective concentration, since high concentrations

238   of auxin are almost always detrimental to the production of secondary metabolites (Kim et al.

239   2007).

240   In the case of the selection of cytokinins it is advisable to use BAP at 2.5 µM, since when

241   combined with auxin callogenesis is potentiated, which did not occur with other cytokinins. This

242   coincides with the study of Kalidass et al. (2010). They studied the effect of BAP in the

243   production of *Catharanthus roseus* callus and found that increasing the levels of BAP resulted in

244   higher dry weight of callus. In other studies with explants of *J. curcas* effective concentrations of

245   BAP for callogenesis were found, similar to the findings in the present study (Datta et al. 2007;

246   Rajore & Batra 2007; Wei et al. 2004).

247   In regard to lighting conditions, it does not influence the amount of callus formed, however, it

248   was significant in the differentiation of callus tissue. Since the long-term objective is obtaining

249   dedifferentiated cell cultures that can be maintained in bioreactors, the condition of darkness is

250   preferable. Paz et al. (2006) mention something similar. They found that under lighting, friable

251   calluses could be conditioned to a vitrification process, which they did not observe in the

252   conditions of darkness. Also, Pletsch & Charlwood (1997) found that the production of

253   jatrophone in cultures *in vitro* of *J. elliptica* decreases up to 15 times under continuous lighting.

254   There are other studies which conclude that in the condition of darkness calluses can accumulate

255   more secondary metabolites compared to those grown under light (Banthorpe et al. 1986;

256   Mukundan & Hjortso 1991; Yazaki et al. 2001).

257   It is worth noting that jatrophone was found in the mother plant but not in the calluses, probably

258   because of two main reasons: 1) Calluses do not produce jatrophone. Charlwood et al. (1990)

259   point out it is common that dedifferentiated cells lack the capacity for synthesizing isoprenoids

260   present in the mother plant and this is related to its disorganized nature. Similar results are

261   reported in the biosynthesis of heavier terpenes such as steroids (Lui & Staba 1979). Other

262   possibility is that *in vitro* cultures may have the ability to synthesize the compounds produced by

263   the field plant but lack the capacity to accumulate them (Banthorpe et al. 1986). 2) *In vitro*

264   cultures produce smaller quantities than those detectable by the equipment used (lower than 3

265   μg/mL). In this regard Pletsch & Charlwood (1997) detected minimum amounts of jatrophone (3

266   μg/g of dry weight), which were at the detection limit. Nevertheless, cell calluses accumulated a

267   large amount of the compound **(1)** in relation to that found in the field plant (26 times more),

268   which deserves further studies for identification and biological activity.

269   In this study, a compound similar to jatrophone in root hexane extracts of *J. curcas* grown in the

270   field was identified, while the best treatment for callus induction was the addition of 2, 4-D 5

271   μM, together with BAP 2.5 μM in dark conditions. However, the evaluation of the synergy

272   between 2,4- D and NAA is recommended, as well as the evaluation of BAP in concentrations

273   higher than the ones evaluated in the current optimization. Lastly, when evaluating the possible

274   differences between accessions, ARR-251107-MFG7 turned out to be the one, which produced

275   the greatest amount of callus. In the analyses of extracts of dedifferentiated cells were not

276   detected desirable compounds of interest, like jatrophone or jatropholones, however, this

277   approach could be used towards providing large quantities of a particular starting material.

