Pertanika J. Sci. & Technol. 27 (2): 619 - 632 (2019)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Sustainable Approach using *Carica papaya* Stem for *in vitro* Propagation of *Clinacanthus nutans*

Chia Chay Tay[1, 2]*, Najihah Mohamad Nasir[1], Nur Liyana Jamaluddin[3] and Wan Nurul Hidayah Wan Annuar[4]

[1]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 UiTM, Shah Alam, Selangor, Malaysia
[2]myBioREC, Faculty of Civil Engineering, Universiti Teknologi MARA, 40450 UiTM, Shah Alam, Selangor, Malaysia
[3]Faculty of Applied Sciences, Universti Teknologi MARA, 02600 UiTM, Arau, Perlis, Malaysia
[4]Faculty of Applied Sciences, Universti Teknologi MARA, 35400 UiTM, Tapah, Perak, Malaysia

ABSTRACT

Clinacanthus nutans, known to the locals as *Belalai Gajah*, is a medicinal plant widely used by Malaysians in the belief to cure various diseases including cancer. However, the production of *Belalai Gajah* hardly fulfils the market demand since the growing techniques of stem cutting and tissue culture are inefficient and expensive. Therefore, improvements in tissue culture techniques and materials application are required. Hence, this study tested *Carica papaya* (locally know as papaya) stem, an agricultural waste, to enhance the tissue culture of *C. nutans*. The effects of *C. papaya* stem powder and extract on the shoot proliferation of *C. nutans* parameters were investigated. The average number of shoot, leaves and length of leaves were observed. Phytochemicals screening was also conducted. Overall, *C. papaya* stem extract showed positive performance and 1% extract was found to be the optimum concentration to enhance the shoot proliferation. Meanwhile, *C. papaya* stem powder inhibited the shoot proliferation. It was also found that flavonoids, glycosides, steroid and terpenoid contributed in the shoot proliferation. In conclusion, the papaya stem extract is a potential *in-vitro* supplement for tissue culture studies. This study gained insights in sustainable green economy and showed that

ARTICLE INFO

Article history:
Received: 05 January 2018
Accepted: 26 November 2018
Published: 25 April 2019

E-mail addresses:
taychiay@gmail.com, taychiay@salam.uitm.edu.my (Chia Chay Tay)
najihahnasir94@gmail.com (Najihah Mohamad Nasir)
liyana@gmail.com (Nur Liyana Jamaluddin)
wnhwa@gmail.com (Wan Nurul Hidayah Wan Annuar)
* Corresponding author

ISSN: 0128-7680
e-ISSN: 2231-8526

zero waste can be attained by converting local papaya stem waste to valuable product. In addition, sustainable technology in pharmaceutical approach could be addressed through *in-vitro* low cost large-scale production of *Belalai Gajah* medicinal plant.

Keywords: *Clinacanthus nutans*, papaya stem waste, phytochemicals, shoot proliferation, supplement, tissue culture

INTRODUCTION

The *Clinacanthus nutans* or '*Belalai Gajah*' plant is useful as herbal medicine in Malaysia. This is due to its active biological and phytochemicals contents (Alam et al., 2016; Bertrand et al., 2014). *Belalai Gajah* is traditionally used to assist cancer, gout, kidney syndrome, uterine fibroid, skin inflammations and lesion (Ali et al., 2014; Arullappan et al., 2014; Fong et al., 2014; Shahzad et al., 2015; Tu et al., 2014). *C. nutans* products are commercially available in the market in the forms of cream, lotions, capsule, tablet, herbal tea, concentrated extract. However, manufacturers are not able to fulfil the market demands due to the inefficient stem cuttings growth method. Thus a new growth method needs to be addressed.

Plant tissue culture technique has been reported to have produced high quality plantlets rapidly (Asghari & Ahmadvand, 2018; Chen et al., 2015; Kataria et al., 2013). Plantlets produced through plant tissue culture have high resistance to diseases and stress when transferred to soil (Loyola-Vargas & Ochoa-Alejo, 2012; Hussain et al., 2012; Deepthi & Satheeshkumar, 2017; Muda & Awal, 2017; Rathore et al., 2014; Syaiful et al., 2009). However, this method requires application of expensive synthetic growth hormone, 6-benzylaminopurine (BAP). Thus low-cost agricultural waste should be extensively explored as a substitute to enhance shoot proliferation and reduce cost (Rattana & Sangchanjiradet, 2017; Tay et al., 2016).

In another perspective, various tissue culture studies had reported successful application of agricultural waste of *Carica papaya* (locally know as papaya) juice (Daud et al., 2011), banana *Musa acuminate* peel (Daud et al., 2011; Molnár et al., 2011; Swamy et al., 2014), coconut *Cocos nucifera* husk (Deb & Pongener, 2013; Gnasekaran et al., 2010) leaf litter and sugarcane *Saccharum officinarum* bagasse (Basirat, 2011) as a substitute of growth hormone.

Hence, agricultural waste is a promising tissue culture supplement because it contains natural phytochemicals (Anjusha & Gangaprasad, 2016; Jamal et al., 2017). Phytochemicals in plants include alkaloids (Aravind et al., 2013), flavonoids (Milind & Gurditta, 2011), saponin, tannin, glycoside, steroid, terpenoid, carotenoids (Aravind et al., 2013), minerals and vitamins (Ward et al., 2017). The phytochemicals play important roles in shoot proliferation, plant defence against pathogens and nutrient absorption.

