Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Effect of Media Types on the Growth of Callus Culture in Kumis Kucing *Orthosiphon aristatus* (Blume) Miq

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Abstract

The lack of conventional availability of plant Kumis Kucing makes tissue culture techniques used as a solution to overcome this problem. In tissue culture, media is a major factor in producing a good crop of plantlets. Media Murashige & Skoog (MS), Lloyd & McCown Woody Plant (WPM) media was used in the induction of *Orthosiphon aristatus* (Blume) Miq cat whiskers callus culture, in this study also used growth regulators in the form of 2,4-D added to each-individual media. The results showed the best callus growth occurred in Murashige & Skoog (MS) media compared to the Lloyd & McCown Woody Plant (WPM) media, where the callus produced was 3.28 g on MS media.

Keywords: Kumis Kucing, callus culture, growth media

Submitted: 16 August 2019 **Accepted**: 03 November 2019 **DOI**: https://doi.org/10.25026/jtpc.v5i1.210

Introduction

Orthosiphon aristatus (Blume) Miq. is a plant that is widely spread in Southeast Asia and has been used in traditional medicine, especially in Indonesia [1]. The community uses this plant as a medicine for diabetes, hypertension, and kidney stones [2]. Some of the results of the bioactivity tests that have been done, leaf extract of Kumis Kucing have activities as diuresis [3], analgesics [4], antipyretics [5], antioxidants [6], anti-hyperglycemia [7], antihypertensive [8]. The active compounds contained in it include Sinensetin, Salvagenin [9], Euporatin [10], Rosmarinic Acid, Caffeic Acid [11]. At present some Indonesian herbal medicine industries have produced standardized herbal medicine (OHT) preparations from leaf extract of Kumis Kucing with several properties for degenerative diseases.

The Indonesian Herbal Medicine industry has problems in providing sources of raw material for kumis kucing plants. As much as 60% of the 54 tons of raw material available for kumis kucing are not from cultivation [12], this has an impact on

the quality and quantity of the products produced [13]. Intercropping system results in the quality of raw materials obtained still not meeting the standards of the quality of a pharmaceutical industry [14]. Therefore, tissue culture techniques can be applied to overcome the existing problems. Tissue culture techniques have the several advantages, namely in tissue culture techniques to produce metabolites are big and fast, can produce plantlet in large quantities and does not require large amounts of land, but it also can be used directly in the research of genetic level [15].

Studies in plant tissue culture cannot be separated from media use. The culture medium contains macro and micronutrient elements used for the process of growth of an explant into a plantlet or callus. Callus is an initial stage for making other cultures such as cell suspension culture, root hair culture, and embryo culture used for the production of compounds in the field of pharmaceutical biotechnology [15]. There are many media available and have their respective functions in their uses. MS media used generally for all species of plants, media WPM used in woody plants, B5 media to crop legume species, media Vacin and Went for orchids [15].

The development of kumis kucing plants in the field of tissue culture is still very little and has not been widely carried out regarding the exploration of media used, previous research is only based on the use of one type, MS media [16-21]. On the other hand research in the field of culture proves that the use of various types of media produces different growth responses such as research on acacia plants that have been explored using a variety of media (¾ MS, WPM, B5) and produce different responses for the formation of callus, root & embryos from acacia plants [22]. In addition to the other types of plants such as blueberries have been cultured using media WPM, MS, and MW and produce different shoots of growth of any media used [23]. Therefore the use of the right media for the production of a plant is very important to be investigated further to find media that is suitable for a plant.

The focus of this study is to compare the use of MS media with WPM media to see how the callus growth response from explants of kumis kucing plants, so that good media is obtained in a culture of kumis kucing plants.

Materials ad Methods

Selection Explant

Kumis Kucing plant samples were collected and obtained around the yard of the Mulawarman University Environmental Research Center Samarinda, East Kalimantan. The sample was successfully identified under the scientific name *Orthosiphon aristatus* (Blume) Miq by the Mulawarman University Dendrology and Forest Ecology Laboratory.

Explant Preparation

Explat preparation was carried out at the Mulawarman University Faculty of Pharmacy Tissue Culture Laboratory, by taking young leaves at the top of the leaves, then the leaves washed with running water and cleaned from dirt, after that the leaves were placed into culture bottles and explants were ready to be sterilized.

Explant Sterilization

Explant sterilization is carried out aseptically, by immersing the explants into several sterilizing solutions, the solution used consists of a 10-minute soap solution; bactericidal 0.05% 5 minutes; fungicide 0.1% 5 minutes; 70% alcohol 2 minutes; Sodium hypochlorite solution (5.25% NaOCl + Tween 80 0.1 mL) 5 minutes then the explants were rinsed with sterile aquades three times, then cut the explants with a size of ± 0.25-5 cm^2, then placed on the surface of the basal media, then incubated at 22°C temperature conditions, 4000 Lux, and photoperiod phase 8/16. [24].

