

Effect of IAA and GA₃ toward the growing and saponin content of purwaceng (*Pimpinella alpina*)

DASIYEM FATHONAH^{1,♥}, SUGIYARTO²

¹SMA Negeri 1 Sapuran, Jl. Purworejo Km. 20 Sapuran, Wonosobo 56373, Jawa Tengah, Indonesia. Tel/Fax.: +92-286-611173, ♥email: sma1sapuran@yahoo.co.id

² Bioscience Program, School of Graduates, Sebelas Maret University, Surakarta 57126, Central Java, Indonesia

Manuscript received: 23 December 2008. Revision accepted: 4 February 2009.

Abstract. Fathonah D, Sugiyarto. 2009. Effect of IAA and GA₃ toward the growing and saponin content of purwaceng (*Pimpinella alpina*). *Nusantara Bioscience 1*: 17-22. The aims of this research are to examine (i) the effect of IAA and GA₃ in different concentrations to the growth of the plants and (ii) the saponin contained inside the *P. alpina*, leaves. The research was done in Sikunang Village, Kejajar Subdistrict, Wonosobo District, Central Java from July to November 2007. The experiment methods were used the *Completely Random Design* with two factors were used to analyze this experiment. First treatment gives IAA and GA₃, second was done by giving different IAA and GA₃ concentration. These experiments were repeated three times. Variables measured in this research were the growth of plant which is consisted of the number of leaves, their height, width, wet weight as well as dry weight. The chemical compound of the secondary metabolite in the form of leave saponin was employed. The result was analyzed by Analysis of Variance (ANOVA), then continued to Duncan Multiple Range Test in 5% level to analyze the real difference between those treatments. The result showed that giving IAA and GA₃ differently affect the growth *P. alpina*. In variable of the height, the optimal wet weight and dry weight of the plant in GA₃ treatment was 50 ppm; optimum number of leaves in GA₃ treatment was 50 ppm where as the leave width in IAA treatment was 200 ppm and GA₃ treatment was 75 ppm and optimum saponin treatment was IAA 200 ppm and GA₃ 25 ppm.

Key words: *Pimpinella alpina*, IAA, GA, growth, saponin, Dieng.

Abstrak. Fathonah D, Sugiyarto. 2009. Pengaruh IAA dan GA₃ terhadap pertumbuhan dan kandungan saponin purwaceng (*Pimpinella alpina*). *Nusantara Bioscience 1*: 17-22. Tujuan penelitian ini adalah untuk mengkaji (i) pengaruh IAA dan GA₃ dengan konsentrasi yang berbeda untuk pertumbuhan tanaman dan (ii) saponin yang terkandung di dalam daun *Pimpinella alpina*. Penelitian dilakukan di Desa Sikunang, Kecamatan Kejajar, Kabupaten Wonosobo, Jawa Tengah pada Juli-November 2007. Metode percobaan menggunakan Rancangan Acak Lengkap dengan dua faktor digunakan untuk menganalisis percobaan ini. Pertama memberikan perlakuan IAA dan GA₃, kedua memberikan perlakuan IAA dan GA₃ dengan konsentrasi berbeda. Percobaan diulang tiga kali. Variabel yang diukur adalah pertumbuhan tanaman yang terdiri dari jumlah daun, tinggi tanaman, lebar daun, berat basah maupun berat kering; serta senyawa kimia metabolit sekunder dalam bentuk saponin. Hasil penelitian ditelaah dengan Analisis Varian (ANOVA), kemudian dilanjutkan ke Uji Jarak Berganda Duncan pada tingkat 5% untuk mengetahui perbedaan nyata antara perlakuan. Hasil penelitian menunjukkan bahwa pemberian IAA dan GA₃ yang berbeda mempengaruhi pertumbuhan *P. alpina*. Pada variabel tinggi, berat basah dan berat kering tanaman perlakuan GA₃ yang optimal adalah 50 ppm, jumlah daun optimal dalam perlakuan GA₃ adalah 50 ppm dimana lebar daun optimal pada perlakuan IAA adalah 200 ppm dan pada perlakuan GA₃ adalah 75 ppm, sedangkan kadar saponin optimal adalah perlakuan IAA 200 ppm dan GA₃ 25 ppm.

Kata kunci: *Pimpinella alpina*, IAA, GA, pertumbuhan, saponin, Dieng.