Previous study on *C. nutans* tissue culture demonstrated that spent mushroom compost (Tay et al., 2016) and corn *Zea mays* stem (Tay et al., 2017) had enhanced shoot proliferation of the plant. For application of *C. papaya* as supplement, it is noted that the studies were only limited to papaya fruit juice. The papaya fruit juice served as a supplement in tissue culture, where it enhanced the production of *Celosia* sp. (Daud et al., 2011) and orchid *Doritaenopsis* (Rahman et al., 2004). Since the application of the *C. papaya* stem powder and extract on *C. nutans* is yet to be addressed, this study focuses on *C. papaya* stem as a potential supplement to induce the tissue culture of *C. nutans*.

The objective of this study was to determine the performance of the *in-vitro C. nutans* shoot proliferation using papaya stem powder and extract. The phytochemicals compounds of the papaya stem were investigated to gauge its ability in enhancing the *C. nutans* shoot proliferation.

MATERIALS AND METHODS

C. papaya Stem Powder and Extract Preparation

Papaya stems at aged of 2 with size 5 inches long from top to bottom of the main stem were collected from the UiTM Perlis farm. It was dried, ground and sieved through a 710 µm size sieve. The powder was also used to prepare the *C. papaya* stem extract. A water extraction method was conducted to obtain the extract. An approximate 10 g of *C. papaya* stem powder was mixed with 50 ml of distilled water. The mixture was incubated in an incubator shaker (Infors HT Ecotron) at 200 rpm and 25 ± 2°C for 2 hours. Then, the mixture was centrifuged at 5000 rpm (4458 × *g*) for 10 minutes to obtain the supernatant. The separated supernatant (extract) was then stored at 4°C.

Preparation of the Culture Medium

Distilled water was mixed with 3% β-D-Fructofuranosyl-α-D-glucopyranoside (sucrose) powder (Sigma Aldrich™), 0.4% Murashige and Skoog (MS) powder (Duchefa Biochemie™), 0.01% 1,2,3,4,5,6-Hexahydroxycyclohexane (myo-inositol) powder (Duchefa Biochemie™), 0.005% of prepared 0.1 µM 6-benzylaminopurine (BAP) (Duchefa Biochemie™) solution. After that, the pH of the MS solution was adjusted to 5.8. The prepared MS solution was heated using a hot plate and 0.3% polysaccharide (gelrite) powder (Duchefa Biochemie™) was added. Then the *C. papaya* stem powder or extract was added followed by polysaccharide powder. The completed mixture (MS solution) was poured into jam jars and the medium were left to cool. The jam jars containing MS solution were autoclaved at 15 lbs psi and 121°C for 15 minutes (Das et al., 2015; Tay et al., 2017).

Surface Sterilisation

The autoclavable apparatus was autoclaved and the non-autoclavable materials were cleaned with 70% ethanol. The nodal segments of the *C. nutans* were excised and washed with tap water for five minutes to remove particles that might lead to contamination. All apparatus and materials were put in a laminar flow. Then the nodal segments of *C. nutans* were soaked in 70% ethanol for three minutes, followed by 30% sodium hypochlorite (Sigma Aldrich™) for three minutes, 0.1% mercuric chloride (Sigma Aldrich™) and sterile distilled water. Lastly, the nodal segments were air dried before the shoot induction.

Shoot Induction

The nodal segments of *C. nutans* were sub-cultured on a fresh MS medium. One nodal segment was placed in each jam jar. The culture was incubated in an incubator at 25 ± 2°C under 16 hours of cool white fluorescent light and 8 hours dark photoperiod.

The nodal segments of *C. nutans* from a sterile explants stock were subcultured in three conditions. The first condition only contained an MS medium, which served as a control. The second condition contained the *C. papaya* stem powder and the third, had the *C. papaya* stem extract.

Then 1% of the (m/v) powder, 2% (m/v) powder, 4% (m/v) powder, 1% (v/v) extract, 2% (v/v) extract and 4% (v/v) extract were added into each of the MS medium (Tay et al., 2017). The average number of shoot and leaves, and the average length of leaves were observed and recorded for 6 consecutive weeks. The samples were duplicated for each condition.

Phytochemicals Screening

The aqueous extract of *C. papaya* stem was prepared using an amount of 4 g of *C. papaya* stem powder in 100 ml distilled water agitated in an incubator shaker at 200 rpm at 25 ± 2°C for 24 hours. After 15 minutes of evaporation through a rotary evaporator, approximately 2 mL the *C. papaya* stem extract was obtained. These steps were repeated seven times. The extract was subjected to different phytochemicals tests including alkaloid test using mercuric iodide reagent (Ikeyi et al., 2013), sodium hydroxide test for flavonoids (Usman et al., 2009), saponins test (Ikeyi et al., 2013), ferric chloride test for tannins (Ikeyi et al., 2013), Keller-Killani test for glycosides (Emmanuel et al., 2014), steroids test (Njoku & Obi, 2009) and terpenoids test (Njoku & Obi, 2009).

