Conclusion

Immature zygotic embryo explants excised from *Carica papaya* L. variety Eksotika placed on callus induction (CI) medium supplemented with 250 mg/L carbenicillin induced callus at 93.3 ±11.8% compared to 80.7 ±7.1 % on medium without 250 mg/L carbenicillin after 2 weeks of culture**.**

Using double staining method, nuclei of embryogenic cells were stained as intense bright red whereas blue stained cells showed non-embryogenic callus. Embryogenic cells were selected and used for cell suspension cultures.

Further verification was showed through the histological structure of the embryogenic cells. Groups of small meristematic cells in the whitish parenchymatic microcalli were observed followed by embryo development. The best media for the induction of somatic embryos was 10 mg/L 2,4-D and 250 mg/L carbenicillin supplemented in basal medium where 80.4 % of the callus gave rise to somatic embryos

Cells in suspension cultures were multiplied in liquid multiplication (LM) medium with 250 mg/L carbenicillin. A growth pattern was observed where lag phase was observed from day 10 to day 19, followed by an exponential growth phase from 20 day to 30 day.

Cell viability was assessed by using fluorescence diacetate (FDA) solution and this showed that only cells with dense cytoplasm fluoresced under ultraviolet (UV) light excitation. These cells were small, rounded and with distinct nucleus.

Germination (G) media supplemented with 0.2 mg/L 6-benzylaminopurine (BAP) and 0.2 mg/L naphthaleneacetic acid (NAA) was the optimal culture media for somatic embryo germination (100%). Plantlets were regenerated from germinating embryos on regeneration (R) medium supplemented with 1 mg/L gibberellic acid (GA_3), 0.5 mg/L indole butyric acid (IBA) and 0.37 mg/L riboflavin and rooted in MS medium supplemented with 2.0 mg/L IBA.

For acclimatization, when the first set of matured tri-lobed leaves appeared, plantlets were transferred to the nursery for one to two months before transplanting to the field.

The alkaloid, carpaine, was extracted from various part of *Carica papaya* L. var. Eksotika tree namely the leaves, petiole and fruit peel, and cell cultures namely leaves, petiole, suspension cells and suspension liquid with one impurity compound detected namely dehydrocarpaine II. In *in vitro* samples, petiole gave the highest carpaine content and the lowest content found in the leaves. Meanwhile, in *in vivo* samples, leaves gave the highest carpaine content and the lowest content detected in the fruit peel. Supercritical fluid extraction (SFE) was identified as the more efficient method for getting pure and high yield of carpaine compared to conventional acid/base extraction method.

The ratio of ethanol/water/acetic acid used at 94.5:5:0.5 (v/v/v) was confirmed to be a better solvent system for carpaine extraction compared to the system ethanol/water/acetic acid used at 89:10:1 (v/v/v) used by Coke and Rice, 1968 . However, carbon dioxide used as supercritical fluid (SF) in SFE compared to water seemed to furnish pure and

higher yield of carpaine. Additionally, centrifugation may also contributed to the greater yields of the carpaine extracted observed.