INTRODUCTION

Lately, traditional medicinal plants become popular and are wanted by modern society (the city) because it is believed that the effects of traditional medicines are relatively small when compared to modern medicines. But one of the weaknesses of traditional medicine is not much information about their chemical constituents and compounds which is responsible for biological activity. Traditional medicine is the medicine where the ingredients are derived from nature either from plant, animal or mineral materials (MoH 1981). Purwaceng or purwoceng (*Pimpinella alpina* Molk.; previously named *Pimpinella pruatjan* Molk.) is one of the plants which has a property as

a traditional medicine that its natural existence is already scarce in Dieng Plateau, its natural habitat, along with the loss of protected forest in the region as a result of the uncontrolled forest encroachment activities by the local community. In Wonosobo District, purwaceng is naturally found only in the Villages of Sikunang, Siterus and Dieng, District of Kejajar. Even according Rahardjo (2003) and Shaheed et al. (2004) these plants exist only on a very narrow area of cultivation in Sikunang Village, no longer found in their natural habitat. Basically, this plant can grow in any area in the Dieng Plateau and planted anytime during the dry season even though it does not rain for a long time because it needs no watering as much as in potato cultivation. The morphology of *P. alpina* illustrated

in Figure 1.



Figure 1. Morphology of *P. alpina*

Purwoceng as medicinal plants contains active compounds which give the effect of warmth on the body and increase the emotion. This crude drug has been known as a sexual desire arousing (aphrodisiac) and urine laxative drug (diuretic) (Astuti 2005). Purwaceng contains major phytochemical groups of alkaloids, polyphenols, flavonoids and saponins. Bergapten, isobergapten, and sphondin belong to the group of furanokumarin (Sidik et al. 1975), also coumarins, saponins, sterols, alkaloids, and several kinds of sugar compounds (oligosaccharide) (Caropeboka and Lopez 1975), stigmasterol (Suzery et al. (2004), bergapten, marmesin, 4-hydroxy coumarin, umbeliferon, and psoralen (Hernani and Rostiana (2004).

Saponins have many roles, on healthy plants it functions as an anti-fungal (Zehavi et al. 1993; Bowyer et al. 1995) and anti-virus (Wu et al. 2007). Saponins also have significant anti-microbial effects (Papadopoulou et al. 1999). These molecules also act to overcome a heart attack. Some saponins are also known as active cure against virus attacks (Zao et al. 2008). Commercially, saponins are used to inhibit tumor cell growth and to lower blood cholesterol (Ridker 2005). Low cholesterol on blood serum of East African people, who consume food products of animal which have many fat and cholesterol, because it is counterbalanced by eating the herb-rich with saponins (Oleszek and Marston 2000; Davidson 2004).

By looking at the potential of saponin which is so much in helping the body's physiologic functions, it would require further research on the chemical content of saponins in Purwaceng plants. Because purwaceng are plants that have low flexibility in terms of adaptation, it is necessary to the cultivation of purwaceng crops by manipulating the environment in order to obtain optimal results. Optimal environmental factors are expected to increase purwaceng growth and chemical content of secondary metabolites, so that purwaceng production is expected to increase economic value for people who cultivate it.

According to Salisbury and Ross (1995), high light intensity increases karotinoid content and nitrogen content, resulting in leaf surface becomes more open, but on the other hand, a very high light intensity can reduce leaf chlorophyll content. Some of the knowledge required in the cultivation technique is related to factors of light, knowledge of plants, spacing and the use of crops cover.

In addition for its fast-growing, Purwaceng is managed to contain higher secondary metabolites, one with the application of growth regulators. Growth regulator (PGR), known as plant hormones (phytohormones) is the "regulator" produced by the plant itself and at low levels, it regulates plant biochemical, physiological and morphological processes. Therefore, the effort to improve crop yields of purwaceng is necessary with the use of PGR, so it is expected that it will be more optimal for growing Purwaceng as well as increasing the content of chemical compounds. This study examines how the effect of IAA and GA₃ on the growing and the saponin content of plant leaves of Purwaceng and how to influence the interaction of IAA and GA₃ on growing and the saponin content of plant leaves of Purwaceng.

MATERIALS AND METHODS

Plant material

Materials used in this study are obtained from Purwaceng plants from Dieng Plateau on the border of Wonosobo and Banjarnegara Districts, Central Java Province.