Statistical Analysis

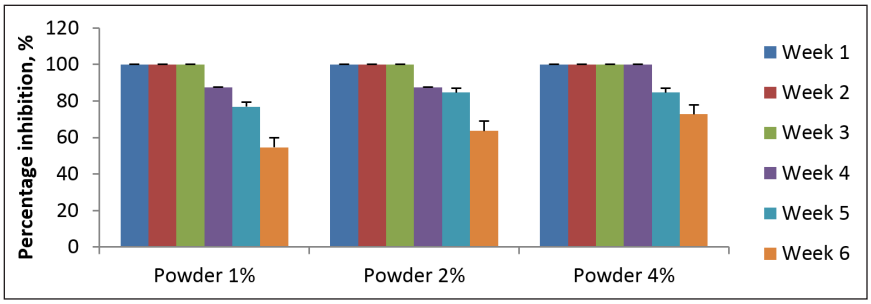
The recorded data were analysed using Microsoft Excel 2010. The obtained results were expressed in mean ± standard deviation (n=2). Data then were further analysed using one-way analysis of variance (ANOVA) with 95% level of confidence.

This study was conducted in 2017 at the Faculty of Applied Sciences, UiTM, Arau, Malaysia. To note, University Teknologi MARA, (UiTM), Malaysia, has its branch campuses in all states of Malaysia, including at Arau, the state of Perlis.

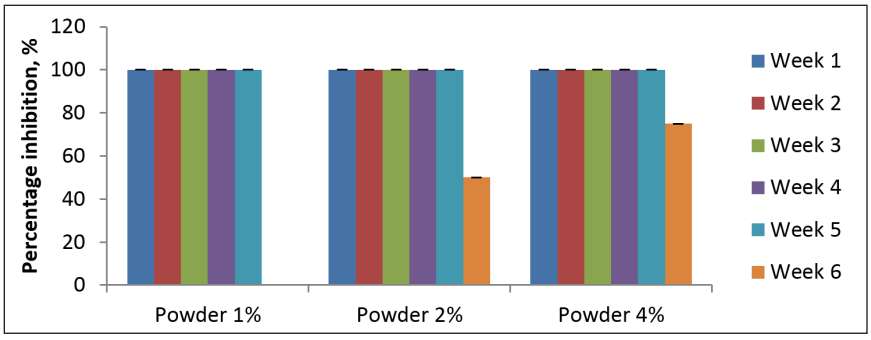
RESULT AND DISCUSSION

Effects of the *C. papaya* Stem Powder on Shoot Proliferation of the *C. nutans*

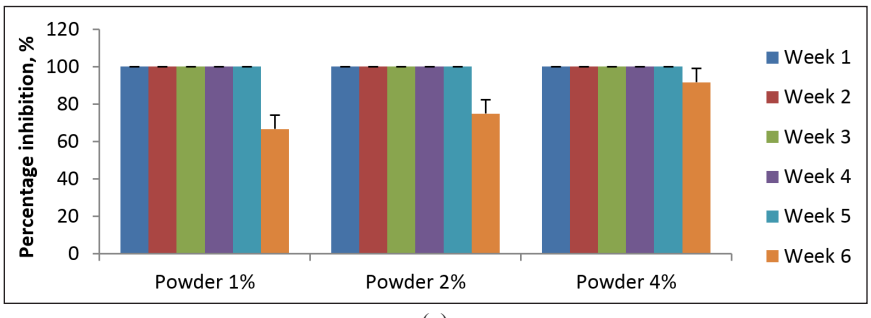
Figures 1(a), (b) and (c) show the inhibition of *C. nutans* shoot proliferation compared to the control. At the end of week 6, the control showed 50% higher number of shoots, number of leaves and length of leaves ($p>0.05$), compared to the samples treated with the



(a)



(b)



(c)

Figure 1. Effects of *C. papaya* stem powder on shoot proliferation of the *C. nutans* (a) average number of shoots, (b) average number of leaves, and (c) average length of leaves

powder. An increase of the powder concentration significantly decreased the measured parameters thus inhibiting the number of shoots and length of leaves. However, 2% (m/v) of the powder treatment recorded the lowest inhibition, which is 50% for the number of leaves compared to 1% and 4 % (m/v).

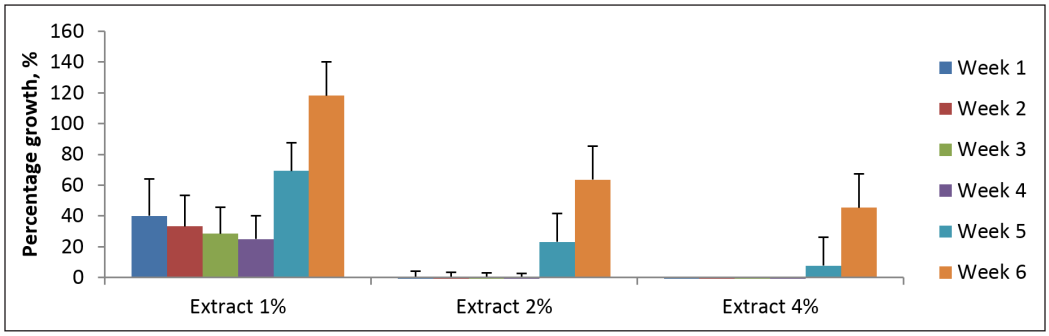
It was seen that a 100% inhibition had occurred in the first few weeks in the *C. papaya* stem powder treatment. This inhibition occurred because the *C. papaya* powder had absorbed the nutrients from the surrounding environment and therefore limiting the nutrients for *C. nutans*. This then led to the inhibition of the shoot proliferation. The shoot proliferation occurred at the later weeks as the *C. papaya* stem powder released its nutrients to the surrounding environment due to the concentration gradient. As a result, the plant utilised the nutrients and the shoot proliferation started. Mercy and Jenifer (2014) also reported a similar observation and explaination that the application of *Punica granatum* pomegranate peel powder inhibited the shoot proliferation of the *Vinca rosea*.

