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Detection of Essential Oils of Patchouli Leaves (*Pogostemon cablin* Benth) with Combination of 2,4-Dichlorofenoxyacetate and Coconut Water In Vitro

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Abstract. The study of essential oils detection of patchouli leaves (Pogostemon cablin Benth) with combination of 2,4-Dichlorofenoxyacetate and coconut water which produced calli had been done at Departement of Food Crops and Horticulture, Tissue Culture Laboratory, North Sumatra and the Organic Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, University of Sumatera Utara. The aim of the study was to determine the concentration of the combination of 2.4-D and coconut water which added in MS media for detection essential oils in patchouli calli in vitro. The experiment used factorial Completely Randomized Design (CRD) with two factors. The first factor was coconut water with concentrations of 0, 10 and 20%. The second factor is 2.4-D with a concentrations of 0, 0.5 and 1 ppm.The results showed that the optimum combination of 2,4-D and coconut water was capable to induce calli initiation after 13 DAP (days after planting), 80% of calli production, green color, nonfriable, and calli weight of 0,28 gram. The TLC (Thin Layer Chromatography) result showed that the treatment of 0.5 ppm 2,4-D and 10% coconut water produced the same Rf value as Rf standard (patchouli oil) which was 0.87

Keyword: Essential oil,coconut water, Patchouli, TLC.

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1 Introduction

The agricultural sector with all the output produced is a fairly high sector compared to other sectors. This has been tested when Indonesia is hit by an economic crisis. Products from the agricultural sector actually become one of the sources of foreign exchange income for the country. Generally these commodities come from plantations, one of which is the result of plantations in the form of essential oil. Essential oils are traditional plant outputs that are widely used in the chemical industry as fragrances (perfumes), cosmetics, medicines, and other basic industrial needs. Of the 70 types of essential oils traded on the international market, about 9-12 types of essential oils including fragrant lemongrass oil, patchouli, fragrant root, mementos, eucalyptus,

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cloves, pepper, and jasmine oil are supplied from Indonesia. Of these types of oil, 70% of the world market is controlled by patchouli oil [1].

Patchouli oil is one of Indonesia's potential essential oil export commodities. According to ISO 3757:2002, the quality of patchouli oil is controlled by the indicator of patchouli alcohol level at the standard limit of 27%-35%. But the world market demand requires a patchouli alcohol content of at least 31%. In previous studies conducted by Setyanto and Rahman the content of patchouli alcohol in the resulting patchouli oil was still not good with an average of 28.92% [2].

The increase in essential oils in patchouli plants can be done with various studies. The process of producing secondary metabolites such as essential oils through in vitro plant tissue culture is one aspect of growth [3]. According to [4], in vitro culture has an important role in obtaining results that are impossible to achieve through in vivo techniques. According to [5], in vitro culture techniques have an advantage in the production of secondary metabolites when compared to whole plants due to the speed of cell growth and biosynthesis in cultures that starts from very high explans in a very short period of time. According to [6], the use of plant in vitro cultures maintained under controlled environmental conditions, nutrients, and growing regulatory substances will produce metabolites continuously. One culture commonly used to produce secondary metabolites is callus culture.

A callus is a group of undifferentiated cells. In theory, all plant tissues whose cells are still alive can form calluses in vitro. However, young plant tissue (no liignifiance in the cell wall), or meristematic young tissue will more easily produce calluses. Calluses will form if the explant is planted in a culture media containing amoxine and cytokinin of the same ratio or a medium containing 2,4-D (Dichlorophenoxyaceticacid) [7]. This growing regulatory substance is stable because it is not easily damaged by light or heating at the time of sterilization [8]. Concentrations of 2.4-D 1 ppm and 2 ppm provide the best results for callus formation in micropropagasi strawberry plants [9] In this study the researchers used young coconut water as a substitute for cytokines. Cytokinin plays a role in spurring cell division, proliferation of final meristems, and encouraging the formation of chlorophyll in callus [1].

From the description above it can be known that in vitro plant culture with the addition of young coconut water and 2,4-Dichloroenoxiacetat in MS media has a good influence on plant growth in vitro, for it needs to be added to MS media i.e. the combination of coconut water and 2,4-Dichloroenoxiacetat to detect the essential oil content in pogostemon cablin Benth patchouli callus culture. Patchouli oil production in Indonesia has not produced maximum results so it is necessary to conduct research on the process of producing secondary metabolites of essential oil through plant tissue culture detection regarding essential oils with the addition of 2,4-Dichlorophysoxyacetate and young coconut water in MS media through callus culture

2. Research Methods

2.1. Time and Location of Research

The study was conducted from September 2017 to October 2018 at the Food and Horticulture Plant Tissue Culture Laboratory, Medan, North Sumatra and at the Organic Chemistry Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of North Sumatra.

2.2. Research Methods

2.2.1 Research Plan

This study used a completely randomized design (CRD) experimental method with 2 observation factors, the first factor was the concentration of young coconut water and the second factor was the concentration of 2,4 dichlorophenoxyacetate.

Factor 1

(A): Treatment of MS media with the addition of young coconut water

A0: Concentration 0%

A1: Concentration 10%

A2: 20% concentration

Factor 2 (D): Treatment of MS media with the addition of 2,4-D

D0: Concentration 0 ppm

D1: Concentration 0.5 ppm

D2: Concentration 1ppm

Thus obtained 9 treatment combinations between the concentration of young coconut water and a concentration of 2,4 Dichlorophenoxyacetate, namely:

$A_0 D_0$	$A_1 D_0$	$A_2 D_0$
$A_0 D_1$	$A_1 D_1$	$A_2 D_1$
$A_0 D_2$	$A_1 D_2$	$A_2 D_2$

2.2.2 Explan Preparation

The plant material used is the leaves of Benth Pogostemon patchouli cablin obtained from farmers' land in Sidikalang Dairi Regency, North Sumatra. Young patchouli plants are moved from the soil to the polybeg. The patchouli plant is then moved to a shady place and awaited about four weeks until the patchouli plant has many new shoots. At the age of three weeks the patchouli plant is sprayed with fungicides. The explant used is the second leaf and the third leaf.