Preparation of stock solution of 2,4-D

As much as 0.05 g 2,4-D, put it in a beaker glass, add a few drops of KOH 1 N until dissolved, then transfer it to a 100 ml volumetric flask, enough to mark the limit, then homogenize, store the stock of solution in bottle storage.

Media Preparation

The MS and WPM media used were Murashige & Skoog Basal Medium with Vitamins and Lloyd & McCown's media Woody Plant Basal Medium with Vitamins obtained from PhytoTechnology Laboratories®. By making according to the composition of the media, namely, 4.43 g/ L MS and 2.41 g/ L WPM. Dissolve the media in distilled water then add

sucrose with a concentration of 3%, pH of the medium 5,6-5,8. If the pH is not appropriate add a few drops of HCl/KOH solution (1N HCl to reduce pH and 1N KOH to increase pH). Then the media added a solidifying agent in the form of 0.7% while stirring and heated to dissolve, add ZPT 2,4 D with a variety of concentrations (0.5; 1; 1.5; 2; and 3) ppm in each media, then the media is divided into culture bottles. Sterilize with Autoclave 121ºC ± 15 minutes, remove and store the media in the incubation room before use.

Induction of Callus Culture

The explants were sterilized was added to each medium with various concentrations of PGR (0.5, 1, 1.5, 2, and 3) ppm, conducted three replication for each of the various concentrations of PGR. In one bottle the media contains 3 leaf explants. Incubation of explants for 28 days, with conditions of 22 °C, lighting 4000 Lux, and photoperiod 8/16.

Data analysis

Analysis of the data in this study was in the form of an assessment of the ideal callus criteria by looking at the callus morphology (color and texture) and callus growth obtained from the callus mass for 28 days. The results of the data presented in the form of images and graphics to determine the media that are good in the culture callus of kumis kucing explant.

■ Results and Discussion

The effect of MS and WPM media on callus induction uses 2.4 D

Media and plant growth regulators play a role in the induction of plant explants into callus cells that have irregular shapes. Callus induction from MS media and WPM media was carried out by adding several concentrations of growth regulator 2.4 D. The 2.4 D concentration used in MS media and WPM media was 0.5 ppm; 1 ppm; 1.5 ppm; 2 ppm and 3 ppm. Callus cell growth from the type of media given growth regulator expressed in units of average weight of callus obtained from each week, callus growth is defined as a permanent increase in the size of the callus cell section, where wet weight can represent variable callus cell growth [25].

Giving a concentration of 1.5 ppm 2.4 D on MS media can induce good callus cell growth. WPM media giving a concentration of 3 ppm 2,4 D can induce good callus cell growth. Plant growth regulators and the media play a role in the induction of callus cells. The use of plant growth regulator auxin types (2.4 D) for leaf explants cat whiskers, has been reported to induce callus cells with either [19,20,26].

Callus formation (Figure 1) average occurred on the 7th day on media MS and WPM medium, characterized by there is a set of cells that grow at the edge of the explant with irregular shape, it is consistent with other studies [20] using kumis kucing leaf explants, mention the callus can be induced on the 7th day. The difference in the concentration of 2.4D from MS media and WPM media which can induce the best callus is due to the content of the media that can affect callus cell growth.

Effect of MS and WPM media on callus characteristics at each 2.4 D Growth Regulating Substance concentration

Color Callus

Color is the main indicator in the success of callus cell growth, callus has a variety of colors but in general the color of the callus is white to bright yellow, bright colors that have a callus indicate that the callus condition is still quite good [27] and has not undergone stationary phase [28] The color of callus produced from MS media and WPM media with variations in the concentration of growth regulator 2.4 D resulted in different callus colors.

The initial color of callus on MS media (Table 1) with a concentration of 2.4 D 0.5 ppm and 1 ppm produced callus Cream white while the concentration of other growth regulators was 1.5 ppm; 2ppm; and 3ppm, the initial color of the Pale green colored callus. After 28 days of the overall concentration of the 2.4 D growth regulator on MS, the color of the callus was Dirty white. In contrast to the color of callus on MS media, WPM media (Table 2) which were given several concentrations of growth regulator 2.4 D, produced different callus colors. The color of callus in the WPM media from 4 concentrations of 2.4 D given (1ppm; 1.5ppm; 2 ppm; and 3 ppm) was colored Dirty white, whereas at a

concentration of 0.5 ppm the callus was colored yellow Cream, after 28 days of overall concentration 2.4 D growth regulator on WPM media, the color of the callus turns brown.