Procedures

Experiments are conducted using Completely Randomized Design (CRD), the first factor in the form of IAA and GA₃ and the second factor is the concentration of IAA and GA₃ which are performed differently for three repetitions including the number of leaves, plant's height, leaf's area, fresh weight, dry weight, while the secondary metabolites of saponin content of leaves and analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at 5% test level to find out the real differences among the treatments.

Table 1. The experimental design

Concentration of IAA (A)	Concentration of GA ₃ (B)			
	0 ppm (B ₀)	25 ppm (B ₁)	50 ppm (B ₂)	75 ppm (B ₃)
0 ppm (A ₀)	A ₀ B ₀	A ₀ B ₁	A ₀ B ₂	A ₀ B ₃
100 ppm (A ₁)	A ₁ B ₀	A ₁ B ₁	A ₁ B ₂	A ₁ B ₃
200 ppm (A ₂)	A ₂ B ₀	A ₂ B ₁	A ₂ B ₂	A ₂ B ₃
300 ppm (A ₃)	A ₃ B ₀	A ₃ B ₁	A ₃ B ₂	A ₃ B ₃

Seeding. Seeding is started from the manufacture of growing media and site preparation on seedling plots of land measuring 100 cm x 400 cm plus compost with a ratio of 3: 1 under protective cover/shelter. Seeding process is started by selecting a good seed that is not broken and at the same size. Sowing seeds is in the morning and in the

early of sowing seeding, the watering is done once in 2 days until the plants are 6 weeks old and ready to be moved into polybags.

Planting. Planting is done when seeds are 6 week old on the planting medium i.e poly bag measuring 9 cm x 15 cm and filled with soil and compost with a ratio of 3: 1. At this stage watering is done every 3 days.

Treatment. Treatment begins after the age of 4 weeks of planting. Treatment includes: spraying IAA and GA₃ in combination with appropriate design of experiments on plants with 10 weeks of age for 8 weeks with 8 times spraying for 1 week in the morning at 09.00 am. Treatments include: controlling plants, spraying of IAA concentration of 0 ppm, 100 ppm 200 ppm and 300 ppm at different polybags, spraying of GA₃ concentration of 25 ppm, 50 ppm, 75 ppm, also at different polybag. Each spraying is done as much as 5 ml with 5 times spraying with the same pressure.

Observations. The variables measured in the observations included leaf number obtained by counting all the existing leaves on the plant, plant's height measured from the base of the stem (leaf midrib) to the tallest part of plants, the leaf was calculated with the method grafimetri (Sitompul and Guritno 1995) which is formulated as follows:

$$LD = W_r \times \frac{LK}{W_t}$$

LD = leaf's area

W_r = weight of paper leaf replica

W_t = total weight of paper

L_k = total area of paper

The next variable is the wet weight of plants that are weighed with an analytical balance, dry weight of plants which is calculated by, first, the plants are harvested and immediately measured and placed in a paper bag and then roasted at a temperature of 60°C for 5 days to achieve a constant dry weight, then weighed by analytical scales. Then the leaf saponin content analysis is conducted after harvest (plants are 2 months old) with UV-spectrophotometric method with following steps, first, the extraction phase, the dry leaves are crushed with a mortar till they become powder, then 0.1 grams of powder of them are extracted with 10 milliliters of ethanol 70% above the water steam bath with a temperature of 80° C for 15 minutes, then, the phase of making a standard curve that is made by Merck Saponin standard solution with concentration 2.5, 5.0, 7.5, 10 ppm and then the absorbance is measured using UV-Vis spectrophotometer at a wavelength of 365 nm in order to obtain a standard curve of saponin (Stahl 1985). The phase of counting saponin content of the leaves, extracted leaves are counted their saponin levels using UV-Vis spectrophotometer based on the standard curve of saponin Merck. The levels obtained then is converted into mg/g dry weight of leaves (Suskendriyati et al. 2004) with the following formula:

$$S = \frac{\text{Saponin content of sample} \times \text{volume of dilution}}{\text{Sample weigh of leaves}}$$

S = concentration of saponin

RESULTS AND DISCUSSION

Plant growth

According to Abidin, plant growth regulator or hormone is believed to regulate plant physiological processes (1994), because hormones can affect protein synthesis and regulation of enzyme activity. An increase in protein synthesis as raw material constituent enzymes in plant metabolic processes would enhance growth. This process can enhance the future growth can increase the biosynthesis of secondary metabolites (Taiz and Zeiger 2006).