2.2.3 Sterilization of tools

Research tools in the form of a set of desecil tools (tweezers, skapels, scissors and knives), culture bottles and petri dishes, washed and cleaned with soap, rinsed with running water then put in an autoclave and heated to temperature 121°C for 1 hour.

2.2.4 Creation of Stock Solutions

The manufacture of stock solutions is done by weighing chemicals, macronutrients, micronutrients, vitamins and ZPT according to the composition of MS media for patchouli plants. The ingredients are dissolved with sterile a akuade and then stirred until homogeneous using a magnetic stirrer, then put into a bottle labeled according to the treatment. For the stock of hormone 2.4 D is done by weighing the hormone as much as 0.1 grams then dissolved in aade streril 100 ml and then stirred homogeneously, then put in a labeled bottle and stored in the refrigerator.

2.2.5 Media Creation

MS media creation is done by entering the composition of MS media Is a stock solution consisting of stock solutions A, B, C, D, E, F, and vitamins as needed. The mixture of stock solution is put into the erlenmeyer, then added aquades up to a volume of 1 liter. Next, the solution is added 40 g/l of sugar and measures the acidity of the solution using a pH meter. The required media pH is 5.8. If it is too alkaline or acidic then add HCl or NaOH to get a pH of 5.8. After the pH measurement, the solution is put into a pot that already contains gelatin.-agar and heated while stirring well until the solution boils. For the treatment media used is a mixture of MS media material with hormone 2,4-D. Mixing is done with existing treatments according to the concentration of each needed, then the media is poured into the erlenmeyer and covered using aluminum foil and plastic seal, then sterilized autoclave at a pressure of 17.5 Psi, temperature 121□C for 20 minutes. The media that has been poured into a 5 ml petri dish is then covered and glued again with a plastic seal. The poured media is then stored in the cultural space before the media is used for planting. This storage aims to find out if there is contamination in the culture media before it is used to plant explants.

2.2.6 Sterilization and Planting of Explan

Some pogostemons leave cablin Benth patchouli. Cleaned with running water then soaked with liquid detergent for 10 minutes and rinsed under running water 3 times. Then soaked in a fungicide solution 1% plus 2 drops of tween 80 for 10 minutes. Then soak in an antibiotic solution plus 2 drops of tween 80 for 30 minutes, then rinsed with sterile aquedy aquedy three times. After that the explant is inserted into Laminar Air Flow to avoid contamination, then explan is soaked in a solution of $HgCl_2$. 0.5% for 1 minute then rinsed with a akuades three times. Front Soaked in a solution of clorox% for 5 minutes then rinsed with sterile aquedade as much as yiga time. Finally,

explan is soaked in 70% alcohol and then rinsed three times using sterile a akuade, placed in a sterile petri dish then cut into pieces using a skapel with a size of 1x1 cm. Then the explant that has been cut is soaked in betadine that has been dissolved in water with a ratio of 10 drops of betadine in 10 ml of water. After that, dredged on filter paper for 5 minutes. Explant is implanted using sterile tweezers into a bottle that already contains a treatment medium. Bottles that have been planted are covered and diselotip with plastic seals then stored in the culture room and stored at a temperature of 25°C. Observe the growth of calluses for 50 days.

2.2.7 Parameters of Callus Observation

Observations are made every day starting from the day after planting (HST) during the 50 days. Parameters observed among others [10]

a. Time begins to grow callus

Calculated from the day after planting (HST).

b. Percentage culture forms callus:

$$\text{Percentage culture forms callus} = \frac{\text{Number of plants growing}}{Number\ of\ repetitions} x100\ \% \quad (1)$$

c. Wet weight of callus (gram)

The weight of wet culture can be known by weighing the culture at the end of adaming.

d. Color of Callus

e. Texture of Callus explan

2.2.8 Making Of Nilam Callus Extract

Patchouli calluses are drenned to dry. Dried calluses are made powder by pounding using mortar and pastels. Then weighed 0.5 grams and is accelerated 3 days with ethyl acetate 5 ml. The results of maceration are then evaporated using waterbath until a thick extract is obtained [11].

2.2.9 Detection of Essential Oils

The identification reaction of patchouli leaf essential oil is the chromatography analysis of a thin layer using GF254 silica gel as a stationary phase and a phase of motion of hexane-Ethyl acetate (8.5:1,5) by development methods. Spotting is observed with UV light. The reagents used for spraying are vanilin–H_2SO_4. The positive response contains essential oils in the form of purple patches with Rf of about 0.75-0.95 [11].

2.2.10 Phytochemical Test

a. Alkaloid Test

The callus extract and essential oil were each put into two test plate holes, as many as 3 drops each, the sample was tested with dragendorf reagents, and Wagner. The presence of alkaloids is characterized by the formation of white deposits with Mayer reagents, orange red deposits with Dragendorf reagents and brown deposits with Wagner reagents.

b. Terpenoid test

3 drops of callus extract and essential oil are each put into the test plate hole, added liebermand-Buchard reagent (mixture of 3 drops of anhindrate CH_3COOH a with 1 drop of concentrated H_2SO_4). If the orange color is red or purple is positive for terpenoids, if the green color is positive for steroids, if the above test is negative, then the sample added methanol dripping on the test plate

c. Flavonoid Test

A sample of 5 drops is put into a test tube, then added ethanol and heated for 15 minutes. Then evaporate the solvent until it remains slight and adds concentrated HCl and 0.2 g of Mg powder, when a positive red color appears in the presence of flavonoids.

2.3 Data Analysis

The data obtained is analyzed using ANOVA (Analysis Of Variance). If the treatment has any real effect then continue with the DMRT (Duncan New Multiple Range Test) test at a rate of 5% with the help of SPSS version software. 22.