It was also observed that the shoot proliferation in the control samples had increased in the early weeks but decreased later. Such condition implied that the senescence stage of the *C. nutans* and the depletion of nutrients and growth hormone in the medium had occurred. Hence, this resulted in the loss of leaves of the *C. nutans*. Zwack and Rashotte (2013) supported this study by explaining that the senescence of leaves occurred due to the depletion of the cytokinin growth hormone in medium.

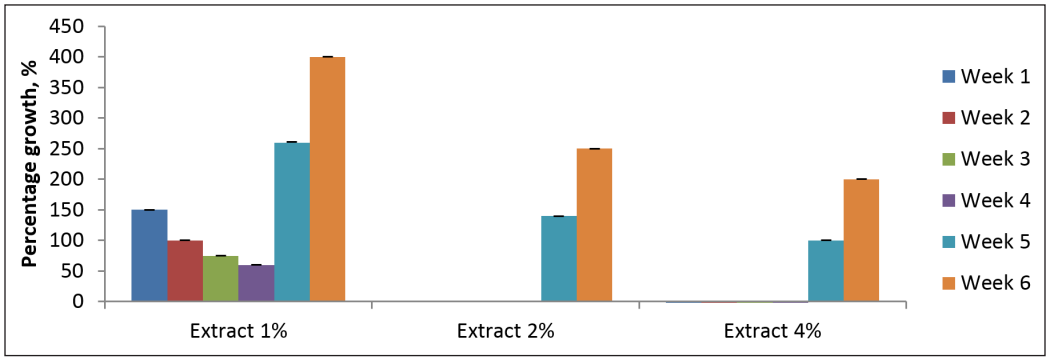
Effects of *C. Papaya* Stem Extract on Shoot Proliferation of the *C. nutans*

The effects of the papaya stem extract on the *C. nutans* shoot proliferation are shown in Figures 2(a), (b) and (c). The percentage of shoot proliferation growth, ranging from 32% to 400%, was recorded at the end of week 6. Statistically, when the extract concentration increases, it significantly decreases the measured shoot proliferation parameters ($p<0.05$).

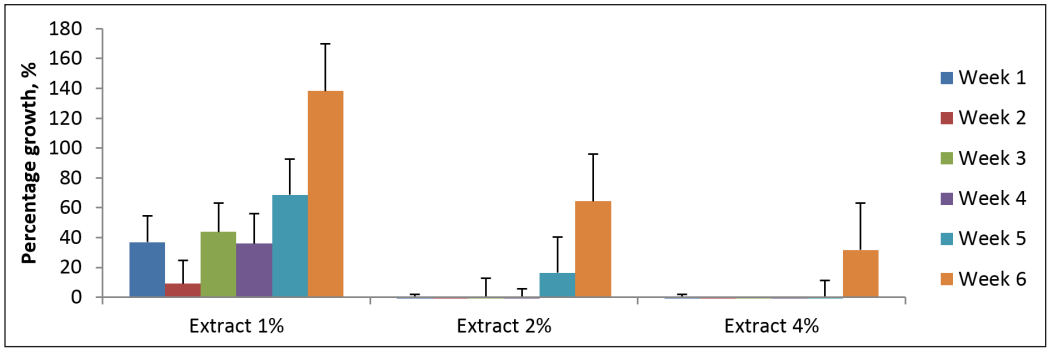
Only 1% (v/v) of the extract exhibited positive and the most efficient shoot proliferation. This was due to the *C. papaya* stem extract containing high soluble nutrients in aqueous liquid form, which easily absorbed and utilised by the *C. nutans*. Contrary, the shoot proliferation of the *C. nutans* in the 2% and 4% extract were lower than the control in the early weeks. Such observation occured due to addition of high concentration of nutrients causing excessive growth nutrients and osmotic pressure in the plants, thus turning the plant dry and brown (Al-Khateeb, 2008). Daud et al. (2011) and Swamy et al. (2014) also reported similar results with our finding, where lowest concentration of papaya fruit extract efficiently promoted shoot proliferation of the *Celosia* sp. and *Pogestemon cablin*.