Figure 1. Callus formation (left and right)

Table 1. Callus morphology in MS media

PGR (2,4 D)	Callus day seen	Color of Callus		Texture of callus
		21 Day's	28 Day's	
0,5ppm	7 Day	*Cream white*	*Dirty white*	Compact
1ppm	7 Day	*Cream white*	*Dirty white*	Compact
1,5ppm	7 Day	*Pale green*	*Dirty white*	Compact
2ppm	7 Day	*Pale green*	*Dirty white*	Compact
3ppm	7 Day	*Pale green*	*Dirty white*	Compact

Table 2. Callus morphology in WPM media

PGR (2,4 D)	Callus day seen	Color of Callus		Texture of callus
		21 Day's	28 Day's	
0,5ppm	7 Day	*Cream yellow*	*Brown*	Compact
1ppm	7 Day	*Dirty white*	*Brown*	Compact
1,5ppm	7 Day	*Dirty white*	*Brown*	Compact
2ppm	7 Day	*Dirty white*	*Brown*	Compact
3ppm	7 Day	*Dirty white*	*Brown*	Compact

Callus color on MS media (Figure 2), with variations in the concentration of regulated substances growing 2.4 D (0.5 ppm; 1 ppm; 1.5 ppm; 2 ppm; and 3 ppm), on the 7-21 day is a good callus. While on WPM medium (Figure 3), callus was good only at a concentration of 0.5 ppm 2,4 D, therefore it can be said MS medium good medium in growing callus from kumis kucing leaf explants, because of the results obtained initial color callus on MS medium entry in the indicator as a good callus.

The brown color of the callus found in the explants of the Kumis Kucing leaves shows that the callus undergoes a stationary process, wherein this phase the callus does not carry out cell division [27]. Browning is a natural event that can occur in callus caused by the influence of other biochemical compounds that often occur in parts of plants [16]. Brown color on the callus indicates that the growth of callus decreased [20], so that good callus has the colors tend to be bright.

Callus Texture

In addition to color, callus texture is used as an indicator in ideal callus assessment. Callus texture is divided into two types, namely compact and crumbs. This study produced a compact callus texture from both media used, namely MS media and WPM media. The compact callus texture shows that the callus contains more

secondary metabolites than the crumb texture [25]. Nutritional elements from the media play a role in the formation of callus textures. Callus texture of Kumis Kucing leaf explants (*Orthosiphon aristatus* (Blume) Miq) produced in this study is different from previous studies [29], where the results of other studies state that kumis kucing leaf explants have crumby callus texture, this difference is caused by callus texture influenced by the type of plants used, the composition of nutrient media, growth regulators and environmental conditions of the culture [30].