3. Result and Discussion

Detection of the essential oil content of patchouli leaf callus (Pogostemon cablin Benth) was given treatment 2.4 Dichloroenoxiacetat and young coconut water were randomly selected from nine combination variations in MS media. Data at the time of callus initiation, percentage of explan growth, callus color, callus texture, weight of callus, secondary metabolite content (alkaloids, bouncoids, and flavonoids) and essential oil content in patchouli extract have been obtained.

3.1. Initiation Time Patchouli Leaf Explan Pogostenon cablin Benth On MS Media with Combination of 2.4 Dichlorophyxiacetat and Young Coconut Water

The initiation time of callus is calculated manually from the day after planting (HST) to the formation of callus. Statistical test results showed the administration of young coconut water, 2,4

Dichloroenoxiacetatate, and the combination of the two had no noticeable effect at the time of calus initiation (p>0.05). The average value of initiation time of statistical test results can be seen in Table 1.

Table 4.1 Time Initiation Callus (Day) Explan Nilam Leaves Pogostenon cablin Benth In MS Media with a Combination of 2.4 Dichlorophyxiacetics and Water Young Coconut

Water concentration of young coconuts (%)	D_0 (0)	D_1 (0,5)	D_2 (1)
A0 (0)	17±8,08	15±8,21	14±6,26
A1 (10)	15,6±9,04	15±7,03	13,2±6,06
A2 (20)	14,5±7,94	12±6,57	16±8,84

Note: A0 : water concentration of young coconuts, A1 : 10% water concentration of young coconuts, A2 : 20% water concentration of young coconuts, D0 : 0 ppm 2,4-D, D1 : 0,5 ppm 2,4-D, D2 : 1 ppm 2,4-D.

Table 4.1 shows that the treatment of young coconut water, 2,4-D and the combination of the two has no noticeable effect at the time of callus initiation. The above results show that the treatment of A2D1 is MS media + 20% young coconut water + 0.5 ppm 2.4-D has the best effect on callus formation so that callus. It can grow faster. According to [12] 2,4-D combined with the addition of coconut water is 20% good for inducing green grape callus (Vitis vinifera L.). According to [13] coconut water can have a better effect on callus growth if in the media also given amenosin such as 2,4-D because coconut water contains urea diphenyl which has cytokinin-like properties. Coconut water is used to promote tissue growth, while 2,4-D for cell differentiation stimulates cell division and enlargement in explan thus triggering callus formation and growth.

In this study the fastest callus growth was on the 12th day. 3) in A2D1 treatment with a young coconut water supply of 20% and 0.5 ppm 2.4 Dichlorophysioxiacetat. This is because the administration of 2,4-D with low concentrations exerts a good influence and response in inducing callus. According [12] giving 2,4-D at a concentration of 0.5 ppm can induce callus with a relatively fast time of -2 weeks. This is supported by the statement [8], the use of regulatory substances that grow by 2.4 D is usually used in small amounts and is able to induce callus in a short time between 2-4 weeks.

In this study, the longest initiation time of callus growth was daily to 12 on A0D0 treatment. This is because in the treatment of A0D0 there is no addition of 2,4-Dichloroenoxiacetat or young coconut water so it experiences the slowest callus growth among all treatments. According to [3] the existence of regulatory substances that grow amuksin such as 2,4-D in tissue culture activities is required, growing regulatory substances is required as a media component for growth and differentiation, without the addition of regulatory substances that grow in the media, growth is severely inhibited and may not even grow at all.According to [14] that there are three stages of callus, namely induction, cell division and cell differentiation. The callus produced in this study

has not reached the differentiation stage because the callus formed only in the area of the former softener during cutting and showed the activity of swollen cells then forming calluses. The initiation time of Pogostemon cablin Benth patchouli leaf callus with treatment of young coconut water given singularly to MS media had no noticeable effect on the formation of pogostemon Benth patchouli leaf calluses.

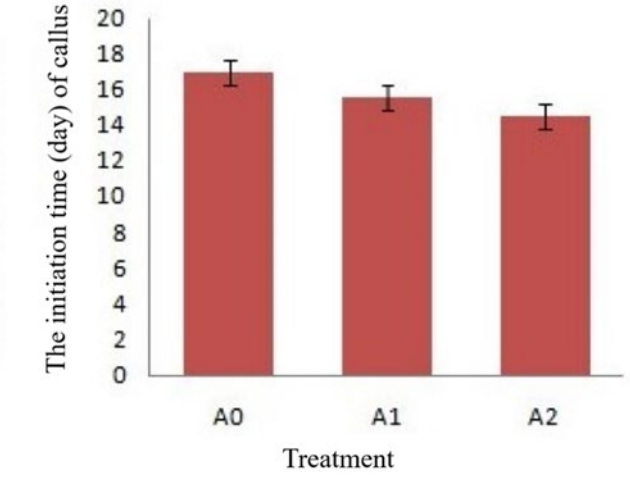

Figure 1 The initiation time (day) of callus from leaves *Pogostemon cablin* Benth treated young coconut water with various concentrations, A0 : 0 %, A1 : 10%, A2 : 20%.

In Figure 1 it can be seen that coconut water given singularly to MS media has no noticeable influence on the initiation of callused cablin benth benth leaves. The initiation time of pogostemon cablin benth patchouli leaf callus with the treatment of 2.4 Dichloroenoxiacetat given singularly to MS media also had no noticeable effect on the formation of pogostemon cablin benth patchouli leaf callus. This can be seen in figure 2..

