KnE Energy
ISSN 2413-5453 Volume 1 (2015) 125-128
Renewable Energy and Energy Conversion Conference and Exhibition
(The 2nd Indo EBTKE-CONEX 2013)

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

Randi Abdur Rohman[a*], Dwi Yono, Nurita Toruan-Mathius[a] and Roy Hendroko

[a] *PT Sinar Mas Agro Resources and Technology, Bogor, Indonesia*

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 phorbolester 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 phorbolesters 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

* Corresponding Author: Telp: +62-881-1233870
Email: randi.abdur.rohman@gmail.com

Peer-review under responsibility of EBTKE-CONEX 2013
DOI http://dx.doi.org/10.18502/ken.v1i1.340

is an attempt to measure phorbolester 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* fruits were separated and dried. Analysis of phrobolester has been done by UPLC.

2.2 Determination of phorbolester

The extraction of phorbolester using modified Makkar et al. (1997) method. Briefly, 0.25 g of the sample was sonificated two times with methanol. Phorbolester was determined on reverse phase (Acquity UPLC BEH C18, endcapped 1.7 µm) 50x2.1 mm. The phorbolester peak was identified at 280 nm. Phosphoric acid 0.14% and acetonitle (25:75) used as mobile phase. A total of 1 µL of sample solution was injected. Phrobol-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 o 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 phorbolester 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 phorbolester content within the toxic and non-toxic fruit seed and capsule of *Jatropha curcas* showed difference in first and fifth week. Phorbolester 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 phorbolester content of *Jatropha curcas* genotypes of PT Bumi Mas were lower than Makkar and Becker's genotypes.

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

The graph (Figure 4) indicate that biosynthesis of phorbolester occured in initiation (first week) and maturation (fifth week) of fruit. Phorbolester level in seventh week decreased. It is possible that phorbolester has degraded due to fruit maturity.

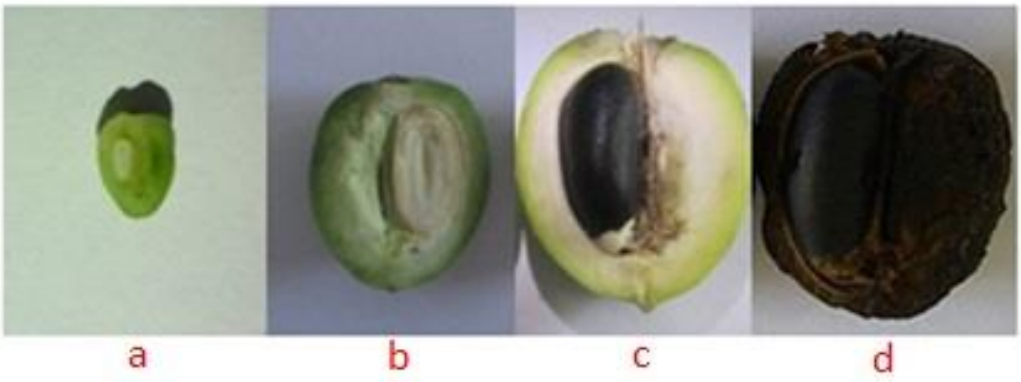

Fig. 1 *Jatropha curcas* fruits in the first week (a), third week (b), fifth week (c), and seventh week (d). .

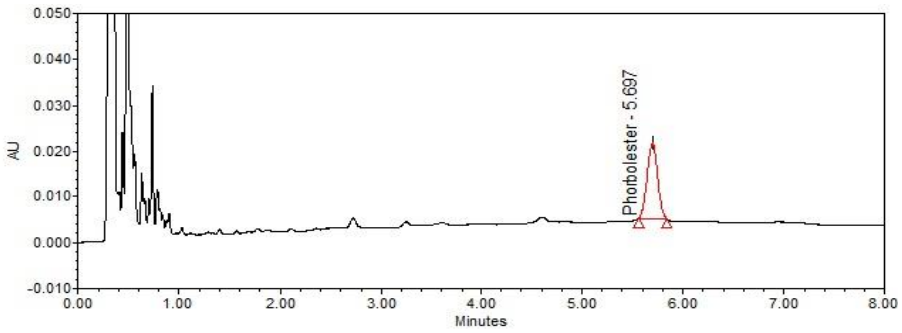

Fig. 2 UPLC chromatogram of standar phorbolester.

