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Phorbol Ester Quantitation in Dehulled Seed Structural Parts of Eight Accessions of *Jatropha curcas* from Latin America

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ABSTRACT

Phorbol esters (PE) present in *Jatropha curcas* seed are heat stable toxic compounds causing activation of promoter's kinase C and cancer. The objective of this study was to quantify and compare the amount (mg/g) of PE in structural parts (cotyledons, tegument and embryo) of eight accessions from Mexico, El Salvador, Brasil and Honduras. PE were extracted and quantified by high pressure liquid chromatography. A split plot design was used where the main plots were the accessions and the subplots were the structural parts of the seed. An LSMeans mean separation test was performed to determine significant differences (P<0.05) between accessions and structural parts. All accessions were toxic because they exceeded the value considered non-toxic (PE 0.10 mg/g of dry seed). Accessions with the highest content of PE were Mexican and India Salvadoreña with 7.56±0.36 and 7.36±0.16 mg/g seed respectively and the lowest was Puebla (3.15±0.10 mg/g seed). The cotyledons had significantly higher values of PE (6 mg/g) than tegument (0.9 mg/g) and embryo (0.7 mg/g). The higher concentration of PE in the cotyledons may discard the possibility of reducing toxicity of *Jatropha* seed oil and meal by mechanical removal of the tegument as previously suggested.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN
A split plot design with three replications was used. Phorbol ester (PE) content (mg/g) was measured in three dehulled seed structural parts (tegument, cotyledon and embryo) from eight accessions from Honduras (Arturo Araujo and 111), Brazil (Bravo x Mali and EMBRAPA), El Salvador (Criolla Salvadorena and India Salvadorena) and Mexico (Mexican and Puebla). Seed samples were obtained on three harvest dates (July 3rd and 26th, and August 19th 2013). ANOVA and LSMEANS were performed with SAS® v. 9.3.

DEHULLED SEED STRUCTURE
100g samples of dehulled seeds were taken as experimental units. Structural parts were separated manually with scalpel and forceps and weighed with an Ohaus Explorer analytical balance to the nearest 0.1 mg (Figure 1).

MOISTURE OF SEEDS
AOAC 952.08 method was used to measure moisture content (g/100g) of dehulled *Jatropha* seeds.

PHORBOL ESTER EXTRACTION
300 mg of sample and 50 µg of phorbol 12-myristate, 13-acetate (PMA, internal standard) were mixed and extracted with 4 ml of a hexane-isopropanol (3:2) solution following the method by King *et al.* (2009). The extract was disolved in acetonitrile, defatted with hexane, passed through a 0.45µm PFTE filter and concentrated under a stream of N_2 to 300 µl in vials for HPLC injection.

PHORBOL ESTER QUANTITATION
PE and PMA were identified and quantified (mg PE/g sample, dry basis) with an Agilent Eclipse Plus C_{18} column (150 mm x 4.6 mm x 5 µm) by HPLC-DAD at 240 nm following the method by King *et al.* (2009)

RESULTS

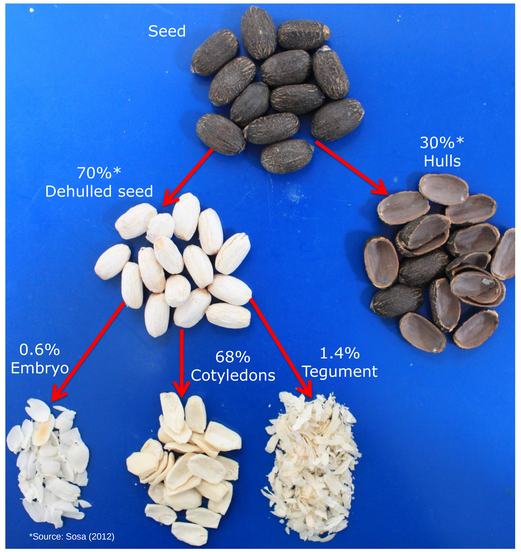

Figure 1. Seed structural parts of *Jatropha curcas*

Table 1. Yield of structural parts from dehulled seed of eight accessions of *Jatropha curcas*