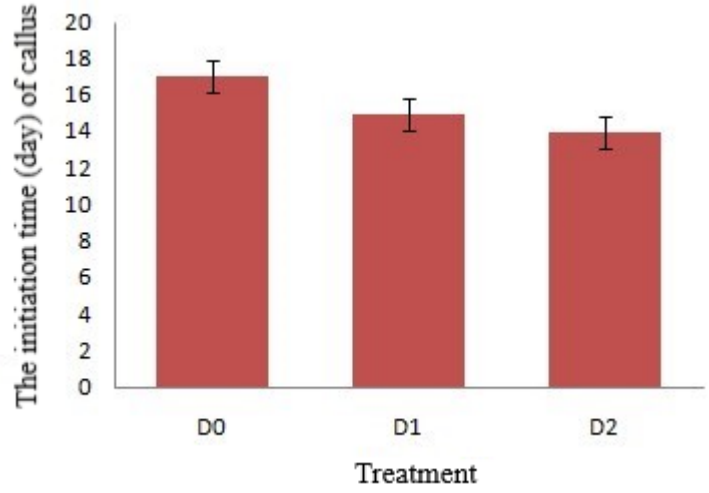

Figure 2 Initiation time (day) *Pogostemon cablin* benth patchouli leaf callus treated 2.4 Dichlorophysioxiacetat with various concentrations, D0:0ppm, D1:0.5 ppm, D2:1ppm.

In Figure 2 it can be seen that 2.4 Dichloroenoxiacetats given singularly to MS media has no noticeable effect on the initiation of pogostemon cablin Benth patchouli leaf kalus. According to [15], the administration of amokinin and cytokinin alone is not able to compensate for or even inhibit endogenous amokiin and cytokinin in explan so it takes a combination of both.

3.2 Percentage of Explants Grow in MS Media by a Combination of 2.4 Dichloroenosiasetat and Young Coconut Water

The growth of calluses is observed daily for 50 days. The success of callus growth is expressed by the percentage of excess in forming calluses on observation 50 days after planting. Observational data the percentage of patchouli callus can be seen in Figure 3.

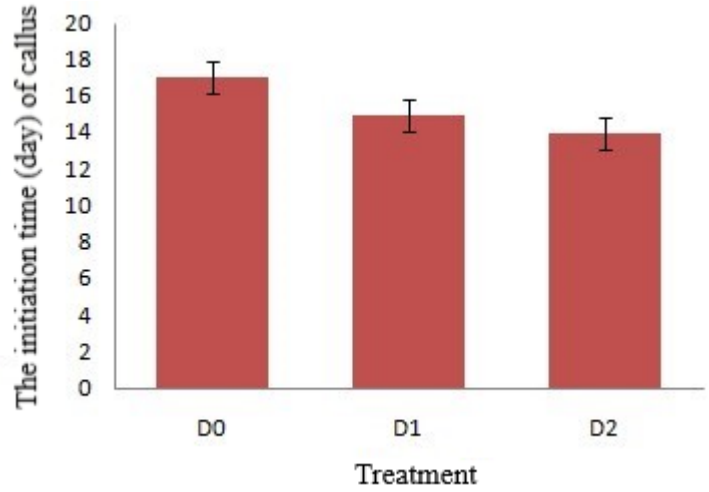

Figure 3 Percentage of explants grown in MS media with combination 2.4 Dichloroofenosiasetat and young coconut water. A0D0 : control, A0D1: MS +0.5 ppm 2.4-D, A0D2: MS +1 ppm 2.4-D, A1D0: MS+10% young coconut water, A1D1: MS+10% young coconut water+0.5 ppm 2.4-A1D2: MS+10% young coconut water+1 ppm 2.4-D, A2D0: MS+20% young coconut water, A2D1: MS+20% young coconut water+0.5 ppm 2.4-D, A2D2: MS +20% young coconut water +1 ppm 2.4-D.

In Figure 3 it can be seen that the percentage of explan that grows the most is 80% in the treatment of A1D1 and A1D2 i.e. explants grow with hormone concentrations of 0.5 ppm 2.4-D and 10% young coconut water and concentration 1 ppm of 2.4 D hormone and 10% young coconut water. This is because the provision of young coconut water containing urea diphenyl which like cytokinin added 10% to the medium is able to induce callus to the maximum. According to [12] the administration of various concentrations of 2,4-D with 10% young coconut water produces a high percentage of growing calluses.

The lowest percentage of explants is 20% in the treatment of A0D2 with a homon concentration of 1 ppm 2.4-D. This is thought to be due to the concentration of auxin-growing regulatory substances used in the study of 2.4-Dichlorophysioacetate given for the treatment of A0D2 is inappropriate in inducing callus, thus inhibiting the growth of callus in explan. Inhibition of callus formation due to endogenous and exogenous hormones contained in explan can not stimulate the growth of callus quickly, in addition also because the condition of explant that has begun to decline or almost wither so that forming calluses takes a relatively slow time.

According to [15] concentrations of regulatory substances that grow differently give different responses to callus induction. Calluses that do not appear are possible because explant has a low ampogenous amolynin and cytokinin content, so it still requires more amofin or cytokinin redogenously.

The rapid onset of callus is influenced by the work of the hormones auxin and endogenous cytokinin and exogenous correlations. As [5] revealed that the addition of auxin and exogenous cytokinin will change the concentration of regulatory substances that grow endogenous cells. The effectiveness of auxin-growing regulatory substances and exogenous cytokinin depends on the concentration of endogenous hormones in plant tissues.

3.3 Cultural Weight Of Kalus Nilam Pogostemon cablin Benth Di MS Media with a Combination of 2.4 Dichloroenosiasetat and Young Coconut Water

Growth is a permanent increase in the size of an organism or part of a plant that is the result of an increase in the number and size of cells. Growth is characterized by irreversible weight gain, so callused weight measurements can represent the growth of callused variables derived from leaves of patchouli explant. In this study the weight of calluses was weighed 50 days after planting. Statistical test results showed the administration of young coconut water, 2.4 Dichlorophysyacetic and the combination of both have a noticeable effect on the weight of callus ($p < 0.05$). The average value of callused weight from statistical test results can be seen in Table 2.

