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Yeast Extract Stimulate Growth and Production of Phenol, 2, 4-Bis (1,1-Dimethylethyl) of Tissue Culture Orthosiphon Stamineus

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ABSTRACT

Yeast extract is a weapon component used to elicit the production of natural defensive compound as well as improves growth of shoots and roots in tissue culture Orthosiphon stamineus. Five different concentrations of yeast extract (0 mg/L, 0.5 mg/L, 2.0 mg/L, 3.5 mg/L and 5.0 mg/L) had been tested with internodes serve as explant. O. stamineus were cultured 2, 4 and 6 weeks under MS media supplemented with or without yeast extracts. By different culturing time, O. stamineus were extracted using methanol and filtrate analyzed with Gas Chromatography-Mass Spectrometry (GC-MS) for detection. The percentage composition of Phenol, 2, 4-bis (1,1-dimethylethyl) in O. stamineus had been recorded for different incubation culture (two until six weeks). The result shown, the growth rates shoot and root of O. stamineus increases by the weeks. The growth rate of shoots highest compare to roots in MS media supplemented with 5.0mg/L of yeast extract and the mean values were (24.50 ± 0.215) and (13.50 ± 0.554) respectively after 6 weeks cultured. Phenol, 2, 4-bis (1,1-dimethylethyl) was identified present at 30.26 of retention time with highest percentage 31.42% also after 6 weeks cultured. Therefore, the addition of yeast extract in media tissue culture gave the stimulation effect on the production of phenolic compound as well as the growth of shoots and roots.

KEYWORDS: Growth, Orthosiphon Stamineus, Phenol, 2, 4-Bis (1, 1-Dimethylethyl), Tissue Culture, Yeast Extract.

INTRODUCTION

Orthosiphon stamineus Benth. or 'cat whisker' is a medicinal plant that belongs to the family Lamiaceae that full enrichment with bioactive compounds such as betulinic acid and caffeic acid derivatives (rosmarinic acid) [10-11]. According to [7], leaves of Orthosiphon stamineus is extensively used as antihypertensive, anti-inflammatory, anti-allergic, antibacterial and anti-cancer.

Their various medicinal values trigger O. stamineus to have high market demand, however its conventionally propagated vegetatively by stem cutting [11]. This propagation is low rate of germination and they produced inconsistent of bioactive compounds. Therefore, plant tissue culture technique offer plant improvement such as can reduce the period of plant growth compare to conventional way [15].

The exposures to the plant tissue culture have been recognized recent years with manipulated their bioactive compounds production using different plant growth regulators and elicitors [9]. To increase the amounts of bioactive compound as well as growth rate of plants, yeast extract was introduced. Yeast extract is an example of attention fungi elicitor that was used in plant tissue culture due to their ability to stimulate the defence mechanism, which leads to increase the bioactive compounds production [2]. In plant tissue culture, yeast extract is also used as a supplement in order to promote plant growth, due to its high amino acid content [16]. In [5] claimed rosmarinic acid elicit by yeast extract in Orthosiphon aristatus that was identified and correlated as antioxidant compound. However, Phenol, 2,4-bis (1,1-dimethylethyl) compound in tissue culture of Orthosiphon stamineusis limited explore. As stated by [12], Phenol, 2, 4-bis (1, 1-dimethylethyl)- derivative is present in various plants and is known for its antibacterial and anti-inflammatory activities. Therefore, the aims of this study are to determine the growth and the production of Phenol, 2, 4-bis (1, 1-dimethylethyl) elicit by yeast extract.

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METHODOLOGY

Collection of Orthosiphon Stamineus

The naturally planted of O. stamineus were obtained from the plant nursery. The internode was further cut off and surface sterilization was applied on the explant to culture onto the medium.

Surface Sterilization of Explant

The size internodes of O. stamineus about 0.5 cm long were used as explants. Explants were first washed under running tap water for 30 minutes, followed by immersing in 70 % ethanol solution and then surface sterilization with 15 % clorox solution with addition of Tween 20 and was agitated at 180 rpm in the orbital shaker. The sterilized explants were then rinsed with sterile distilled water for three times to remove any bleach traces.