Country	Accession	Cotyledons g part/100 g dehulled seed Mean±SD*	Tegument Mean±SD*	Embryo Mean±SD*
Mexico	Mexicana	96.5^{cd} ±0.4	2.4^{a} ±0.3	1.1^{ab}±0.1
	Puebla	96.8^{bcd}±0.1	2.1^{ab} ±0.1	1.1^{ab}±0.1
El Salvador	Criolla Salvadorena	97.5^{a} ±0.2	1.7^{cd} ±0.2	0.9^{b} ±0.1
	India Salvadorena	97.5^{ab} ±0.2	1.6^{c} ±0.1	1.0^{b} ±0.1
Brazil	Embrapa	97.1^{abc}±0.3	2.0^{abc}±0.2	0.9^{b} ±0.1
	Bravo x Mali	96.2^{d} ±0.4	2.4^{a} ±0.3	1.4^{a} ±0.2
Honduras	111	96.6^{bcd}±0.3	2.1^{ab} ±0.2	1.2^{ab}±0.1
	Arturo Araujo	97.1^{abc}±0.2	1.9^{abc}±0.1	1.0^{b} ±0.1

[a]to[d] different letters on the same column indicate significant difference among varieties at (P<0.05).
*SD=Standard Deviation.
*%CV = Percent Coefficient of Variation

Table 2. ANOVA summary for phorbol ester content in seed structural parts of eight accessions of *Jatropha curcas*

Source of Variation	F	Pr>F*
Accession	50.16	<0.0001
Part	441.79	<0.0001
Accession × Part	72.05	<0.0001

*Statistically significant at P <0.05.

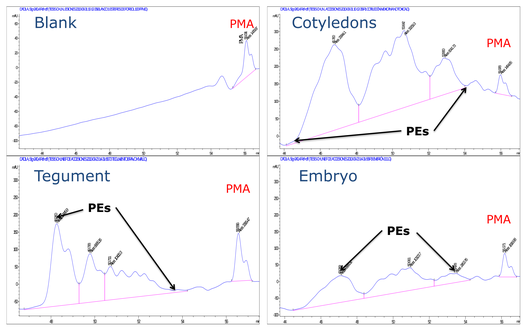

Figure 2. Phorbol esters in chromatograms from dehulled seed structural parts from a Mexican variety of *Jatropha curcas*

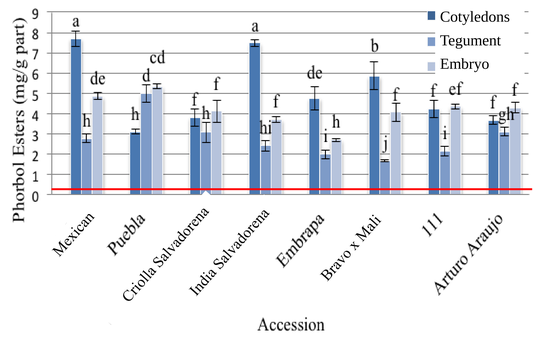

Figure 3. Phorbol ester content of dehulled seed structural parts of eight accessions of *Jatropha curcas*

DISCUSSION

DEHULLED SEED STRUCTURE
Proportions of dehulled Jatropha seed structural components varied significantly among accessions (Table 1). Cotyledons comprised 96.2-97.5%, tegument 1.6-2.4% and embryo represented 0.9-1.4% of the dehulled seed. Devappa *et al.* 2011 reported similar proportions in Jatropha seeds.

PHORBOL ESTER DISTRIBUTION
Significant differences in dehulled seed PE content were observed among accessions (P<0.01), being Mexican the highest (7.6 mg/g) and Puebla the lowest (3.2 mg/g). Variability among seed structural parts was eight times higher than among accessions (Table 2). Phorbol esters were detected in all dehulled seed structural parts from all accessions (Figure 2). King *et al.* (2009) found that most of PEs were concentrated in the tegument. However, all accessions had >97% of PEs concentrated in cotyledons and only 0.5-3.4% in the tegument, with the rest in the embryo (Table 1, Figure 3). This discards the possibility of mechanically detoxifying Jatropha seeds by removing the tegument and make them edible.

DEHULLED SEED TOXICITY
All structural parts from all eight accessions were considered toxic (>0.1 mg PE/g) (Figure 3) according to Verdugo et al. (2010). Non-toxic Jatropha varieties have been found in south and western Mexico (0.03-0.08 mg PE/g).

CONCLUSIONS

- All eight accessions of *Jatropha curcas* had toxic PE concentrations in dehulled seed and its structural components.
- Most PE were concentrated in the cotyledon. Mechanical removal of tegument did not reduce PE in *Jatropha curcas* dehulled seed below toxic levels.

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