(a)



(b)



(c)

Figure 2. Effects of *C. papaya* stem extract on shoot proliferation of the *C. nutans* (a) average number of shoots, (b) average number of leaves, and (c) average length of leaves

Comparison between The Effects of *C. Papaya* Stem Powder and Extract on Shoot Proliferation of the *C. nutans*

Table 1 shows that 1% (v/v) of *C. papaya* stem extract was most effective to promote the *C. nutans* shoot proliferation. Comparison of the *C. papaya* stem powder and extract on the *C. nutans* shoot proliferation over time is illustrated in Figures 3(a), (b) and (c). Statistical analysis shows there was significant difference for all treatments ($p < 0.05$).

All parameters showed a similar pattern where in earlier weeks, the *C. nutans* shoot proliferation in the medium with the extract and control had increased. Meanwhile, there was no growth in the medium with the *C. papaya* stem powder. This was due to the soluble nutrients in the liquid extract that enhanced the shoot proliferation. However, the solid *C. papaya* stem powder absorbed the nutrients required for the *C. nutans* shoot proliferation, thus inhibiting its growth.

Table 2 shows the comparison of plant-based extract on various plant growth performances in tissue culture. The plant extracts were the *Carica papaya*, banana *Musa acuminate*, corn *Zea mays*, tomato *Solanum lycopersium*, carrot *Daucus carota* and coconut *Cocos nucifera*. A low concentrations of 5% to 30% of plant extracts was optimum for plants growth. The optimum concentration of water-based extracts was dependent on the sources of extracts and types of plant (Daud et al., 2011; Swamy et al., 2014; Gnasekaran et al., 2010; Molnár et al., 2011; Chukwuka et al., 2014; Deb & Pongener, 2013).

Table 1
The most effective treatment using C. papaya *stem for shoot proliferation of the* C. nutans

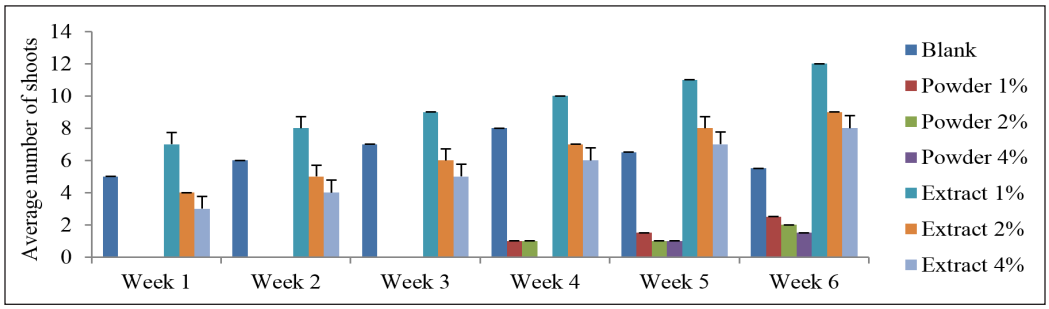
Parameters of Shoot Proliferation	Optimum Condition
Average Number of Shoots	1% (v/v) extract
Average Number of Leaves	1% (v/v) extract
Average Length of Leaves	1% (v/v) extract

Table 2
Comparison of plant-based extracts on various plant growth performances in tissue culture

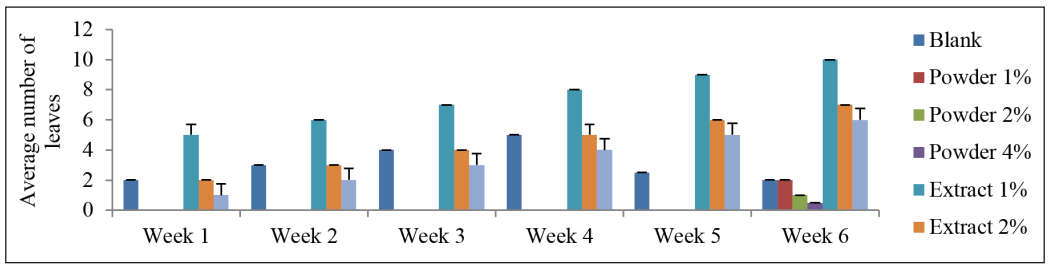
Extract source	Type of extract	Optimum concentration	Plant	References
Papaya *Carica papaya* fruit	Juice/water extract	2% (2%, 3%, 5%, 7%)	*Celosia* sp.	Daud et al., 2011
Banana *Musa acuminate*	Juice/water extract	5% (2%, 3%, 5%, 7%)	*Celosia* sp.	Daud et al., 2011
	MS liquid extract	10% (5%, 10%, 20%)	*Pogostemon cablin*	Swamy et al., 2014
	Water extract	5% (0%, 5%, 10%, 20%, 30%)	*Phalenopsis violacea*	Gnasekaran et al., 2010
	Water extract	Not mentioned	*Pisum sativum, Nicotiana tabacum*	Molnár et al., 2011
Tithonia diversifolia	Water extract	50% (50%, 100%)	Corn *Zea mays*	Chukwuka et al., 2014
Tomato *Solanum lycopersium*	Water extract	5% (2%, 3%, 5% and 7%)	*Celosia* sp.	Daud et al., 2011
	Extract with MS liquid	10% (5%, 10%, 20%)	*Pogostemon cablin*	Swamy et al., 2014
	Water extract	10% (0%, 5%, 10%, 20%, 30%)	*Phalenopsis violacea*	Gnasekaran et al., 2010