Preparation of Yeast Extract

The stock solutions of yeast extract were prepared at different concentrations which are 0.5 mgL^{-1}, 2.0 mgL^{-1}, 3.5 mgL^{-1} and 5.0 mgL^{-1}. Yeast extract in powder form were weighted first depends on concentration of yeast extract needed. These different concentrations of yeast extract powder were added into the MS medium together with sucrose and myo-inositol. The solution was mixed by using hot plate and magnetic stirrer until it is fully dissolved.

Preparation of Media and Explant

About 4.4 g of MS media was used and supplemented with 3 % (w/v) sucrose that used as carbon source, gelled with 2.2 % (w/v) gelrite for medium solidify, 0.1 g of myo-inositol for nutrient supply and difference concentrations of yeast extract (0.5 mgL^{-1}, 2.0 mgL^{-1}, 3.5 mgL^{-1}, and 5.0 mgL^{-1}). The solution was mixed by using a magnetic stirrer on a hot plate until it is fully dissolved. Distilled water was be added to justify the volume till 1 L. The pH value of media then was measured by using pH meter to 5.8 ± 0.1 by using 0.1 M of NaOH or 0.1 M of HCl. The medium was autoclaved at 121 ºC, 15 lbs for 15 minutes. The autoclaved medium was poured into each of the pill box and allowed medium to solidify. Cultured explant was incubated in a growth chamber and be maintained at 25 ± 2ºC under cool white fluorescent light with 16 hours of light and 8 hours of dark photoperiod.

Experimental Design

The five different concentrations of yeast extract with eight replicates for each treatment of different concentration. Every 2 weeks, 4 weeks and 6 weeks the plants cultures were observed based on proliferation of shoots, roots and height of stem as well as measurement of the level phenolic compounds (Phenol-2,4-bis(1,1-dimethylethyl) in O. stamineus that were analysed with GC-MS by using soaking method for the extraction process.

Extraction of Tissue Culture Orthosiphon Stamineus

The whole part of O. stamineus were dried in a dry oven for 24 hours at 55 ºC. The dried sample were ground into fine powder and was stored at 25 ºC until further extraction. The biomass dried sample of each treatment in every two weeks was standardized for further extraction process (week 2, 4, 8). The 0.1 g of dried sample was extracted by soak in 2 ml of aqueous methanol. The extracts were filtered through filter paper into 100 ml volumetric flask to get the fitrate extract of O. stamineus.

Identification of Phenol, 2, 4-bis (1,1-Dimethylethyl) by Using GC-MS

The filtrate was manually injected into GC-MS machine. For GC-MS/MS detection, an electron ionization system with ionization energy of 70 eV was used. The GC-MS/MS analysis was performed using waters GC-MS (model 800) equipped with ZB-1 MS-fused silica capillary column (30 m x 0.25 m i.d., film thickness 0.25µm). Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 220 ºC and 290 ºC respectively. The oven temperature was programmed from 50 ºC to 150 ºC at 3 ºC/min, held isothermal for 10 min and finally raised to 250 ºC at 10 ºC/min. The relative percentage of Phenol, 2, 4-bis (1,1-dimethylethyl) was expressed by peak area normalization.