278

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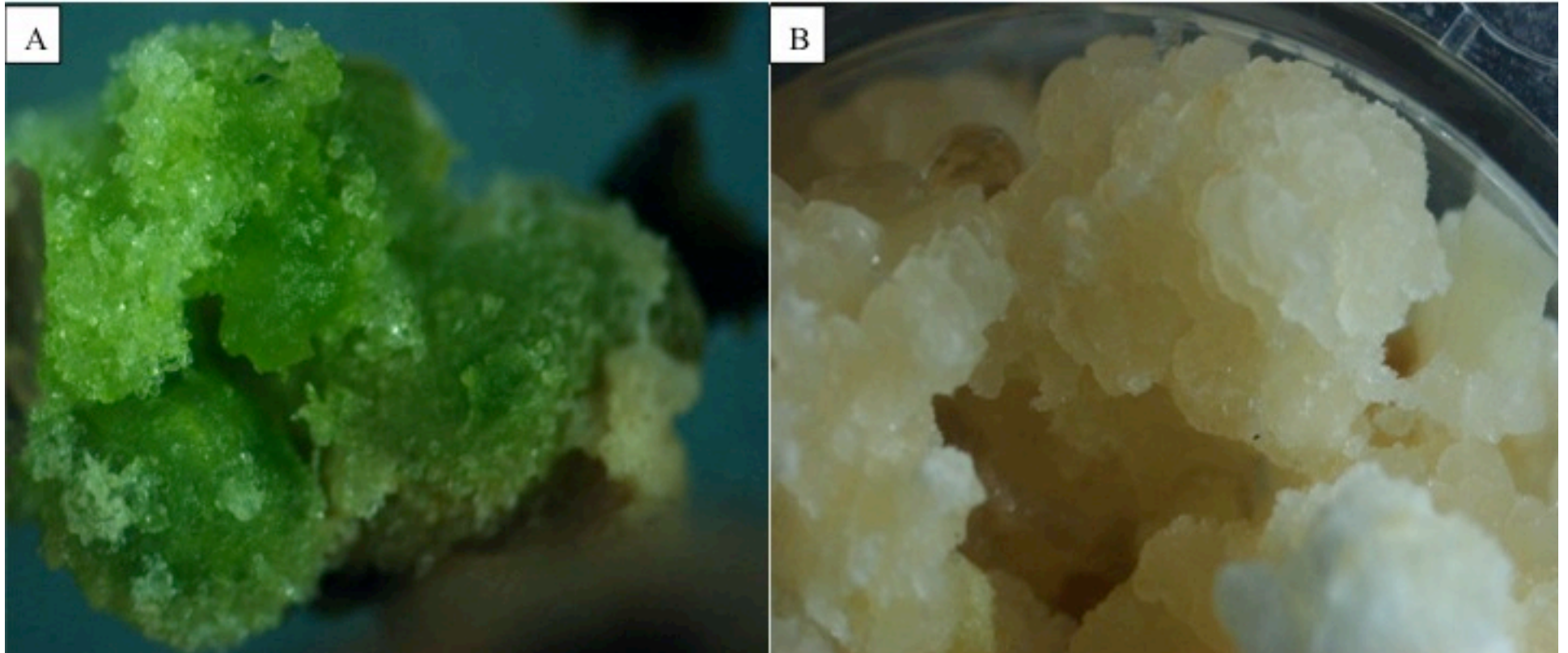
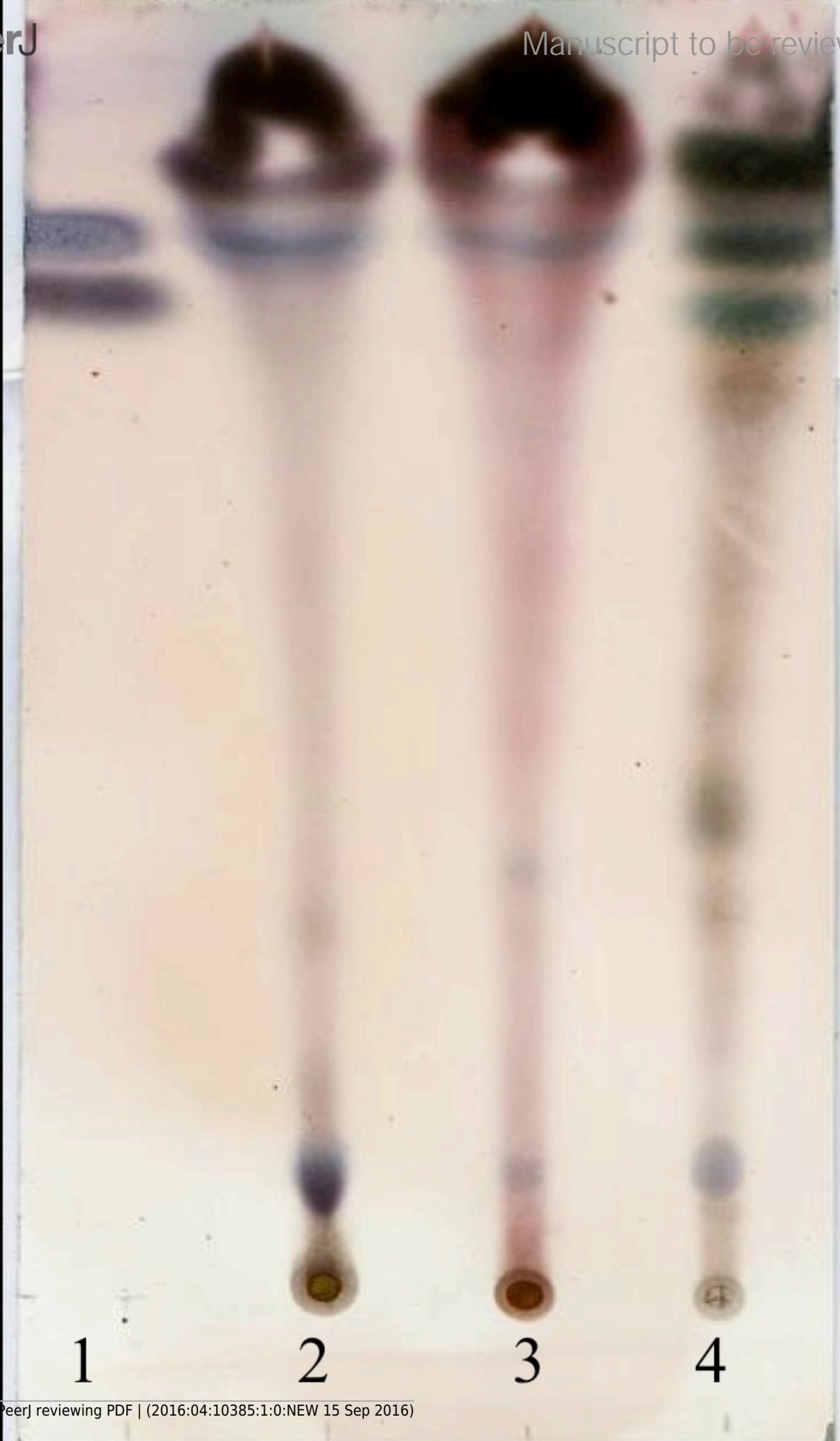