Plant growth is influenced by several factors, including external and internal factors. Internal factors that influence the growth including auxin (IAA) and gibberellin (GA₃). Some effects of hormones on plant cell growth were as follows: stimulating effect on the growth hormone was highly inhibited by the actinomyosin D antibiotic. This substance use its influence on the cell with a very precise manner i.e. by binding DNA nucleus and prevent the two DNA bands to split up so that DNA cannot be used as a mold for manufacturing of, whether additional DNA molecules or RNA molecules. Without additional new RNA, protein synthesis by the cells freezes quickly (Kimball 1991). This understanding can be used as a base on exogenous hormone use with a certain concentration to stimulate or inhibit growth. Gene activity begins with the transcription of DNA into mRNA. mRNA comes out from the nucleus to the cytosol and is translated at the ribosomes, resulting a protein synthesis. Protein synthesis forms new enzymes and activates certain enzymes that affect metabolism. A series of metabolic processes will affect plant growth (Salisbury and Ross 1995).

Growing variable in the study includes number of leaves, leaf's area, plant's height, wet weight, dry weight and saponin content. The Treatment with various concentrations of IAA in this study is given to 3-month-old purwaceng plants until they reach young harvest time, namely at age of 5 months. The results are shown in Table 2.

Number of leaves

Based on Table 2, it is known that the treatment will give real effect at a concentration of IAA 0 ppm GA₃ 50 ppm. For the overall provision of IAA, GA₃, or a combination of IAA and GA₃ will increase the number of leaves, except at a concentration of IAA 300 ppm GA₃ 0 ppm. It is possible because the provision of growth hormone IAA 300 ppm would hamper growth. The experiment of Noggle and Fritz (1983) show that exogenous IAA plays a role in inhibiting the mother leaves bone, and then the inhibition of mother leaves bone formation will also inhibit the formation of the leaf itself.

Leaf's area

Leaf is one of the growth parameters that can be observed due to environmental changes. A change in the leaf is very sensitive to environmental changes. Leaf growth is closely associated with water availability in the environment. The development of the leaves is very

Table 2. The plant growth of *P. alpina* with the IAA and GA₃ treatment

Concentration of IAA (ppm)	Concentration of GA ₃ (ppm)			
	0	25	50	75
Number of leaves				
0	7.67 ^a	9,00 ^{cd}	10,33 ^e	8,67 ^{cd}
100	9.67 ^{de}	9,00 ^{cd}	8,67 ^{cd}	8,00 ^{bc}
200	8.67 ^{cd}	9,00 ^{cd}	9,67 ^{de}	9,67 ^{de}
300	7.00 ^{ab}	8,33 ^{bc}	8,33 ^{bc}	8,00 ^{bc}
Plant leaf's area				
0	24.20 ^{bc}	21,60 ^{bc}	27,60 ^f	26,40 ^{eg}
100	24.80 ^{bf}	25,20 ^{cf}	27,60 ^f	26,60 ^{ef}
200	25.40 ^{df}	22,40 ^{bd}	24,60 ^{bf}	31,40 ^g
300	19.00 ^a	22,00 ^{bc}	26,40 ^{ef}	22,40 ^b d
Plant's height				
0	12.00 ^{ab}	14,33 ^{cd}	20,50 ^e	15,00 ^{de}
100	15.17 ^{dc}	12,17 ^{de}	15,83 ^d	11,83 ^{ab}
200	16.67 ^c	15,33 ^d	13,83 ^{ce}	16,00 ^{de}
300	10.83 ^a	11,83 ^a	11,67 ^{ab}	12,67 ^{ac}
Plant's fresh weight				
0	2.57 ^a	4,12 ^{gh}	5,82 ⁱ	3,88 ^{fg}
100	2.92 ^{ac}	3,60 ^{ef}	4,27 ^h	2,93 ^{ac}
200	2.77 ^{ab}	2,77 ^{ab}	3,35 ^{de}	3,91 ^{fg}
300	2.63 ^a	3,25 ^{ee}	3,26 ^{ce}	3,10 ^{bd}
Plant's dry weight				
0	0.38 ^{ab}	0,53 ^{eg}	0,74 ^j	0,51 ^e
100	0.49 ^{dc}	0,55 th	0,57 ^{gi}	0,52 ^{eg}
200	0.41 ^{bc}	0,62 ⁱ	0,42 ^{bc}	0,59 ^{hi}
300	0.35 ^a	0,40 ^{ac}	0,43 ^{bc}	0,45 ^{cd}
Saponin content of plant				
0	9.59 ^e	8,92 ^{bc}	8,92 ^{bc}	8,78 ^b
100	9.28 ^d	8,54 ^a	11,16 ^j	10,68 ^h
200	9.05 ^c	12,00 ^l	10,83 ⁱ	10,54 ^g
300	8.93 ^b	11,53 ^k	10,85 ⁱ	10,38 ^f