RESULTS AND DISCUSSION

The Growth of Tissue Culture Orthosiphon Stamineus

The proliferation of shoots and roots of Orthosiphon stamineus after two weeks being cultured in MS medium containing difference concentration of yeast extract. Figure 1 shows the proliferation of shoots of O. stamineus is highest compare to roots which at treatment 5.0mg/L the mean values were (8.6259 ± 0.3750) and (0.2500± 0.1637) respectively. These might be due to the explants that used in these culturing process is internode that shown the highest capability to induce shoot first compare to others part of plant. When the direct plant regeneration was at high frequency of 92% in internodes, 88% in leaf segments and 43% in root segments. This led to the formation of multiple shoot clusters on established culture media with rapid proliferation rates. The lowest shoots proliferation of O. stamineus shown in MSO that act as a control with no addition of yeast extract and its value of mean is (5.0000 ± 0.4226). These results shown that the addition of yeast extract effect the proliferation of shoots in tissue culture of O. stamineus. According to [18], addition of yeast extract to culture medium enhances the shoot proliferation rate in vitro. The promotive effect of yeast on shoot proliferation was reported earlier in Lavandulalatifolia in vitro studies. Moreover, in [13] obtained the yeast extract as an elicitor expression defense response by establishment of actively growing cell cultures of Astragaluschrysochlorus cells.

The different proliferation of shoots and roots tissue culture Orthosiphon stamineus when treated with difference concentration of yeast extract after fourth weeks being cultured were illustrated in Figure 2. When 5.0mg/L concentration of yeast extract was treated in tissue culture of O. stamineus the results shown the highest growth of shoots and roots where the mean values were (17.3750 ± 2.6988) and (8.5000 ± 1.954) respectively. Figure 3-4 shown the effect of different concentration of yeast extract on the growth of tissue culture O. stamineus in week sixth. Same as week 2 and week 4, at 5.0mg/L concentration of yeast extract shown the highest proliferation of shoots and roots. Proliferation of shoots still shown the highest proliferation compare to roots. In the observation from week 2 until week 6, at 5.0mg/L concentration of yeast extract the proliferation of roots increases drastic and continuously from the values of mean are (0.2500 ± 0.1637), (8.5000 ± 1.9548) and (13.5000 ± 1.0522). In weeks 6, 5.0mg/L concentration of Ye also shown the highest proliferation of shoots (24.5000 ± 2.5355), compare to proliferation of roots where the mean values are 13.5000 ± 1.0522.

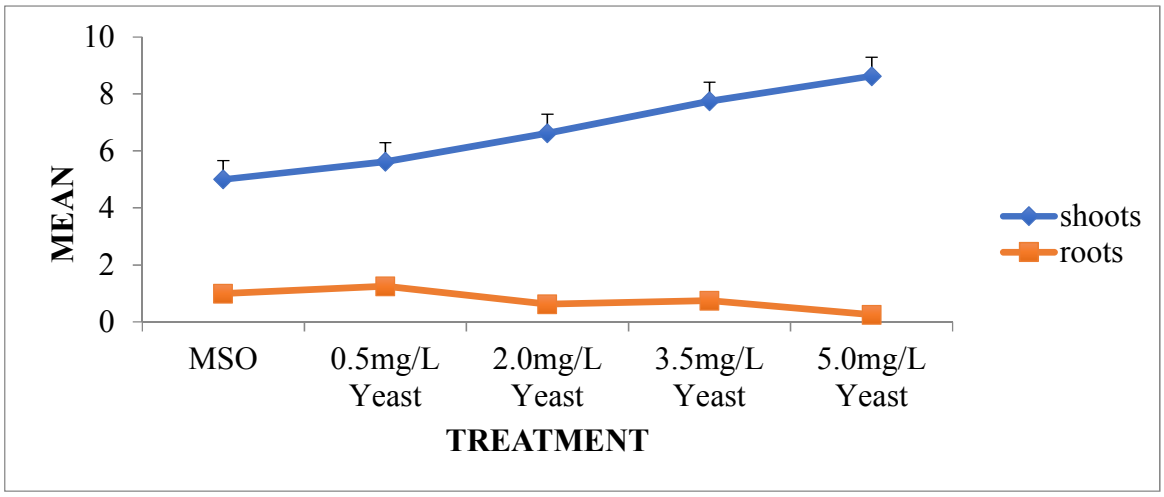

Figure 1: The proliferation of shoots and roots of Orthosiphon stamineus after two weeks cultured