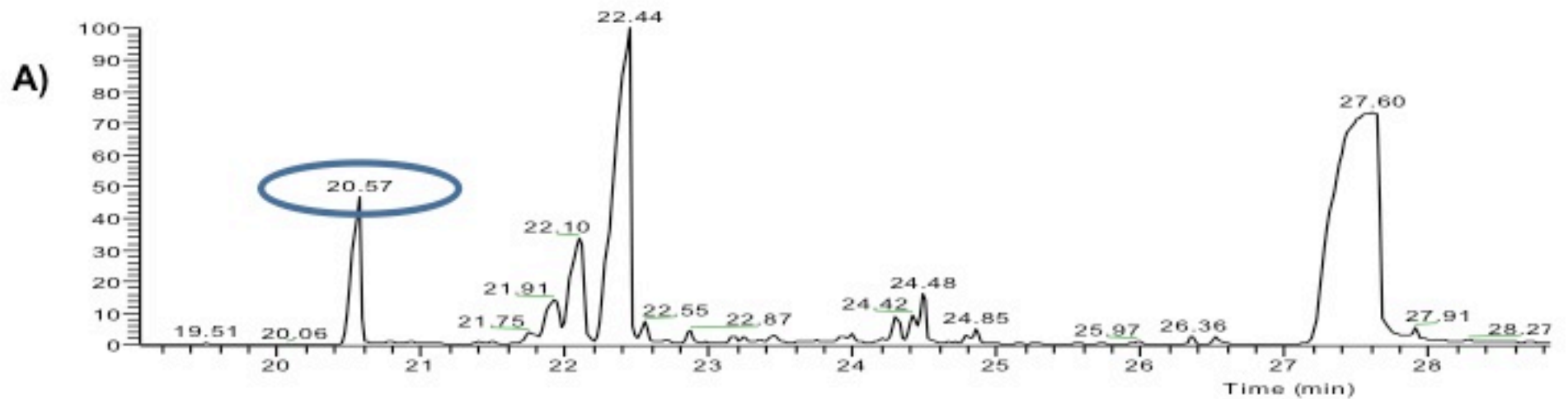
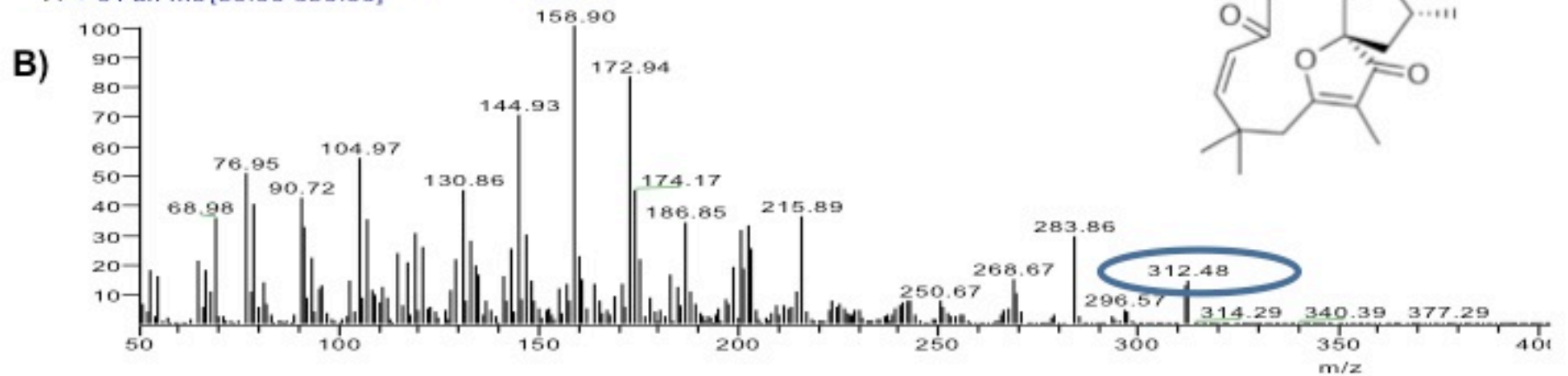
Influence of illumination on the appearance of Jatropha curcas calli