sensitive to environmental changes and also to growth hormone either exogenous or endogenous. The following table shows the results of studies of leaf's area of *P. alpina* plant on different IAA and GA₃ treatment. By providing a combination of IAA and GA₃, it is expected to give positive effect on changes in leaf's area. Table 2 notes that the treatment combination of IAA and GA₃ has a significant influence on leaf's area. The highest leaf's area is at the treatment of IAA 200 ppm GA₃ 75 ppm, so the capacity indicates that it is the best response of the optimal growth of leaf's area compared to other treatments. At the treatment of IAA 300 ppm GA₃ 25 ppm, the leaves area are, in average, at least number, this shows that to this treatment the response of plant for exogenous hormones has the character of inhibiting the growth of leaves area.

Plant's height

Plant's height is very sensitive to water availability in the soil. Plant's height is the parameter most frequently observed to measure the influence of the environment (Sitompul and Guritno 1995). Treatment of plant's cells

with auxin causes an increase not only in the synthesis of RNA but also in protein synthesis. At first, the application of synthetic gibberellin to plant cells leads to the explosion of RNA synthesis which is then followed by a synthesis of various hydrolytic enzymes (Kimball 1999). These activities encourage good growth processes of roots, stems and leaves. The following table is the results of research on plant's height of *P. alpina* on different IAA and GA₃ treatment. From Table 4, it is noted that the combination treatment of IAA and GA₃ has a significant influence on plant's height. The average plant's height is seen in combination IAA 0 ppm GA₃ 50 ppm. This shows that this combination is best treatment for variable height. By giving IAA 300 ppm GA₃ 0 ppm, the lowest plant's height is created, this is possible that in such combinations there are negative feedback so that the plant suffered intrauterine growth retardation.

Plant fresh weight

All synthetic plant hormones or compounds that have physiological and biochemical properties similar to plant hormones are plant growth regulators (plant growth regulator substances). Plant hormones and plant growth regulator in general encourage the growth and development occurs. The effect of the plant growth regulator (PGR) depends on plant species, the PGR site of action on plants, plant growth stage and concentration of PGR. One PGR does not work alone in influencing the growth and development of plants. In general, equilibrium of concentration of some PGR will control the growth and development of plants (Kusumo 1989). It is noted in Table 2 that the treatment combination of IAA and GA₃ have a significant influence on fresh weight of plants. The lowest fresh weight is obtained in the combination treatment of IAA 0 ppm GA₃ 0 ppm. The highest fresh weight occurs in the treatment of IAA 0 ppm GA₃ 50 ppm. This means that the plant growth regulator provided at that concentration affects optimally on the growth of almost all aspects of growing except the leaf's area growth.

Dry weight of plant

Plant's dry weight depends on the speed capability of cells to divide, enlarged and elongated. The speed of cell activity can be influenced by growth of hormones such as auxin and cytokinin endogenous. The addition of some exogenous growth hormone is expected to accelerate the growth process. Auxin affects stem length increment, growth, differentiation and branching roots. While the provision of gibberellins promotes bud development, stem elongation and leaf growth, influencing growth and differentiation are also roots, plant dry weight of *P. alpina*. Table 2 notes that the treatment combination of IAA and GA₃ have a significant influence on plant dry weight. The highest plant dry weight was obtained at treatment combinations of IAA 0 ppm GA₃ 50 ppm, this indicates that the combination is optimal growth. The lowest dry weight contained in the IAA treatment 300 ppm GA₃ 0 ppm. This shows that the combination treatment does not occur in an optimal growth due to metabolic disorders.