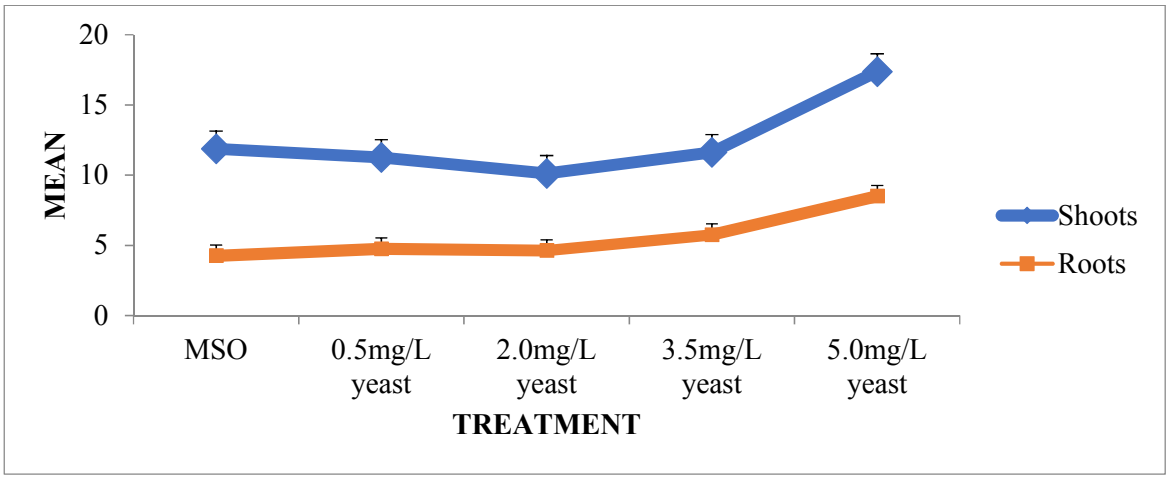

Figure 2:The proliferation of shoots and roots of Orthosiphon stamineus after four weeks cultured

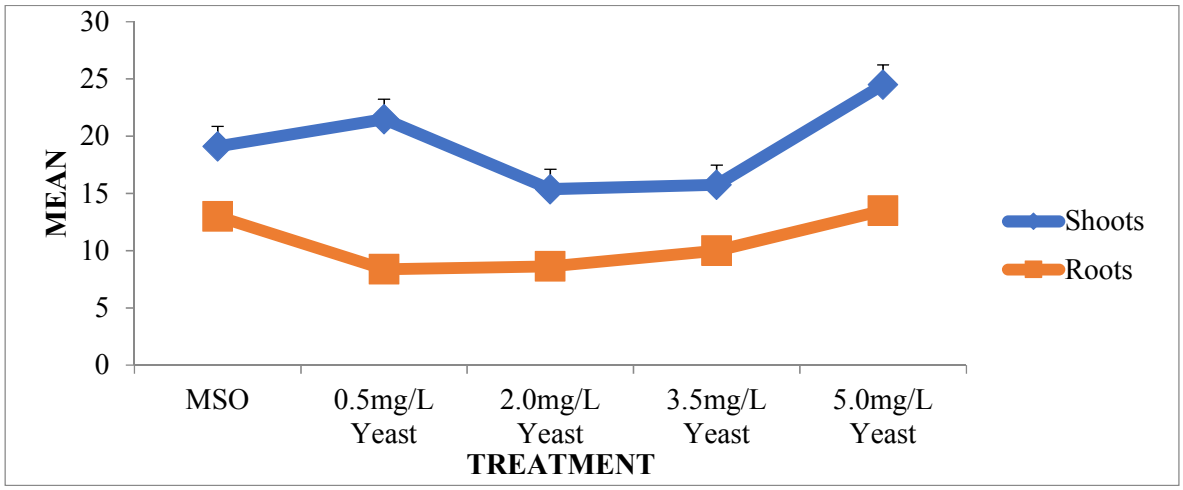

Figure 3: Proliferation of shoots and roots of Orthosiphon stamineusafter sixth weeks cultured

Figure 4: Orthosiphonstamineus at week 6 supplemented with 5.0mg/L concentration of yeast extract