Table 2 *(continue)*

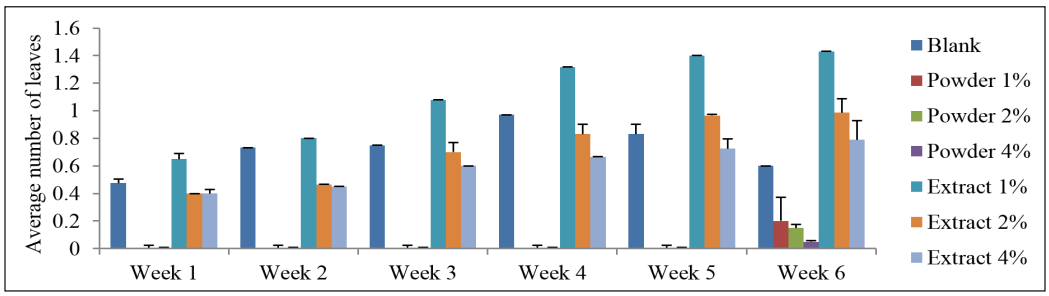
Extract source	Type of extract	Optimum concentration	Plant	References
Carrot *Daucus carota*	Extract with MS liquid	10% (5%, 10%, 20%)	*Pogostemon cablin*	Swamy et al., 2014
Coconut *Cocos nucifera*	Water extract	7% (2%, 3%, 5% and 7%)	*Celosia* sp.	Daud et al., 2011
	Water extract	10% (0%, 5%, 10%, 20%, 30%)	*Phalenopsis violacea*	Gnasekaran et al., 2010
	Not mentioned	Not mentioned	*Cymbidium iridioides*	Deb and Pongener, 2013
	Water extract	Not mentioned	*Pisum sativum, Nicotiana tabacum*	Molnár et al., 2011
Papaya *C. papaya*	Water extract	1% (1%, 2% and 4%)	*C. nutans*	This study



(a)



(b)



(c)

Figure 3. Comparison of the effects of *C. papaya* stem powder and extract on shoot proliferation of the *C. nutans* (a) average number of shoots, (b) average number of leaves, and (c) average length of leaves

Phytochemicals Screening of *C. papaya* Stem

The phytochemicals screening of the *C. papaya* stem showed a presence of flavonoids, glycoside, steroid and terpenoid (Table 3). Researchers explained that the flavonoids functioned as plant defence system against ecological and physiological pressures such as pathogen and insect attack (Khoddami et al., 2013). Grabkowska et al. (2014) reported that the glycoside was able to enhance *Harpagophytum procumbens* shoot proliferation. Meanwhile, a study conducted by Kandelinskaya et al. (2007) recorded that steroid had increased the protein content in various types of lupine plants and resulted in better light signaling. Hence, presence of steroid in the *C. papaya* stem increased the protein content in the *C. nutans* and thus promoting its shoot proliferation. An application of terpenoid for *in vitro* culture had also enhanced the multiplication of shoots and root (Khan et al., 2016). The root multiplication had increased the nutrients absorption and produced higher number of shoots. The identified phytochemicals in *C. papaya* stem are important where phytochemicals contributed to *C. nutans* shoot proliferation.

Table 3
The phytochemicals screening result of the C. papaya *stem*

Phytochemicals parameters	Result
Flavonoids	+
Glycoside	+
Steroid	+
Terpenoid	+

CONCLUSION

This study concluded that agricultural waste, *C. papaya stem* extract has potential to be a sustainable supplement for *C. nutans* tissue culture. *C. papaya* stem is an agriculture waste and easily procured, thus the application is cost-effective for commercial utilisation. The 1% extract of the *C. papaya* stem resulted in the highest average number of shoot, leaves and average length of leaves. The one-way ANOVA demonstrated significant difference ($p<0.05$) among all conditions of the powder and extract treatments. Meanwhile, the *C. papaya* stem powder inhibited the *C. nutans* shoot proliferation up to the fourth week. It contains flavonoids, glycoside, steroid and terpenoid, showing that phytochemicals support *C. nutans* shoot proliferation. This study had also provided vital and fundamental information that supports future application of *papaya* stem extract in the *in-vivo* growth of *C. nutans*. In this case, it is concluded that the *C. papaya* stem extract can be a sustainable supplement for *C. nutans* tissue culture. Therefore, this study not only shed light into waste-to-product and zero waste concepts, but also contributed to the green economy. The papaya stem, an agriculture waste is easily procured in many tropical countries, hence can

be viable for commercial production. Through application of agricultural waste as for tissue culture supplement, the waste is recycled. At the same time, the demand for plant-based drug is fulfilled and environmental pollution is minimised.

ACKNOWLEDGEMENTS

This work was supported and funded by the Universiti Teknologi MARA grant, 600-RMI/IRAGS 5/3(13/2015). The authors would like to thank Associate Professor Dr. Megawati Omar for language editing of this paper.

REFERENCES

Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A. S., Khatib, A., & Sarker, Z. I. (2016). *Clinacanthus nutans*: a review of the medicinal uses, pharmacology and phytochemistry. *Asian Pacific Journal of Tropical Medicine*, *9*(4), 402–409.

