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SHORT COMMUNICATION

Pharmaceutical potential of phorbol esters from *Jatropha curcas* oil

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Phorbol esters (PEs) are diterpenes present in *Jatropha curcas* L. seeds and have a myriad of biological activities. Since PEs are toxic, they are considered to be futile in *Jatropha*-based biodiesel production chain. In the present study, the extracted PEs from *Jatropha* oil were used as a starting material to synthesise pharmacologically important compound, prostratin. The prostratin synthesised from *Jatropha* showed identical mass with that of the reference standard prostratin, as determined by Nano-LC-ESI-MS/MS. Considering the rapid growth in *Jatropha* biodiesel industry, potential exists to harness large amount of PEs which can be further utilised to synthesise prostratin as a value added product.

Keywords: by-product; *Jatropha*; phorbol esters; prostratin; toxicity

1. Introduction

Phorbol esters (PEs) are tigliane diterpenes present in the genus Euphorbiaceae. These groups of compounds have an array of biological activities, exhibiting toxicity at higher doses and beneficial or pharmaceutical effects at lower doses (Goel, Makkar, Francis, & Becker, 2007). In *Jatropha curcas* L. (Euphorbiaceae), six different types of PEs (*Jatropha* factors C_1–C_6) are present (Haas, Sterk, & Mittlebach, 2002). The Jatropha kernel, screw pressed oil and screw pressed cake generally contains PEs in the range of 2–6 g kg^{-1}, 3–6 g kg^{-1} and 0.5–4 g kg^{-1}, respectively (Makkar & Becker, 2009; and unpublished data from our laboratory). Although present in lower concentrations, they exhibit severe toxicological symptoms upon oral and topical exposure to different parts of *Jatropha* plant including seeds. Several studies report the need to remove these compounds to increase the potential use of byproducts such as protein rich seed cake (Goel et al., 2007; Makkar, Becker, Sporer, & Wink, 1997). Due to toxicity, these compounds are generally regarded as futile compounds in the *Jatropha* biodiesel production chain. In addition, the removal of PEs from *Jatropha*-based products and their further utilisation in agropharmaceutical applications could enhance the economic sustainability of the *Jatropha* biodiesel production chain. In the present study, the pharmaceutical potential of PEs obtained from *Jatropha* oil was evaluated by converting them into a high-valued compound, which is nontoxic and has high biological activity. The studies were focused on the synthesis of prostratin from *Jatropha* PEs.

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Prostratin (12-deoxyphorbol-13-acetate) is a tigliane diterpene, and its presence is reported in plants such as *Pimelea prostrate*, *Euphorbia cornigera* and *Homalanthus nutans* (Miana, Bashir, & Evans, 1985). Similar to phorbol-12-myristate-13-acetate (PMA; synonym, 12-O-tetradecanoylphorbol-13-acetate), the prostratin is a protein kinase C (PKC) activator. However, prostratin is a potent anti tumour compound while PMA a tumour promoter. In addition, prostratin was found to inhibit PMA-stimulated tumour formation (Szallasi & Blumberg, 1991; Szallasi, Krausz, & Blumberg, 1992; Szallasi, Krsmanovic, & Blumberg, 1993). Thus, it belongs to a discrete subclass of PKC activators differing in its biological activities when compared with the tumour-promoting PEs, such as PMA (Ventura & Maioli, 2001). Prostratin has also been found to be a promising adjuvant in antiviral therapy. Many antiviral treatments have been successful in decreasing the active viral pool in AIDS patients (HAART). However, the persistence of latent viral reservoirs limits the complete viral eradication. Prostratin activates this latent virus pool (Wender, Kee, & Warrington, 2008) in turn acting as an adjuvant in HIV therapy. Prostratin have been found to induce HIV expression in latently infected cell lines and primary cells *in vitro* and also to inhibit HIV entry into target cells by down regulating CD4 and CXCR4 receptors (Gustafson et al., 1992; Kulkosky et al., 2001).

The prostratin was reported to be the active component present in Samoan mamala tree (*Homalanthus nutans*) bark extract which is used for treating hepatitis (Cox, 2001; Johnson, Banack, & Cox, 2008). In *H. nutans*, its concentration has been found to vary from 0.2–52.6 $\mu g\,g^{-1}$ fresh stem wood (Johnson et al., 2008). However, the presence of low amounts of prostratin in plants, difficult purification steps and the lesser yield limits its potential to economically harvest in large quantities. In addition, establishment of large-scale plantation by replacing the native ecosystem to obtain substantial amounts of prostratin could have significant ecological effects (Johnson et al., 2008; Wender, Warrington, & Kee, 2009).