Saponin content in leaf

Secondary metabolites in plant cells accumulate in different amounts. Secondary metabolism contributes to survival, one of which was in self defense (Manito 1992). Saponin is one class of terpenoid secondary metabolites which are synthesized through the acid path mevalonate of respiration. From Table 2, it is noted that the combination treatment of IAA and GA₃ has a significant influence on levels of leaf saponins. The highest saponin content present in treatment of IAA 200 ppm GA₃ 25 ppm and saponin content of the lowest in the treatment of IAA 100 ppm GA₃ 25 ppm.

Discussion

The results of this study show giving ZPT on the various treatments had significant effect on growth and saponin content of plant *P. alpina*. The following table calculates the average dry weight and saponin content of each crop in each treatment (Table 3).

The effect of IAA

IAA at a concentration of 100-200 ppm affects various growth parameters including leaf number, plant's height, leaf's area and plant fresh weight. Giving IAA at low concentrations of 100 ppm gives a real difference to the number of leaves formed. The number of leaves is strongly influenced by genetic factors (Goldworthy and Fisher 1992). In this experiment, 300 ppm IAA treatment produces the least number of leaves, although no significant difference with control plants. The higher concentration of IAA is given, the fewer leaves are formed.

Table 3. Average dry weight calculations and saponin content of each crop in each treatment

Treatment (ppm)	Dry weigh	Saponin content (mg/g)	Saponin content of each plant
IAA 0 + GA ₃ 0	0.35	9.59	3.36
IAA 100 + GA ₃ 0	0.49	9.28	4.55**
IAA 200 + GA ₃ 0	0.41	9.05	3.71
IAA 300 + GA ₃ 0	0.38	8.93	3.39
IAA 0 + GA ₃ 25	0.53	8.92	4.23
IAA 0 + GA ₃ 50	0.74	8.85	6.55 **
IAA 0 + GA ₃ 75	0.51	8.79	4.48
IAA 100 + GA ₃ 25	0.55	8.54	4.70
IAA 200 + GA ₃ 25	0.62	12.00	7.44**
IAA 300 + GA ₃ 25	0.40	11.53	4.61
IAA 100 + GA ₃ 50	0.57	11.16	6.36
IAA 200 + GA ₃ 50	0.42	10.82	4.55
IAA 300 + GA ₃ 50	0.43	10.85	4.67
IAA 100 + GA ₃ 75	0.52	10.68	5.55
IAA 200 + GA ₃ 75	0.59	10.54	6.22
IAA 300 + GA ₃ 75	0.45	10.38	4.67

Based on the research it is known that the treatment will give real effect on leaf's area at a concentration of 200 ppm IAA, although not significantly different from a concentration of 100 ppm. In the provision of the 300 ppm IAA, the plant's height is low, although not significantly different from control plants. The provision of IAA which

is not optimal will inhibit the growth of the plant itself (Hopkins 1999). Provision of 200 ppm IAA shows the highest number of leaves, although not significantly different from 100 ppm IAA and control, while the provision of 300 ppm IAA significantly different with the control. Giving the IAA will increase leaf's area formed. IAA triggers the formation of mesophyll tissue so leaf's area which is formed also increases. The provision of the concentration of 100 ppm IAA gives the highest fresh weight, although not significantly different from the concentration of 200 ppm IAA treatment. Giving IAA 300 ppm gives no significant difference in wet weight. IAA plays a role in cell elongation, especially in the vertical direction. Elongation will be followed by cell enlargement and increased wet weight. Increased wet weight is mainly due to higher water uptake by these cells (Noggle and Fritz 1983).

Giving IAA concentration of 100 ppm gives a lower weight control, while the provision of 300 ppm IAA not significantly different from the control. Growth associated with increasing volume and number of cells, the formation of protoplasm, and in subsequent, weight of the dry weight. Drying aims to stop the cellular metabolism of these materials (Sitompul and Guritno 1995). Provision of IAA in plants *P. alpina* has the effect of accelerating growth at a concentration of 100-200 ppm, but not so with the content of saponin. The content of saponin in this study is best in the condition without treatment and then followed at a concentration of 100, 200 and 300 ppm.

The effect of GA₃

The results of this study show that the growth of *P. alpina* is strongly influenced by the provision of GA₃. The provision of GA₃ within 8 weeks had an impact on the process of growth. From the observed parameters, the provision of 50 ppm GA₃ had optimal growing the number of leaves, plant's height, leaf's area, fresh weight, dry weight and this effect is inversely proportional to the saponin content. The higher concentration of GA₃ given, the saponin levels produced decreases. The decrease occurred in the treatment of 75 ppm is possible because the concentration gives a negative feedback effect on the growth of the primary plant.