Table 2 Weight Pogostemon Polin Benth Patchouli Leaves Callus Culture On MS Media Combination of 2.4 Dichlorophysoxyacetic and Young Coconut Water

Water concentration of young coconuts (%)	D_0 (0)	D_1 (0,5)	D_2 (1)
A0 (0)	0,07±0,05[a]	15±8,21	14±6,26
A1 (10)	0,05±0,03[a]	15±7,03	13,2±6,06
A2 (20)	0,13±0,09[a]	12±6,57	16±8,84

Note: A0 : water concentration of young coconuts, A1 : 10% water concentration of young coconuts, A2 : 20% water concentration of young coconuts, D0 : 0 ppm 2,4-D, D1 : 0,5 ppm 2,4-D, D2 : 1 ppm 2,4-D.

Statistical test results showed an interaction between young coconut water and 2.4 Dichlorophysioxiacetats has a noticeable effect on disincinated weight. Table 2 the treatment of A1D2 with the administration of 10% young coconut water and 1 ppm 2.4 Dichlorophysoxyacetate is the best combination for gaining a high callused weight of 0.28 grams (Appendix 5), while in the treatment of A2D1 with the administration of 20% young coconut water and 0.5 ppm 2.4 Dichloroenoxiacetats produce the lowest vessel weight of 0.06 grams.

According to [16], regulatory substances grow like 2.4 Dichlorophysyacetics given in the right amount can have an effect on cultural weight. 2.4 Dichlorophysoxyacetate plays a role in triggering cell enlargement so that cultural weight increases. The interaction of growth substances and growing regulatory substances will increase the number and size of cells in plant tissues. Growing a regulatory substance given at the right concentration can initiate cell division and promote cell growth. [13] stated that heavy wet calluses are caused by their high water content. The resulting wet weight is very dependent. at the speed at which the cells divide, multiply and proceed with an enlarged callus.

Callus weight of patchouli leaf callus Pogostemon cablin Benth with young coconut water treatment given singly on MS media gave a significant effect on callus formation of patchouli leaf *Pogostemon cablin* Benth.

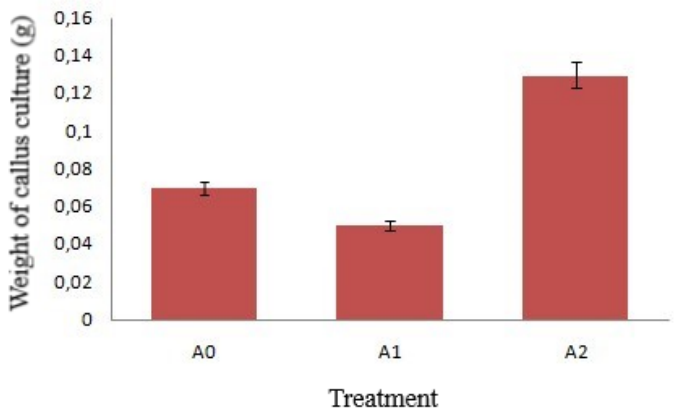

Figure 4 Weight *Pogostemon cablin* benth patchouli leaves of callus culture given treatment of young coconut water with various concentrations, A0 : 0%, A1 :10%, A2: 20%.

In Figure 4 it can be seen that the weight of *Pogostemon cablin* benth patchouli leaf callus culture with treatment of young coconut water given to MS media has a noticeable influence on the weight of *Pogostemon cablin* benth patchouli leaf callus culture. The provision of young coconut water with a concentration of 10% is very different from treatment without young coconut water and is not noticeable in contrast to the provision of young coconut water with a concentration of 20%, but the concentration of 20% of young coconut water can increase the weight value of callus culture, while the weight of pogostemon cablin benth patchouli leaves the callus culture with treatment.2,4 Dichlorophysenoxiacetats given singularly to MS media has no noticeable effect on the formation of cablin benth leaf calluses. The results of pogostemon cablin benth patchouli leaf callus culture severe with treatment 2.4 Dichlorophysoxyacetat given itself to MS media can be seen in Figure 5.

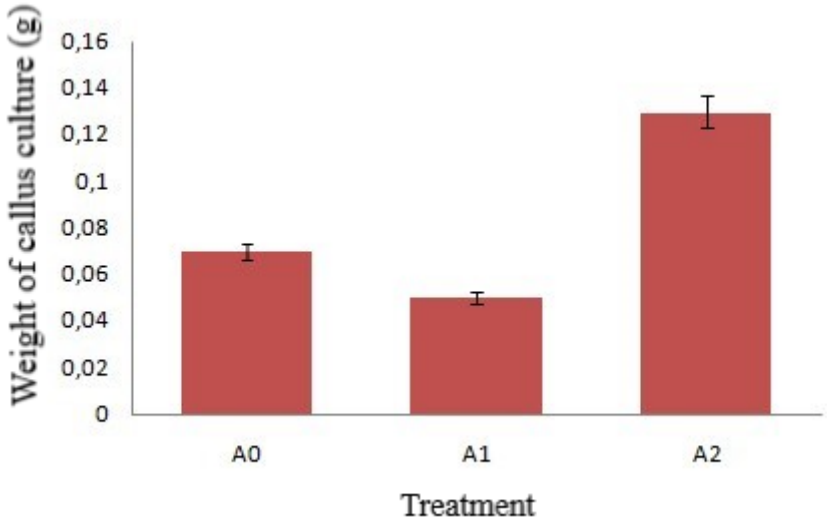

Figure 5 Weight *Pogostemon cablin* benth patchouli leaf callus culture treated 2.4 Dichlorophyxiacetat with various concentrations, A0: 0%, A1 : 10%, A2 : 20%.