Identification and Percentage Composition of Phenol, 2, 4-bis (1,1-Dimethylethyl)

Table 1 shown the identification and percentage composition of Phenol, 2, 4-bis (1,1-dimethylethyl) in tissue cultured Orthosiphon stamineus supplemented with different concentrations of yeast extract within 2, 4, 6 weeks cultured analysed by GC-MS. From the result, in week 6 the percentage composition of Phenol, 2, 4-bis (1,1-dimethylethyl) increased by a week and the highest after culture onto MS media supplemented with 5.0 mg/L yeast extract (31.42%) at retention time 30.26. However, in week 2 and 4, without addition of yeast extract (MSO) shown the highest percentage composition of Phenol, 2, 4-bis (1,1-dimethylethyl) compare to O. stamineus cultured onto MS medium supplemented with yeast extract which the values of percentage composition analyzed were 10.50% and 9.45% respectively. According to [17], secondary metabolites natural products from fungi were reported contained various anticancer, antioxidant and other biological activities including the presence of phenol, 2, 4-bis (1,1- dimethyl ethyl) was observed, where the compound is reported for antibacterial activity. The antibacterial activity of phenol, 2, 4-bis (1,1-dimethyl ethyl) appeared to correlate with the antioxidative activity reported to this compound, because it could inhibit the ROS production in both Aspergillus and Phytophthoracinnamomi [14]. In [6] stated to improve the production of secondary metabolites of in vitro plant cultures, several strategies such as supplementation and elicitation have been employed for instance, the use of yeast extract and jasmonic acid.

Table 1: Percentage composition (%) of Phenol, 2, 4-bis (1,1-dimethylethyl) in different weeks

Retention Time	Conc. of YeastExtract	Weeks	Percentage Composition (%)
30.26	MSO	Week2	10.50
		Week 4	9.46
		Week 6	9.53
	0.5mg/L	Week 2	7.82
		Week 4	6.84
		Week 6	7.36
	2.0mg/L	Week 2	7.72
		Week 4	4.57
		Week 6	20.99
	3.5mg/L	Week 2	6.47
		Week 4	5.26
		Week 6	9.24
	5.0mg/L	Week 2	7.28
		Week 4	5.01
		Week 6	31.42

These results were supported by [8] where the therapeutic effects of Orthosiphon stamineus have been described mainly to its polyphenol, the most dominant constituent in the leaf that be effective in reducing oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems which could lead to some of the chronic diseases such as coronary heart disease. The leaves of Orthosiphon stamineus was reported to have the highest antioxidant properties whereby the phenolic fraction is the most active principle among the phytochemicals studied [4]. Phenol, 2, 4-Bis (1, 1-Dimethyl ethyl) has molecular weight, 206; molecular formula, C H O has good antioxidant 14 22 activity [3]. According to [1], in the Malaysian mango kernel also was identified present Phenol, 2, 4- Bis (1, 1-Dimethyl ethyl) by GCMS and reported to be responsible for antibacterial activity. Moreover, as stated by [5], the yeast extract (5.0mg/L) elicitation resulted in the highest production of bioactive compound (rosmarinic acid) in tissue culture Orthosiphon stamineus.

CONCLUSION

The yeast extract can be a potential fungi elicitor to increase the production of secondary metabolites of tissue culture O. stamineus as well as the proliferation of shoots and roots of plants. Treatment of 5.0mg/L yeast extract also shown the highest effect for growth of O. stamineus which shown the highest proliferation of shoots compare to proliferation of roots (24.50 ± 0.215) and (13.50 ± 0.554) respectively. In week 6, production of Phenol, 2, 4-bis (1,1-dimethylethyl) shown the highest percentage composition (31.42%) in the treatment supplemented with 5.0mg/L concentration of yeast extract. The lowest production of Phenol, 2, 4-bis (1,1-dimethylethyl) shown in tissue culture Orthosiphon stamineus that containing 2.0mg/L yeast extract which the percentage composition is 4.57%.

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