Taiz and Zeiger (2006) explains that the provision of high GA₃ will cause a decrease transcription of GA₂₀ oxides which is a major target in the regulation of feedback. If transcription of these compounds decreased, there will be biosynthesis hindrance of GA₃ which will cause activity of GA₃ to decrease. The results are consistent with reports of Chairani (1988) that the application of concentration of 50 ppm GA₃ gives good effect in increasing the biomass of leaves of *Mentha piperita* plants. At a concentration of 50 ppm, leaf dry weight is 56% higher than the controls and is different from the results of research Khristyana et al. (2005) that the concentration of 75 ppm GA₃ treatment in *Plantago major* shows significant difference with control.

Results analysis of variance of plant *P. alpina* shows that GA₃ provide significant different levels of saponins at level of 5% in the test. Based on researched data, the

highest levels of saponin was in the control and the levels decreased along with the increasing concentrations of GA₃. GA₃ affects nucleic acid metabolism that play a role in protein synthesis and regulate the activity of enzymes for plant growth. Increased protein synthesis as a raw material constituent enzymes in plant metabolic processes may increase the biosynthesis of secondary metabolites, including saponins at a later time (Martin 1998).

Providing a combination of IAA and GA₃ on various treatments significantly affects the growth and saponin content of plant *P. alpina*. The results of this study show the growth of *P. alpina* is strongly influenced by a combination of IAA and GA₃ treatment. The provision of IAA and GA₃ within 8 weeks affects on the growth process which is shown on its dry weight at the treatment of IAA 200 ppm GA₃ 25 ppm, although no significant difference in the treatment this. In general, the combination of IAA and GA₃ treatment that will increase the growth can be seen from the increasing of wet weight and dry weight. The treatment combination of IAA and GA₃ enables the influence of IAA and GA₃ to be optimal since the IAA is required for maximum effect of GA₃ work. The research shows a combination of IAA 200 ppm GA₃ 25 ppm produced the highest saponin content. It means that the treatment combinations gave a real influence on the growth and on saponin content of *P. alpina* plant on the concentration of IAA 200 ppm GA₃ 25 ppm.

CONCLUSION

The provision of different plant growth regulator (PGR) affects the growth of *P. alpina* and at the variables of plant's height, leaf's number, fresh weight, dry weight which was optimal at GA₃ 50 ppm treatment, the optimal leaf's area growth is at the treatment of IAA 200 GA₃ 75 ppm, while the saponin content is optimal at the treatment of IAA 200 GA₃ 25 ppm. The provision of different plant growth regulator affects the leaf saponin content of *P. alpina*. In a single treatment, saponin content is lower than control plants, whereas the treatment combination of IAA 200 GA₃ 25 ppm increases leaf saponin content of 12 mg/g.