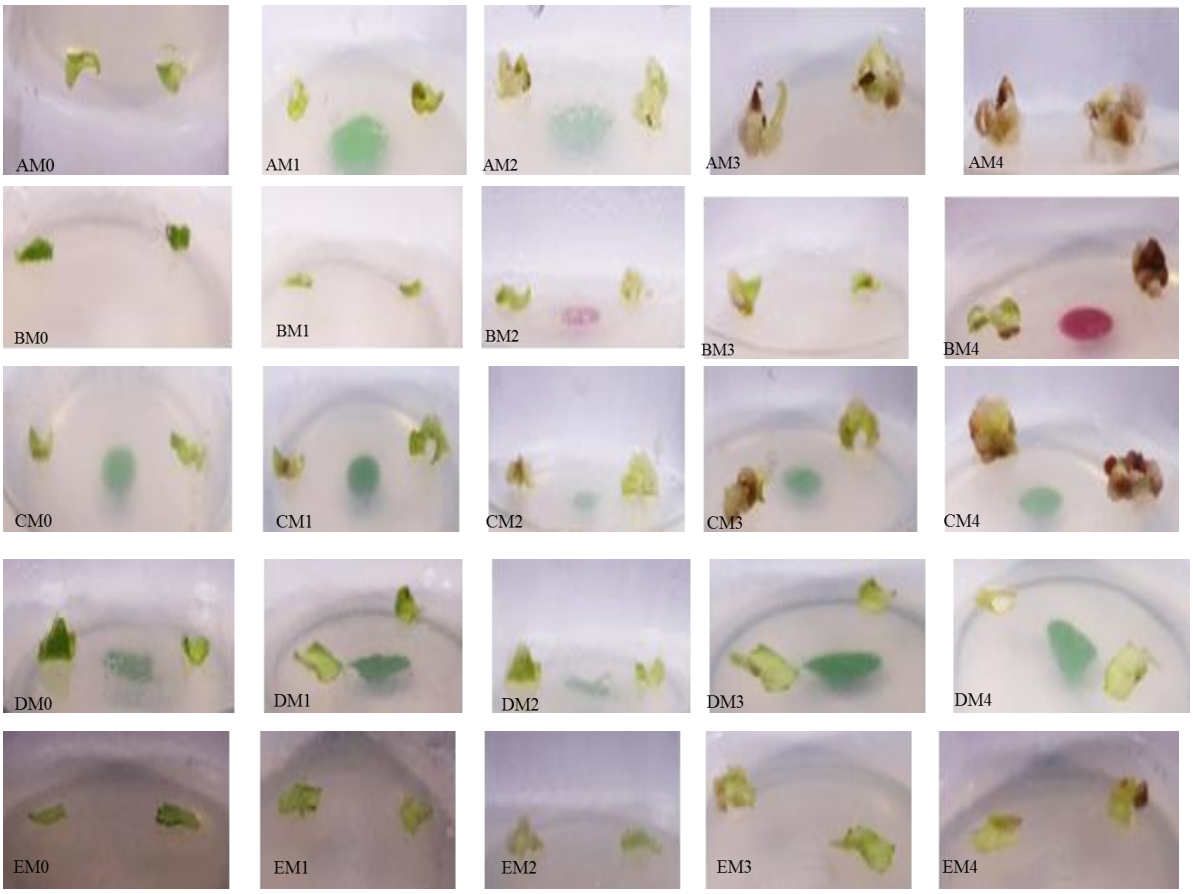

Figure 2. Morphology of callus leaves of cat whiskers explants on MS media, A (0.5ppm 2.4D), B (1ppm 2.4D), C (1.5ppm 2.4D), D (2ppm 2.4D), E (3ppm 2,4D), M (Week's)