Alternatively, prostratin could be obtained by synthetic methods by utilising PEs as a starting material. Industrial oil crop such as *Jatropha curcas* provide a promising feedstock of PEs for prostratin synthesis. Wender et al., (2008) have reported the synthesis of prostratin from phorbol (obtained from croton oil), which is a basic skeleton in the PEs such as PMA. They also presumed that prostratin could be synthesised from *Jatropha* PEs by hydrolysis, but further investigation has not been carried out. However, the *Jatropha* PEs do not contain phorbol as a basic skeleton, instead they contain 12-deoxy 16-hydroxy phorbol as a basic skeleton. Wender et al. (2008) suggested that 12-deoxy 16-hydroxy phorbol obtained from the *Jatropha* PEs could be converted into crotophorbolone, which can be further subjected to synthetic reactions to obtain prostratin (Figure 1). In the present study, the possibility of converting *Jatropha* PEs into crotophorbolone and then into prostratin was investigated.

2. Results and discussion

Wender et al. (2008) postulated that *Jatropha* PEs could be a good candidate for producing highly bioactive compound prostratin. The present study has illustrated that prostratin could be synthesised from *Jatropha* PEs. The experimental section is described in the supplementary material (online only). The synthesis reaction of prostratin from crotophorbolone is shown in Figure 1. As this was a preliminary study to assess whether *Jatropha* PEs from PE-enriched fraction (PEEF) could be used as an intermediate to synthesise prostratin, the material balance was not considered. The oil and the PEEF had 4.7 and 53 $mg\,g^{-1}$ PEs (PMA equivalent) (or 0.11 and 1.3 $mg\,g^{-1}$ as factor C_1 equivalent). After treating the PEEF with barium hydroxide in methanol, crotophorbolone was

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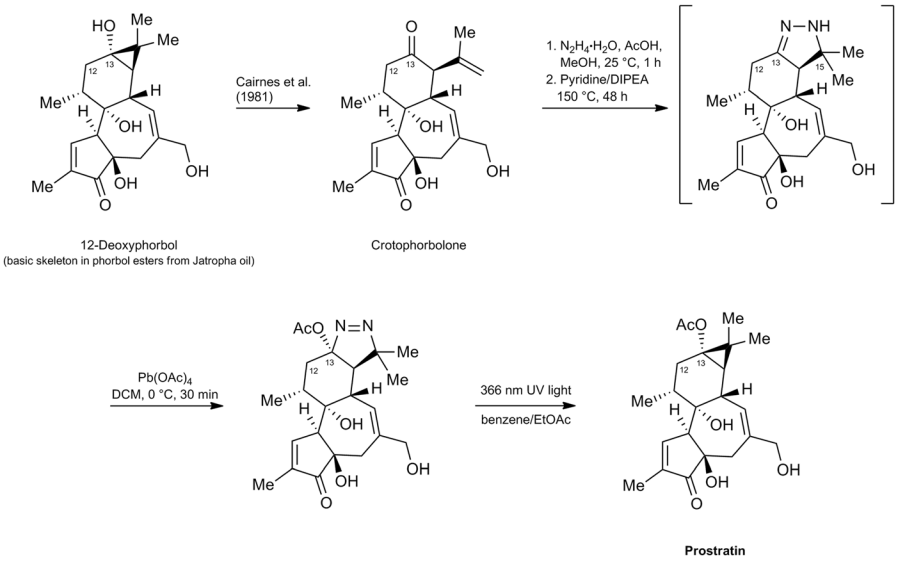

Figure 1. Synthesis of prostratin from *Jatropha* PEs (Cairnes, Mirvish, Wallcave, Nagel, & Smith, 1981; Wender et al., 2008).

obtained (~6–10 mg). Similarly, the crotophorbolone was also obtained from *Jatropha* PEs by reacting with methanolic sodium methoxide treatment for 2 h at room temperature (Hirota et al., 1988). The crotophorbolone was the starting material for the reaction to synthesise prostratin. In our study, prostratin synthesised from crotophorbolone was recovered after separation by preparative TLC, stored at 4°C for 3 weeks and then mass was detected by Nano-LC-ESI-MS/MS. The result showed complete degradation of prostratin obtained from *Jatropha* PEs, which may be due to prolonged storage (3 weeks) in dried form. Due to limited sample availability to test the mass, we further subjected the 'reaction mixture-B' (which was stored in −80°C) directly to Nano-LC-ESI-MS/MS. The results showed similar chromatographic peak pattern and had a similar molecular weight to that of the reference prostratin obtained from Sigma (Supplementary Figure S2 – online only).

3. Conclusion

The results suggest that prostratin can be synthesised from *Jatropha* PEs. However, further optimisation of conditions to have higher yield is needed. Our assumption is that the rapidly growing *Jatropha* biodiesel industry could yield high amounts of oil, about 26 million tons/annum by 2015 (GEXSI, 2008). Considering the average PEs content (our laboratory observation) of 3–6 g kg^{-1} oil (PMA equivalent) or 73–145 mg kg^{-1} oil (*Jatropha* factor C_1 equivalent), there exists a potential of obtaining 79,500–15,900 tonnes or ~1900–3850 tonnes of PEs, respectively. Overall, the PEs could be obtained as a by-product in the process of biodiesel production. This means, there will be no extra burden on the ecosystem for disposing of toxic PEs and even the prostratin synthesised from *Jatropha* PEs could be a value added product to the *Jatropha* biodiesel industry.

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Supplementary material

Figures S1 and S2 and experimental details relating to this paper can be found online.

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