Ali, G., Alireza, N., Hawa, Z. E. J., Ali, B., & Izham, A. (2014). Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of Sabah snake grass (*Clinacanthus nutans*) in relation to plant age. *Molecules Journal*, *19*(11), 17632-17648.

Al-Khateeb, A. (2008). Enhancing the growth of date palm *Phoenix dactylifera in vitro* tissue by adding date syrup to the culture medium. *Scientific Journal of King Faisal University*, *9*(1), 71–85.

Anjusha, S., & Gangaprasad, A. (2016). Callus culture and in vitro production of anthraquinone in *Gynochthodes umbellata*. *Industrial Crops and Products Journal, 95*, 608-614.

Aravind, G., Bhowmik, D., Duraivel, S., & Harish, G. (2013). Traditional and medicinal uses of *Carica papaya*. *Journal of Medicinal Plants Studies*, *1*(1), 7–15.

Arullappan, S., Rajamanickam, P., Thevar, N., & Kodimani, C. (2014). *In vitro* screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (Acanthaceae) leaf extracts. *Tropical Journal of Pharmaceutical Research*, *13*(9), 1455-1461.

Asghari, R., & Ahmadvand, R. (2018). Salinity stress and its impact on morpho-physiological characteristics of *Aloe vera*. *Pertanika Journal of Tropical Agricultural Science*, *41*(1), 411-422.

Basirat, M. (2011). Use of palm waste cellulose as a substitute for common growing medium in Aglaonema growing. *Journal of Ornamental and Horticultural Plants*, *1*(1), 1–11.

Bertrand, S., Donatella, F., Rita, C., Carla, M., Giancarlo, M., & Vittorio, C. (2014). Antioxidant and anti-inflammatory activities of extracts from *Cassia alata, Eleusine indica, Eremomoatax speciosa, Carica papaya* and *Polyscias fulva* medicinal plants collected in Cameroon. *Plos One, 9*(8), 1-10.

Chen, B., Zhang, J., Zhang, W., Zhang, C., & Xiao, Y. (2015). The rapid propagation technique of the medicinal plant *Clinacanthus nutans* by tissue culture. *New York Science Journal, 8*(2), 23-27.

Chukwuka, K. S., Obiakara, M. C., & Ogunsumi, I. A. (2014). Effect of aqueous plant extract and inorganic fertilizer on germination, growth and development of maize (*Zea mays* L.). *Journal of Agricultural Sciences, 59*(3), 243-254.

Das, K., Dang, R., Ghanshala, N., & Rajasekharan, P. E. (2015). Phytochemical investigations of in vitro propagated plant *Taxus wallichiana*: An endangered anticancer medicinal plant of Indian origin. *Annals of Phytomedicine, 4*(2), 59-66.

Daud, N., Taha, R. M., Noor, N. N. M., & Alimon, H. (2011). Effects of different organic additives on in vitro shoot regeneration of *Celosia* sp. *Pakistan Journal of Biological Sciences*, *14*(9), 546–551.

Deb, C. R., & Pongener, A. (2013). A study on the use of low cost substrata against agar for non-symbiotic seed culture of *Cymbidium iridioides*. *Australian Journal of Crop Science*, *7*(5), 642–649.

Deepthi, S., & Satheeshkumar, K. (2017). Effects of major nutrients, growth regulators and inoculum size on enhanced growth and camptothecin production in adventitious root cultures of *Ophiorrhiza mungos*. *Biochemical Engineering Journal, 117*, 198-209.

Emmanuel, C. O., Helmina, O, A., & Egwim, C. E. (2014). Phytochemical constituents of seeds of ripe and unripe *Blighia sapida* and physicochemical properties of the seed oil. *International Journal of Pharmaceutical Science Invention, 3*(9), 31-40.

Fong, S. Y., Terrence, P., Sylvia, U., & Tien, H. (2014). Genetic homogeneity of vegetatively propagated *Clinacanthus nutans*. *Journal of Medicinal Plant Research, 8*(25), 903-914.

Gnasekaran, P., Rathinam, X., Sinniah, U. R., & Subramanium, S. (2010). A study on the use of organic additives on the protocorm like bodies (PLBS) growth of *Phalaenopsis violaceae* orchid. *Journal of Phytology*, *2*(1), 29–33.

Grabkowska, R., Mielicki, W., & Wielanek, M. (2014). Changes of phenylethanoid and iridiod glycoside distributions in various tissues of shoot cultures and regenerated plants of *Harpagophytum procumbens*. *South African Journal of Botany, 95*, 159-164.

Hussain, A., Qarshi, I. A., Nazir, H., & Ullah, I. (2012). Plant tissue culture: Current status and opportunities. In A. Leva & L. M. R. Rinaldi (Eds.), *Recent Advances in Plant in Vitro Culture* (pp. 1-28). Rijeka: InTech.

Ikeyi, A. P., Ogbonna, A. O., & Eze, F. U. (2013). Phytochemical analysis of paw paw (*Carica papaya*) leaves. *International Journal of Life Sciences Biotechnology and Pharma Research, 2*(3), 348-351.