Callus induced in conditions of light and darkness from cotyledon explants of *Jatropha curcas*.
A) Developing photosynthetic callus. B) Developing callus of "sugary" and friable
appearance.

Thin layer chromatogram of crude hexane extracts from different tissues of Jatropha curcas MAP- 011107-G8.

Extracts were diluted to 0.1 g/mL and revealed with sulphuric anisaldehyde (Pertino et al., 2007). Lanes: 1) mixture of standards Jatrophone 10 mM + Jatropholone at 4 mM + *b*; 2) leaf extract; 3) bark extract; 4) root extract. In lane 1 band **"a"** represents the jatropholones (Rf = 0.817); and band **"b,"** the jatrophone (Rf = 0.772).

Analysis of hexane extracts of Jatropha curcas by GC-MS

A) Chromatogram of a fraction of the root hexane extract of *Jatropha curcas* MAP-011107-G8, where the peak corresponding to jatrophone is circled. B) Fragmentogram of jatrophone–retention time 20.57 min, molecular weight is circled 312g • gmol^{-1}.

Effect of phytohormones and lighting conditions on callus dry weight (mg) obtained from cotyledon explants of *Jatropha curcas*.

Each treatment was repeated three times and each replicate consisted of 20-30 cuttings of a seed cotyledon from accession MAP-011107-G8. Means with different letters are significantly different ($p \leq 0.05$). Formulation of Medium: Basal Medium MS + 3% sucrose (p/v) + 500 mg• L^{-1} PVP. Auxins: 2,4-D (2,4-dichlorophenoxyacetic acid), NAA (naphthalene acetic acid), IAA (indole-3-acetic acid). Cytokinins: KIN (kinetin), BAP (6-benzylaminopurine), SAD (adenine sulfate).