In Figure 5 it can be seen the cultural weight of patchouli benth pogostemon cablin leaf callus with the treatment of 2.4 Dichlorophyxiacetat given singularly on MS media has no noticeable effect on the formation of pogostemon leaves cablin benth patchouli. According to [12] a balanced comparison of amthic and cytokinin to explan can result in callusan growth. The regulating substance that grows 2.4 Dichlorophysoxyacetate combined with cytokinin will work synergistically, causing increased and cell division against explant. Administration of amocein and cytokinin is able to stimulate the synthesis of proteins in plant tissues that can lead to increased permeability of the cell wall, thus stimulating cell division and extensions that will affect cell accretion and enlargement.

3.4. Patchouli Leaf Pogostemon Color cablin Benth On MS Media Combination of 2.4 Dichlorophysoxyacetic and Young Coconut Water

Callus color is one of the indicators in tissue culture techniques because at each explan will produce a different callus color. In this study each treatment with a combination of a concentration of 2.4 D and young coconut water produced a different color callus. Combination response 2,4-D and young coconut water against the color of the callus are shown in Figure 6.

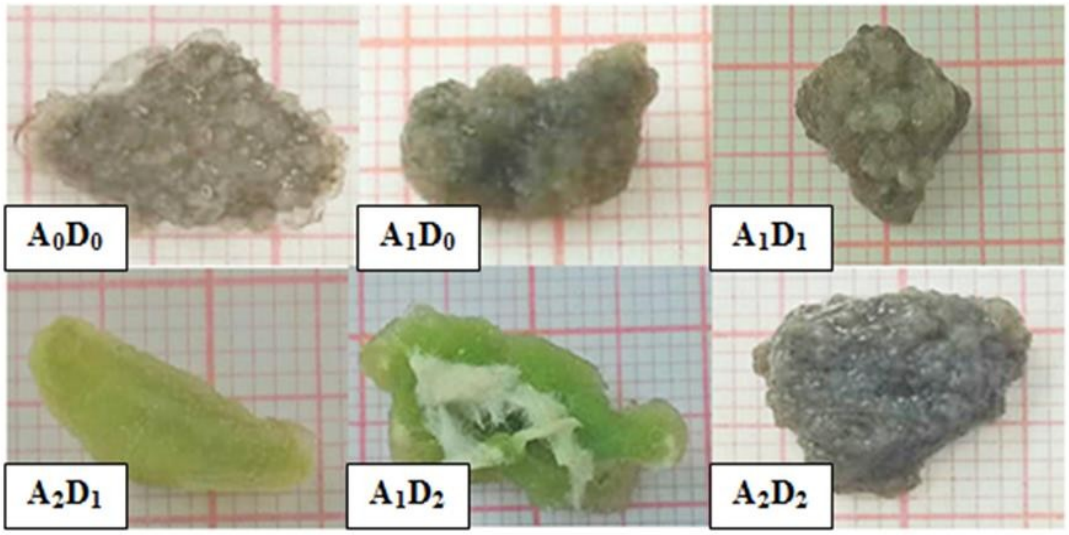

Figure 6 The color of patchouli leaf callus in MS Media with a combination of 2,4-D and Young Coconut Water. A0D0 : control, A1D0: MS +10% young coconut water, A1D1: MS +10% young coconut water + 0.5 ppm 2.4-D, A1D2: MS + 10% young coconut water + 1 ppm 2.4-D, A2D1: MS + 20% young coconut water + 0.5 ppm 2.4-D, A2D2: MS+20% young coconut water+1 ppm 2.4-D.

In figure 6. Each combination of 2,4-D and young coconut water produces a different callus color. In the treatment of A1D2 and A2D1 produce green and brown calluses while in other treatments tend to produce brown calluses. Data on the overall color of calluses produced in each treatment can be seen in Table 3 below.

Table 3 Patchy Color Pogostemon Leaves Cablin Benth At MS MediaCombination of 2.4 Dichlorophysoxyacetic and Young Coconut Water

Treatment	Color of Calus				
	U1	U2	U3	U4	U5
A0D0	0	brown	brown	0	brown
A0D1	0	0	brown	0	brown
A0D2	brown	0	0	0	0
A1D0	brown	brown	0	brown	0
A1D1	Greenish brown	brown	0	brown	brown
A1D2	Greenish brown	Green	0	brown	0
A2D0	brown	0	brown	0	0
A2D1	Green	brown	0	0	0

A2D2	0	brown	brown	brown	0

Description: 0: Explant *Patchouli* leaves do not form calluses. U = Repeat

In this study the resulting callus color was predominantly brown, but in the treatment of A1D2 and A2D1 produced green calluses. In the treatment of A1D2, green calluses are produced at the second repetition while in the treatment of A2D1 green calluses are produced at the first repetition. According to [17] the color of the callus indicates the presence of chlorophyll in the tissues, the greener the color of the callus, the more chlorophyll content.

The color of the brownish callus is found in almost all treatments that form calluses. The brownish color of the callus (browning) is caused by excessive metabolism of phenol compounds, which are often aroused due to the exorcization process [10]. Browning events are actually natural events and processes adaptive changes in parts of plants due to physical influences such as stripping, and cutting but according to [18] browning symptoms are signs of physiological damage from explan. But from the observations, explant pogostemon cablin patchouli leaves have a high brownish resistance, can be seen from the callus that continues to grow even though the explant is brown. This is because callus from the leaves of Nilam Pogostemon cablin Benth contains secondary metabolites in the form of the alcoholic compound Niouli which is the most important content in patchouli oil.

According [19] the brownish color of the callus indicates the synthesis of secondary metabolites. The browning that occurs in callus in addition to phytoalexin accumulation, is also caused by the synthesis of phenolic compounds.

The interaction of growing regulatory substances can affect the color of the callus. According to [13] the higher the concentration of 2,4-D added in the medium affects the decrease in the content of chlorophyll and carotenoids. This decrease in chlorophyll content is thought to occur due to the influence of amoxin on carbohydrate metabolism. The use of 2,4-D in plants can interfere with carbohydrate metabolism. The cystesis of chlorophyll is influenced by carbohydrates that make up the main substances. If the metabolism of the karhidat is disrupted then the synthesis of chlorophyll will be disrupted [20].