REFERENCES

- Abidin. 1994. The basics knowledge about plant growth regulators. Penerbit Angkasa. Bandung. [Indonesia]
- Astuti Y. 2005. Isolation, identification and toxicity testing of methylene chloride fraction of active compounds from purwoceng plants (*Pimpinella alpina* Molk.) [Thesis S1]. Department of Chemistry, State University of Diponegoro. Semarang. [Indonesia]
- Bowyer P, Clarke BR, Lunness P, Daniels MJ, Osbourn AE. 1995. Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science* 267 (5196): 371-374.
- Caroeboka, AM dan I Lubis. 1975. Preliminary examination of the chemical content of *Pimpinella alpina* (purwoceng) roots. Symposium on Medicinal Plants I. Section of Pharmacology, Faculty of Veterinary, Bogor Agricultural University. Bogor, 8-9 December 1975. [Indonesia]
- Chairani, F. 1998. Effect of giberelic acid phytohormon application to the canopy biomass and partition coefficient of photosynthate peppermint plant. *Pemberitaan Penelitian Tanaman Industri* 14 (1-2): 28-33. [Indonesia]
- Davidson, MW. 2008. Saponin. <http://micro.magnet.fsu.edu/phytochemicals/pages/saponin.html> (18 Mei 2008)
- Ministry of Health [MoH]. 1981. Utilization of medicinal plants. 2nd ed. Ministry of Health, GoI. Jakarta. [Indonesia]
- Goldworthy PR, Fisher NM. 1992. Physiology of tropical crops. Gajah Mada University Press. Yogyakarta. [Indonesia]
- Hernani, Rostiana O. 2004. Chemical analysis of root purwoceng (*Pimpinella pruatjan*). Seminar on Indonesian Biopharmaca and Excibition Conference. Yogyakarta, 14-15 July 2004. [Indonesia]
- Hopkins WG. 1999. Introduction to plant physiology. John Wiley and Sons. New York.
- Khrityana L, Anggarwulan E, Marsusi 2005. Growth, levels of saponins and plant tissue nitrogen of *Plantago major* L. in granting giberelic acid (GA₃). *Biofarmasi* 3 (1): 11-15. [Indonesia]
- Kimball JW. 1991. Biology. Penerbit Erlangga. Jakarta. [Indonesia]
- Kusumo S. 1989. Plant growth regulator. Yasa Guna. Jakarta. [Indonesia]
- Manitto, P. 1992. Biosynthesis of natural products. IKIP Press. Semarang. [Indonesia]
- Martin R. 1998. Protein synthesis: methods and protocols. Humana Press. Totowa, NJ.
- Noggle GR, Fritz GJ. 1983. Introductory plant physiology. Prentice Hall. New Jersey.
- Oleszek W, Marston A. 2000. Saponins in food, feedstuffs and medicinal plants. Springer. Amsterdam.
- Papadopoulou K, Melton R E, Leggeff M, Daniels M J, Osbourn AE. 1999. Compromise disease resistances in saponin-deficient plants. *Proc Nat Acad Sci USA* 96 (22): 12923-12928.
- Ridker PM, Nissen SE, Ehrenstein MR, Smith S Jr. 2005. Blood test could help prevent heart deaths. *New England J Med* 352: 20-39.
- Salisbury FB, Ross CW. 1995. Plant physiology. Vol. 3. Penerbit ITB. Bandung. [Indonesia]
- Sidik, Sasongko, Kurnia E, Ursula. 1975. coumarin derivatives isolated from roots purwoceng (*Pimpinella alpina* Molk.) origin of the Dieng plateau. Symposium on Medicinal Plants I. Section of Pharmacology, Faculty of Veterinary, Bogor Agricultural University. Bogor, 8-9 December 1975.
- Sitompul SM, Guritno B. 1995. Analysis of plant growth. Universitas Gadjah Mada Press. Yogyakarta. [Indonesia]
- Stahl E. 1985. Chromatography and microscopic analysis of drug. Penerbit ITB. Bandung. [Indonesia]
- Suskendriyati H, Solichatun, Setyawan AD. 2004. Growth and saponin production *Talinum paniculatum* Gaertn callus cultures. with a variety of carbon sources. *Biosmart* 6 (1): 19-23. [Indonesia]
- Suzery M, Cahyono B, Ngadiwiyan, Nurhasnawati H. 2004. Stigmasterol compounds from *Pimpinella alpina* Molk. *Suplemen* 39 (1): 39-41. [Indonesia]
- Syahid SF, Rostiana O, Rohmah M. 2004. Effect of NAA and IBA on rooting purwoceng (*Pimpinella alpina* Molk.) *in vitro*. Indonesian Biopharmaca Excibition and Conference. Yogyakarta, 14-15 Juli 2004. [Indonesia]
- Taiz L, Zeiger E. 2006. Plant physiology. 4th ed. Sinauer. Sunderland.
- Wu ZJ, Ouyang MA, Wang CZ, Zhang YK, Shen JG. 2007. Anti-Tobacco Mosaic Virus (TMV) triterpenoid saponins from the leaves of *Ilex oblonga*. *J Agric Food Chem* 55 (5): 1712-1717.
- Zehavi U, Ziv-Fecht O, Levy L, Naim M, Evron R, Polacheck I. 1993. Synthesis and antifungal activity of medicagenic acid saponins on plant pathogens: modification of the saccharide moiety and the 23 α substitution. *Carbohydrate Res* 244 (1): 161-169.
- Zhao Y-L, Cai G-M, Hong X, Shan L-M, Xiao X-H. 2008. Anti-hepatitis B virus activities of triterpenoid saponin compound from *Potentilla anserine* L. *Intl J Phytother Phytopharmacol* 15 (4): 253-258.