Treatments	Lighting	
	Light	Darkness
Control	189 ± 8^{abc}	183 ± 37^{abc}
2,4-D	135 ± 36^{c}	136 ± 14^{c}
NAA	168 ± 18^{abc}	190 ± 42^{abc}
AIA	178 ± 12^{abc}	201 ± 30^{abc}
KIN	166 ± 36^{abc}	152 ± 40^{bc}
BAP	168 ± 12^{abc}	207 ± 33^{abc}
SAD	154 ± 37^{bc}	128 ± 8^{c}
2,4-D + KIN	199 ± 33^{abc}	235 ± 52^{abc}
2,4-D + BAP	289 ± 107^{a}	280 ± 13^{ab}
2,4-D + SAD	154 ± 30^{bc}	234 ± 39^{abc}
NAA + KIN	163 ± 18^{abc}	245 ± 34^{abc}
NAA + BAP	170 ± 42^{abc}	239 ± 95^{abc}
NAA + SAD	183 ± 23^{abc}	208 ± 41^{abc}
AIA + KIN	197 ± 46^{abc}	203 ± 52^{abc}
AIA + BAP	156 ± 37^{bc}	205 ± 34^{abc}
AIA + SAD	227 ± 18^{abc}	201 ± 46^{abc}

Combined effect of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and cytokinin 6-benzylaminopurine (BAP) on the formation of cell callus in cotyledons of *Jatropha curcas* accession MAP-011107-G8

The data were taken 30 days after culture in darkness. Each treatment had three replicates and each repetition consisted of 20 to 30 cuttings in a cotyledon. Different letters denote statistical differences revealed by ANOVA and Tukey's tests ($p \leq 0.05$). Formulation of Medium: Basal Medium MS + 3% sucrose (p/v) + 500 mg • L^{-1} PVP.

BAP	2,4 D (µM)					
(µM)	0	1	2.5	5	7.5	10
0	196 ± 10^{efghij}	138 ± 10^{j}	145 ± 7^{ij}	186 ± 20^{ghij}	142 ± 10^{j}	191 ± 20^{efghij}
0.5	194 ± 10^{efghij}	271 ± 110^{bcdefgh}	153 ± 21^{hij}	156 ± 30^{hij}	194 ± 20^{efghij}	208 ± 40^{defghij}
1	152 ± 20^{hij}	199 ± 20^{defghij}	246 ± 31^{cdefghij}	285 ± 30^{abcdefg}	303 ± 10^{abcdefg}	313 ± 20^{abcde}
1.5	140 ± 20^{j}	0.190 ± 20^{fghij}	376 ± 24^{ab}	223 ± 20^{cdefghij}	245 ± 30^{cdefghij}	267 ± 30^{bcdefghi}
2	203 ± 60^{defghij}	0.221 ± 40^{abc}	342 ± 46^{cdefghij}	209 ± 60^{defghij}	**405 ± 11^{a}**	319 ± 20^{abcd}
2.5	211 ± 10^{defgihj}	0.219 ± 30^{defghij}	375 ± 33^{ab}	**402 ± 20^{a}**	311 ± 10^{abcdef}	307 ± 20^{abcdefg}

Jatropha curcas accessions representative of regions in Chiapas State (Mexico) used in this study and their callogenic ability when induced with 2,4-D (5 µM) and BAP (2.5 µM).

*Populations located in Chiapas, Mexico. Source: Ovando-Medina et al. (2011). **Coefficient of variation was 64.9 %. The data were taken 30 days after culture. Each accession had three replicates and each repetition consisted of 20 to 30 cuttings of a cotyledon. Different letters denote statistical differences revealed by ANOVA and Tukey's tests ($p \leq 0.05$). Composition of medium: Medium MS (1962), 3% sucrose p/v and 500 mg•Lof Polyvinylpyrrolidone + 2, 4-D (5 µM) + BAP (2.5 µM).

Accession*	Latitude	Longitude	Population	Callus dry weight (mg)**
ARR-251107-MFG7	16°11.231'	93°54.516'	Isthmus	1014 ± 578^{a}
MAP-011107-G8	15°25.505'	92°53.554'	Soconusco	402 ± 23^{ab}
JIQ-090208-AG1	16°40.012'	93°39.242'	Center	175 ± 31^{b}
PUJ-030 508-S4	16°16.430'	92°17.550'	Frailesca	204 ± 19^{b}
CDCU-030208-F4	15°40.473'	92°00.129'	Border	207 ± 50^{b}

Compounds identified in the root hexane extract of *Jatropha curcas* accession MAP-011 107-G8, by gas chromatography-mass spectrometry.

Yields were calculated based on the areas under the peak curve of purified standards.

Structure	Molecular Weight (g•gmol^{-1})	Retention time (min)	Yield (mg compound•g sample^{-1})
Jatrophone	312	20.57	2.038
Jatropholone *a*	295	22.1	6.331
Jatrotropholone *b*	296	22.3	1.668