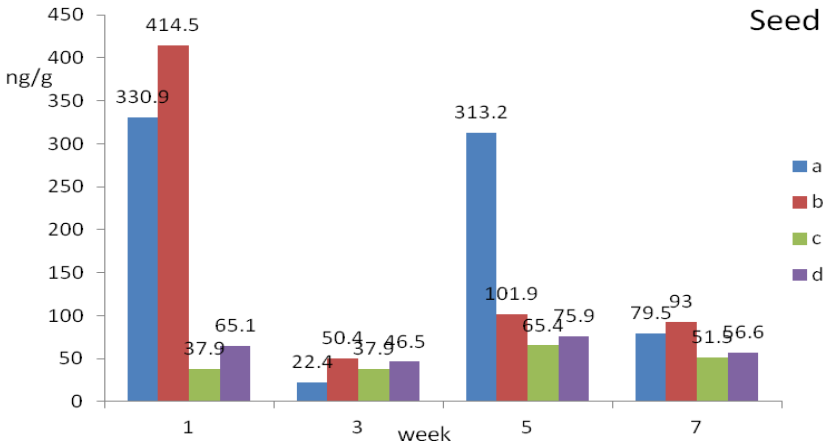

Fig. 3 Phorbolester content in fruit seed.

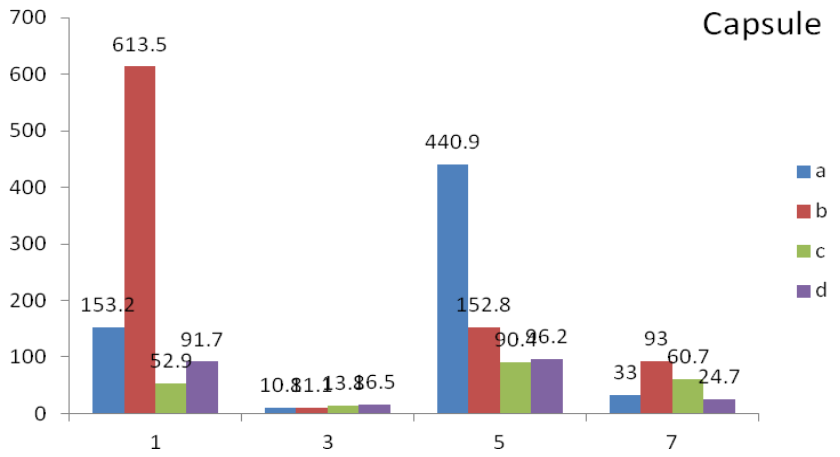

Fig. 4 Phorbolester content in fruit capsule.

4. Conclusion

Phorbolester content in toxic genotypes were higher than non-toxic genotypes appoximately 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 occured in initiation (first week) and maturation (fifth week) of fruit due to biosynthesis of phorbolester.

Acknowledgements

This research is supported by PT Sinar Mas Agro Resources and Technology, Tbk. The author thanked to Mr. Tony Liwang as Division Head of PT SMART, Tbk.

References

Adam, S.E (1974). Toxic effect of *Jatropha curcas* in mice. *Toxicology*, 4,67-76.

Adam, S.E & M. Magzoub. (1975). Toxic effect of *Jatropha curcas* in goats. *Toxicology*, 4,347-354.

David, E.M. & Croteau, R. (2000). Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpene. *Topics in Current Chemistry*, 209, 54-95.

Goel, G. Makkar, H.P.S. Francis, G. Becker, K. (2007). Phorbol esters: structure, biological activity, and toxicity in animal. *International Journal of Toxicology*, 26, 278-288.

Li, C.Y. Devappea, R.K. Liu, J.X. Lv, J.M. Makkar, H.P.S. & Bekker, K. (2010). Toxicity of *Jatropha curcas* phorbol esters in kice. *Elvisier*, 48,620-625.

Makkar H.K.S & Becker, K. (1997). Potential of *Jatropha curcas* seed meal as a protein supplement to livestock feed, constraints to its utilization and possibles stategies to overcome constraits. In: G.M. Gubitz. M. Mittelbach. & M. Trabi, eduitor. *Biofuels and Industrial Product from Jatropha curcas. Proceedings of The Symposium Jatropha* 97:Managua, 23-27 Feb 1997. hlm 190-215.

Makkar, H.P.S. Aderbigle, A.O. & Bekker, K. (1998). Comparative evaluation of nontoxic and toxic varieties of *Jatropha curcas* for chemical compositionj, digestibility, protein degradability and toxic factors. *Food Chem*., 62 (2),207-215.

Makkar, H.P.S. & Bekker, K. (1999). Nutritional studies on rats and fish (carp Cyprinus Carpio) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenace. *Plant Food for Human Nutrition*, 53,183-192.