Analysis of Phorbolester Content During Growth and Development of *Jatropha curcas* Fruits

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ABSTRACT: Utilization of *Jatropha curcas* seed meal as animal feed is limited by the presence of toxic compounds. Phorbolester present in *Jatropha curcas* as a family of compounds known to cause a large number of biological effects such as tumor promoters. The aim of this research is measure phorbolester content during growth and development of *Jatropha curcas* fruit. Phorbolester extracted by sonification method and analysed by UPLC using phosphoric acid and acetonitrile as mobile phase. *Jatropha curcas* fruit has ripe in the fifth week because of yellowing of fruit capsule. Toxic genotypes of *Jatropha curcas* has higher phorobol ester content than non-toxic genotypes approximately 182.1 and 55.2 ng/g respectively. Fruit capsule (122.2 ng/g) has higher phorbolester content than fruit seed (115.2 ng/g). It is due to biosynthesis of diterpene that occur in plastid. Biosynthesis of phorbolester possibly occured in initiation and maturation of fruit because the high level of phoroblester occur in the first and fifth week.

Keywords: *Jatropha curcas*, toxic genotypes, non-toxic genotypes, fruit seed, fruit capsule

1. Introduction

*Jatropha curcas* is a multipurpose bush or a small tree belonging to the family of Euphorbiaceae. The primary use of *J. curcas* seeds is for oil extraction which is a good alternative to biofuel, and has proven success used either independently or by mixing the diesel. *J. curcas* seed contains high amount of oil that can be converted into biodiesel of high quality upon transesterification. Apart from the oil, the seed cake or *J. curcas* seed meal has gained tremendous interest for their utilization in feed formulations (Makkar et al. 1997). *J. curcas* kernel meal is rich in protein and the essential amino acid composition of the protein, except lysine, is comparable to that of soybean meal (Makkar et al. 1998). However, the main toxins present in these by-products are phorbol esters, which prevent their utilization as feed ingredients.

The term 'phorbol' is used to describe the family of naturally occurring compounds that can be referred to as tigliane diterpenes. The structure of the phorbol esters is dependent on the tetracyclic diterpene carbon skeleton known as tigliane. Tigliane is the fundamental alcohol moiety in the phorbol esters (Goel et al. 2007). Biological activity of phorbolestes are reported to be potent tumor promoters. These are responsible for skin irritant effects and tumor promotion because they stimulate protein kinase C, which is involved in signal transduction and developmental processes of most of the cells and tissues, producing a variety of biological effects in a wide range of organisms.

Toxicity of Jatropha seeds has been studied extensively in different animal models like goats, sheep, mice, rats, and fish when fed with phorbol ester–containing feeds (Adam 1974; Makkar and Becker 1999). In most of the studies, the animals were force-fed. Decrease in the glucose level, increase in concentration of arginase, glutamate, and oxaloacetate transaminase in the serum was observed in goats with lack of appetite, reduced water intake, diarrhea, dehydration, and other hemorrhagic effects in different organs (Adam and Magzoub 1975). Li et al. 2010 reported that intragastric administration of *J curcas* seed and oil had LD50 and 95% confidence limits for male mice were 27.34 mg/kg body mass and 24.90–29.89 mg/kg body mass; and the LD5 and LD95 were 18.87 and 39.62 mg/kg body mass, respectively.

No quantitative information is available on the content of phorbolester during growth and development of *Jatropha curcas* fruit. The present study...
is an attempt to measure phorbol ester content to the toxic and non-toxic genotypes of *Jatropha curcas*.

### 2. Material and Method

#### 2.1 Plant material

*Jatropha curcas* genotype Jatromas seed were collected from PT Bumi Mas Eka Persada. *Jatropha curcas* fruits from five trees harvested from April to May 2013. Two genotypes of toxic are *Jatropha curcas* J11 (a) and *Jatropha curcas* J15 (b), whereas the non-toxic are *Jatropha curcas* WNG (c) and *Jatropha curcas* MND (d). *Jatropha curcas* were collected in first, third, fifth, and seventh week. Seed and capsule of *Jatropha curcas* were separated and dried. Analysis of phorbol ester has been done by UPLC.

#### 2.2 Determination of phorbol ester

The extraction of phorbol ester using modified Makkar et al. (1997) method. Briefly, 0.25 g of the sample was sonificated two times with methanol. Phorbol ester was determined on reverse phase (Acquity UPLC BEH C18, endcapped 1.7 µm) 50x2.1 mm. The phorbol ester peak was identified at 280 nm. Phosphoric acid 0.14% and acetonitrile (25:75) used as mobile phase. A total of 1 µL of sample solution was injected. Phorbol-12-myristate 13-acetate used as standard.

### 3. Result and Discussion

Growth and development of *Jatropha curcas* fruits showed in Figure 1. The fruit has ripe in fifth week that showed yellowing of fruit capsule. In the seventh week, fruit looks brownish that indicate the fruit has been rotten.

The analysis in the present work used an isocratic mixture of 75% acetonitrile and 25% phosphoric acid 0.14% which reduced the retention time of the phorbol ester by about 30 min to 6 min compared to HPLC method by Makkar et al. 2009. This reduction of analysis time correlated with high pressure and very tiny stationary phase particles of UPLC (Figure 2). The phorbol ester content within the toxic and non-toxic fruit seed and capsule of *Jatropha curcas* showed difference in first and fifth week. Phorbol ester content in toxic genotypes higher than non-toxic genotypes, fruit seed of toxic genotypes approximately 175.7 ng/g and non-toxic genotypes 54.6 ng/g (Figure 3). On the other hand, fruit capsule of toxic genotypes approximately 188.5 ng/g and non-toxic genotypes 55.9 ng/g (Figure 4). Makkar and Becker (1997) reported that toxic (Cape Verde and Nicaragua) and non-toxic (Mexico) genotype of *Jatropha curcas* approximately 2.44 and 0.11 mg/g kernel. So phorbol ester content of *Jatropha curcas* genotypes of PT Bumi Mas were lower than Makkar and Becker's genotypes.

Fruit capsule has higher phorbol ester content than fruit seed. Average value of phorbol ester content in fruit capsule 122.2 ng/g and fruit seed 115.2 ng/g. Phorbol ester is a diterpene group which is synthesised in plastid (David and Croteau 2000). Therefore, phorbol ester content in fruit capsule higher than fruit seed.

The graph (Figure 4) indicate that biosynthesis of phorbol ester occured in initiation (first week) and maturation (fifth week) of fruit. Phorbol ester level in seventh week decreased. It is possible that phorbol ester has degraded due to fruit maturity.
Fig. 1 *Jatropha curcas* fruits in the first week (a), third week (b), fifth week (c), and seventh week (d).

Fig. 2 UPLC chromatogram of standard phorbol ester.

Fig. 3 Phorbol ester content in fruit seed.
4. Conclusion

Phorbolester content in toxic genotypes were higher than non-toxic genotypes approximately 182.1 and 55.2 ng/g respectively. Fruit capsule (122.2 ng/g) has higher phorbolester content than fruit seed (115.2 ng/g). The high level of phorbolester occurred in initiation (first week) and maturation (fifth week) of fruit due to biosynthesis of phorbolester.

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References


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