3.5 Texture Pogostemon Cablin Benth Nilam Leaf Callus On MS Media Combination of 2.4 Dichlorophysoxyacetic and Young Coconut Water

The texture of callus is one of the indicators in the growth of calluses. A good texture is one that can be interrupted, because the texture of the crumb is easier to separate between one cell and another. In addition to textured crumbs, it can also form a compact texture. The overall texture of the calluses conducted in this study was from a combination of concentrations. 2,4-D and young coconut water that has been observed for 50 days can be seen in Table 4.

Table 4 Textures Pogostemon Cablin Benth Patchouli Leaf Kalus On MS Media Combination of 2.4 Dichlorophysoxyacetic and Young Coconut Water

Treatment	Texture of calus				
	U1	U2	U3	U4	U5
A0D0	0	Compact	Crumb	0	Crumb
A0D1	0	0	compact	0	compact
A0D2	compact	0	0	0	0
A1D0	compact	Compact	0	Crumb	0
A1D1	compact	Compact	0	Compact	compact
A1D2	Crumb	Compact	0	Compact	0
A2D0	compact	0	0	0	0
A2D1	compact	Compact	0	0	0
A2D2	0	Compact	compact	Compact	0

Description: 0: explant *Patchouli* leaves do not form calluses

The results in the chart above show that all treatments formed primarily form compact textured calluses. The formation of compact textured callus according to [10] is driven by the presence of the endogenous hormone auxin produced internally by explans that have been developed to form calluses. Administration of growing regulators can affect the production of secondary metabolites, this is because additional ZPT can cause changes in plant physiology and biochemistry through the regulation of enzyme work. ZPT plays a role in the binding of protein membranes that have the potential for enzyme activity. This binding yield activates the enzyme and converts the substrate into several new products. This newly formed product causes secondary reactions, one of which is the formation of secondary metabolites [21].

According to [3] compact calluses can be caused by several things, including because cells that were initially divided experienced a decrease in proliferation activity. This activity is affected by the natural amxin found in the original explant. The presence of cytokinin in low concentrations can also affect the formation of compact calluses.

3.6 Thin Layer Chromatography Results Patchouli Callus Extract *Pogostemon cabli* Benth

Thin layer chromatography results of essential oil detection in callus extract of patchouli leaf *Pogostemon cablin* Benth can be observed on the purple spots or stains produced on the plate. The purple spots or stains produced are substances that have been separated by the stationary phase and the mobile phase which have been observed under UV light and then sprayed with vanillin-sulfuric acid to produce a purple color

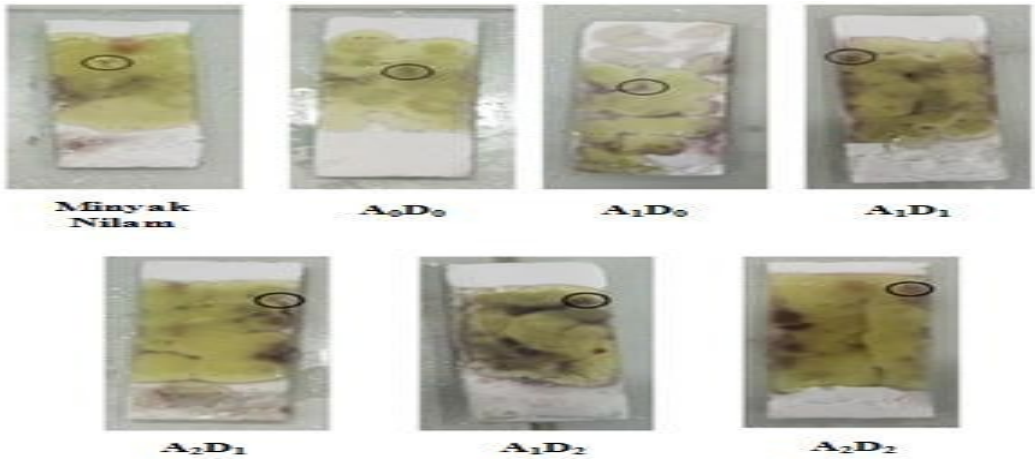

Figure 7 Results of TLC (Thin Layer Chromatography) callus extract of *Pogostemon cablin* Benth patchouli. Circles indicate purple spots that are visible from UV light after being sprayed with vanillin sulfate.

Analysis of patchouli oil with thin layer chromatography results in the separation of components with certain colored stains. The separation that occurs from these components is very closely related to the solvent system used, with the ethyl acetate - hexan solvent system. According to Hernani (1988), if the selected phase of motion had a much different polarity to the substance to be separated, then there would be no good separation. Perhaps the substance still remains at the beginning of the muscle or reaches the limits of development. Thus the success of the separation process in thin layer chromatography is largely determined by the harmony between the stationary phase and the motion phase used.

According to [11] the presence of several stain points seen on each KLT plate can be concluded that in patchouli extract there is a group of compounds that have different levels of polarity. Based on the "like-like" theory, with stationary phases being polar and phases of motion tending to be non-polar, the uppermost stains are a group of non-polar compounds while the lowest stains are a group of polar compounds [22]. The positive response contains essential oils in the form of purple patches with Rf 0.75-0.95 [11] The rf value of essential oils and callus extracts can be seen in Table 5.