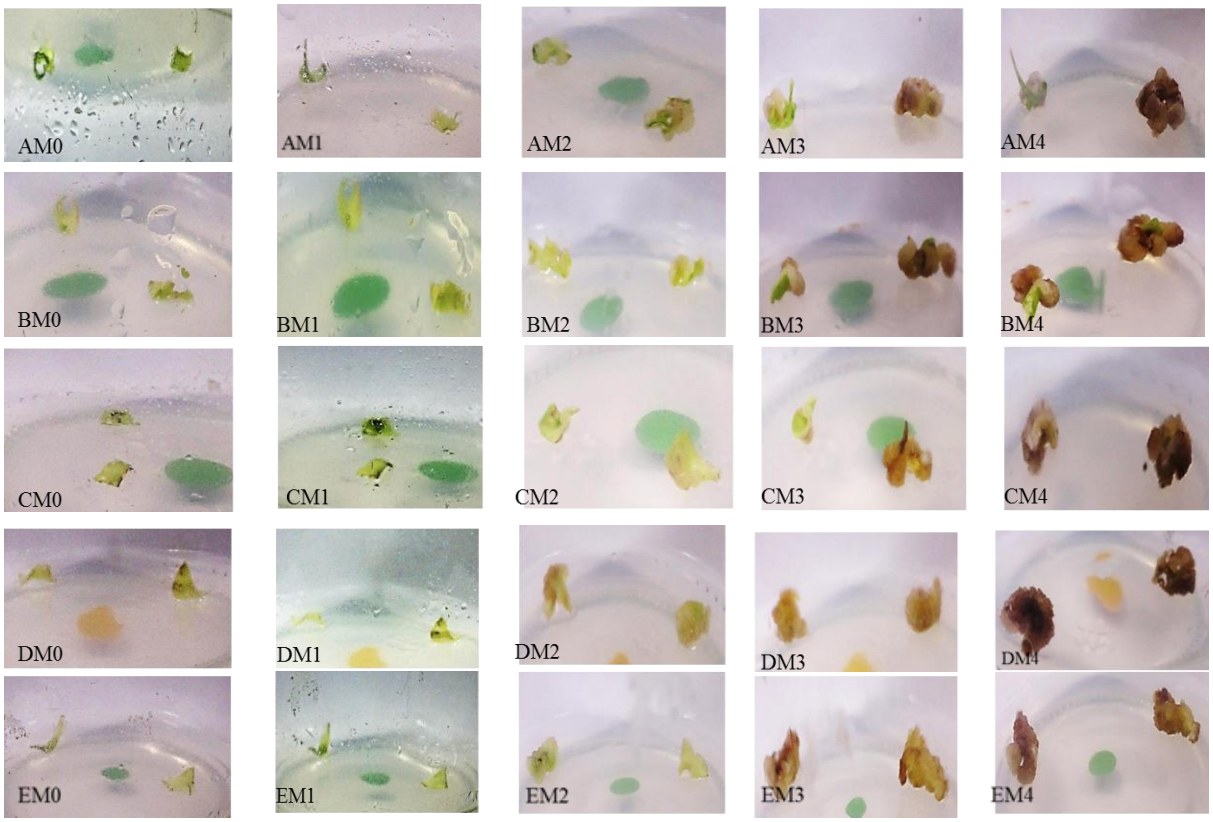

Figure 3. Morphology of callus leaves of cat whiskers explants on MS media, A (0.5ppm 2.4D), B (1ppm 2.4D), C (1.5ppm 2.4D), D (2ppm 2.4D), E (3ppm 2,4D), M (Week's)

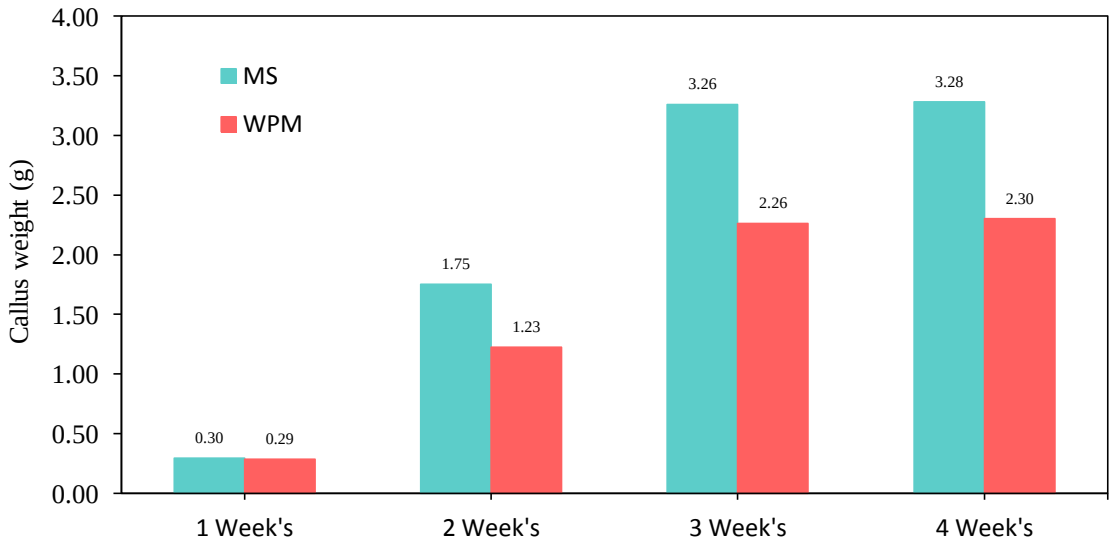

Figure 4. Graph of comparison of callus weight average between MS and WPM media

Effect of MS media and WPM media on callus mass.

Callus mass measured in weight units is used as an indicator of the effect of the successful use of a culture medium. Obtaining a large callus mass can increase the production of secondary metabolites and plantlets from an explant. The callus mass generated from this study (Figure 4), MS media is higher than the WPM media. Callus mass on MS media is 3.28 g and WPM media is 2.30 g. Optimal growth of callus mass from MS media and WPM media occurred at week 3 and began to decline at week 4, this was caused by nutritional factors from different types of media can affect the mass of callus produced.

The nutritional composition of MS and WPM media is very different. Sources of producing nitrogen elements in MS media are higher than WPM media, Nitrogen plays a role in forming glutamine [28] which is used for energy metabolism and cell proliferation [31], if the higher the proliferation, then the callus period formed is also greater. Then the potassium ion (K^+), MS media has a higher potassium (K^+) ion content compared to WPM media, potassium ions have a role in the process of diffusion between cells [32], cell turgor, and stomata movement [28], which play a role in callus cell growth process. From the composition of nutrition media, MS media is a medium that can be used to increase the mass gain of callus from kumis kucing leaf explants.

Conclusion

Orthosiphon aristatus (Blume) Miq Kumis Kucing callus culture from two types of media used, namely MS media and WPM media, seen from the morphology and weight of the callus obtained, that the use of MS media produced better callus growth compared to WPM media.

Acknowledgements

My gratitude, to the pharmacy faculty who have provided research funding through the grants of beginner lecturers (Hibah Dosen Pemula) in 2016 to develop their potential and train the skills of lecturers and researchers

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