Jamal, P., Akbar, I., Jaswir, I., & Zuhanis, Y. (2017). Quantification of total phenolic compounds in papaya fruit peel. *Pertanika Journal of Trropical Agricultural Science*, *40*(1), 87-98.

Kandelinskaya, O. L., Topunov, A. F., & Grishchenko, E. R. (2007). Biochemical aspects of growth-stimulating effects of steroid phytohormones on lupine plants. *Applied Biochemistry and Microbiology*, *43*(3), 324–331.

Kataria, N., Yadav, K., Kumari, S., & Singh, N. (2013). Micropropagation: An important tool for conserving forest trees. *Pertanika Journal of Tropical Agricultural Science, 36*(1), 1-26.

Khan, S., Pandotra, P., Manzoor, M. M., Kushwaha, M., Sharma, R., Jain, S., & Gupta, S. (2016). Terpenoid and flavonoid spectrum of in vitro cultures of *Glycyrrhiza glabra* revealed high chemical heterogeneity: Platform to understand biosynthesis. *Plant Cell, Tissue and Organ Culture*, *124*(3), 507–516.

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328–2375.

Loyola-Vargas, V. M., & Ochoa-Alejo, N. (2012). *Plant cell culture protocols*. Totowa, NJ: Humana Press.

Mercy, S., & Jenifer, I. (2014). Application of different fruit peels formulations as a natural fertilizer for plant growth. *International Journal of Scientific & Technology Research, 3*(1), 300–307.

Milind, P., & Gurditta, G. (2011). Basketful benefits of Papaya. *International ResearchJournalofPharmacy, 2*(7), 6-12.

Molnár, Z., Virág, E., & Ördög, V. (2011). Natural substances in tissue culture medium of higher plants. *Acta Biologica Szegediensis, 55*(1), 123–127.

Muda, N. A., & Awal, A. (2017). Somatic embryogenesis in sugar palm (*Arenga pinnata* wurmb merr.) from zygotic embryo explants. *Pertanika Journal of Science and Technology, 25*(S), 133-144.

Njoku, O. V., & Obi, C. (2009). Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry, 3*(11), 228-233.

Rahman, A. R. M. M., Islam, M. O., Prodhan, A. K. M. A., & Ichihashi, S. (2004). Effects of complex organic extracts on plantlet regeneration from PLBs and plantlet growth in the *Doritaenopsis* orchid. *Japan Agricultural Research Quarterly, 38*(1), 55-59.

Rathore, S., Yadav, K., Singh, N., & Singh, S. K. (2014). Comparative study on callus induction, proliferation and plantlets regeneration in two cultivars of *Stevia rebaundiana* bertoni: The only non-caloric natural sweetener. *Pertanika Journal of Tropical Agricultural Science, 37*(4), 499-508.

Rattana, K., & Sangchanjiradet, S. (2017). Micropropagation of *Dendrobium signatum*. *Pertanika Journal of Tropical Agricultural Science, 40*(4), 577-586.

Shahzad, A., Muhammad, S., & Awang, S. (2015). A review on phytochemical constituents and pharmacological activities of *Clinacanthus nutans*. *International Journal of Pharmacy and Pharmaceutical Sciences, 7*(2), 30-33.

Swamy, M. K., Mohanty, S. K., & Anuradha, M. (2014). Effect of plant growth regulators and natural supplements on *in vitro* propagation of *Pogostemon cablin*. *Journal of Crop Science and Biotechnology*, *17*(2), 1–8.

Syaiful, B. P., Abdullah, S. N. A., Abdul-Aziz, M., Meon, S., & Omar, O. (2009). Somatic embryogenesis from scutellar embryo of *Oryza sativa*. *Pertanika Journal of Agricultural Science, 32*(2), 185-194.

Tay, C. C., Jamaluddin, N., Nasir, N. M., Anuar, W. N. H. W., & Hashim S. N. (2017). *Zea mays* stem agricultural waste for in-vitro shoot propagation of *Clinacanthus nutans*. *Journal of Fundamental and Applied Science, 9*(5S), 673-683.

Tay, C. C., Shaari, M. S., Anuar, W. N. H. W., & Hashim, S. N. (2016). *Pleurotus* spent mushroom compost as green supplementary nutrient in tissue culture. *MATEC Web of Conferences* (Vol. 47, p. 5010). Les Ulis, France: EDP Sciences

Tu, S. F., Liu, R. H., Cheng, Y. B., Hsu, Y. M., Du, Y. C., Mohamed, E., … & Chang, F. R. (2014). Chemical constituents and bioactivities of *Clinacanthus nutans* aerial parts. *Molecules Journal, 19*(12), 20382-20390.

Usman, H., Abdulrahman, F. I., & Usman, A. (2009). Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii*. *African Journal Traditional, Complementary and Alternative Medicines, 6*(3), 289-295.

Ward, S., Petr, K., Mussa, Q., Igor, C., Robert, P. K., Sander, C., … & Karin, L. (2017). cis-Cinamic acid is a novel, naural auxin afflux inhibitor that promotes lateral root formation. *American Society of Plant Biologist, 173*(2017), 552-565.

Zwack, P. J., & Rashotte, A. M. (2013). Cytokinin inhibition of leaf senescence. *Plant Signaling & Behavior*, *8*(7), 1–7.