Table 5 Rf KLT Data Values yield *Pogostemon cablin* Benth patchouli callus extract

No.	Treatment	Value Rf
1.	A0D0	0,82
2.	A1D0	0,85
3.	A1D1	0,87
4.	A2D2	0,92
5.	A1D2	0,92
6.	A2D2	0,97
7.	Patchouli Oil	0,87

In Table 5 it can be seen that from the results of KLT (Thin Layer Chromatography) the results of patchouli oil chromatogram and callus extract produce different colors and different Rf values, except in the treatment of A1D1 produce the same Rf value as patchouli oil which is 0.87. According to [23] if the RF value of an experiment has the same value as the standard RF value of a compound it can be said to have the same or similar characteristics, whereas when rf values differ the compound can be said to be a different compound. In addition, the results are expressed specifically with standard raw materials when color patches between the sample and the standard have an Rf price close to each other with a value difference of about 0.2. So in this study it can be said that all treatments have characteristics that are almost similar to standard RF because all treatments are close to a difference in value of less than 0.2. According to [24] there are several factors that affect the movement of stains on the chromatography of thin layers that affect the value of Rf, namely the chemical structure of separate compounds. The absorbent nature and activity level, thickness and flatness of the absorbent layer, the level of steam saturation in developers, and the number of records used.

3.7 Phytochemical Test Results of Nilam Extract *Pogostemon cablin* Benth Kalus

In this study phytochemical tests included alkaloid, terpenoid and flavonoid tests. Each treatment contains different metabolite compounds. The results of the phytochemical Pogostemon cablin Benth patchouli extract can be seen in Figure 8.

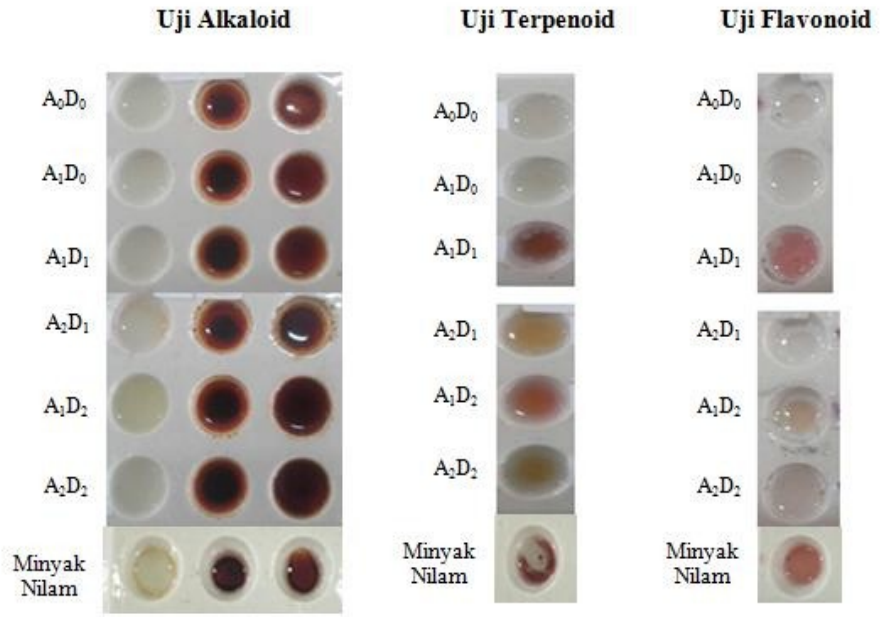

Figure 8 Phytochemical test results patchouli callus extract *Pogostemon cablin* Benth

Phytochemical test results showed that there were white deposits with Mayer reagents, red jinga deposits with Dragendorf reagents and brown deposits with Wagner reagents that showed that in patchouli extract each treatment contained alkaloid compounds.In terpenoid tests on the treatment

of A1D1, A2D1, A1D2 and A2D2 after added there is a discoloration that produces an orange color that indicates the presence of positive terpenoids. In the treatment of A0D0 and A1D0 after the Liebermand-Buchard reagent was added there was no discoloration, this indicated that in the treatment of A0D0 and A1D0 there were no terpenoid compounds.

In flavonoid tests on the treatment of A1D1 and A1D2 produced a red color after added concentrated HCL and powdered 0.2 mg. This indicates the presence of flavonoid compounds in the treatment of A1D1 and A1D2.Of all the concentrations of 2.4 Dichlorophysioxiacetats and young coconut water used in the media did not have a maximum effect on the content of flavonoid compounds. According to [13] it is estimated that the 2,4-D added in the medium is fully used to increase the formation and growth of callus size, resulting in reduced or no formation of flavonoids. In the synthesis of flavonoids, saltin serves to increase the work of the enzyme phenylalanine ammonia lyase (PAL) which produces sinamat from phenylalanine. The next pathway is the formation of flavonoids from malonyl Co-A, if amlea is reduced then the formation of flavonoids is also reduced.

[25] showed that in order to produce phenol compounds in vitro in addition to the addition of growing regulatory substances, it is also necessary to add other elements such as casein hydrolysis, to aid callus growth and chemical compound production. In liquidambar styraciflua culture callus the addition of these elements is effective for increasing the content of phenols.

Flavonoids are a class of secondary metabolites synthesized from pyruvic acid through amino acid metabolism [26]. Flavonoids are phenol compounds, so their color changes when added to bases or ammonia. There are about 10 types of flavonoids: anthocyanins, proantosianidins, flavns, flavns, glycoflavons, biflavonils, khalkon, auron, and isoflavones [27]

4. Conclusion

Based on the results of this study it can be concluded that:

a. The optimal combination of the hormone 2,4-D and young coconut water to induce pogostemon cablin Benth patchouli callus is in the treatment of ms + 1 ppm media. 2.4-D +10% young coconut water with a callus time of 13 HST (days after planting), an average callus percentage of 80%, green callus color, compact (inhospitable) dominant callus texture, and the highest callus weight of 0.28 grams.

b. KlT (Thin Layer Chromatography) results show that ms+0.5 ppm 2.4-D+10% of young coconut water produces the same Rf value as Rf standrad(patchouli oil) of 0.87. The result of the same Rf value can be said that the compound has similar or similar characteristics, and in the treatment of A1D1 contains alkaloids, terpenoids, and flavonoids, the same as the content of patchouli oil which also contains alkaloid compounds, flavonoids and terpenoids.

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