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Effects of Gamma Irradiation on Phenotypic Changes in Vanda Hybrid

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Abstract
Vanda Orchid is one of the most popular ornamental plants. Orchids have slow growth. One way to overcome this weakness is by using gamma-ray irradiation. This research aimed to study the morphological characters of various radiation doses resulting from the crossing of the Vanda Orchid. This study was carried out by observing the characters of vegetative growth for each plant on 0 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy doses of gamma irradiation treatments. The observed variables were the increase in plant height, leaf length, root length, number of roots, number of leaves, leaf width, and leaf color. The results showed that the dose of gamma-ray irradiation were able to increase plant height. 10 Gy and 20 Gy, gamma-ray irradiation doses, resulted in discolored yellowing of orchid leaves. The discoloration of orchid leaves to light green occurs at irradiation doses of 30 Gy, 40 Gy, and 50 Gy. The morphological characteristics of *Vanda* sp. hybrid results gamma irradiation are high at dose 10-40 Gy.

Keywords: characteristics; morphological; orchid

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Introduction

Orchid is one of the prevalent ornamental plants. Hadi et al. (1) stated that orchids have great diversity in both the tropics and sub-tropics. Indonesia has a vast potential for orchids. There are around 5000 types of orchids and more than 200 of them can be commercialized. One of the most popular types of orchids in Indonesia is Vanda Orchid. Vanda Orchid has a unique and attractive shape. Hartati and Darsana (2) stated that the morphological characterization of orchids is necessary to preserve germplasm and select a variety of natural orchid germplasm with superior properties.

The process of making new orchid varieties is conventionally carried out by crossing techniques which are carried out with human assistance. Developed countries have done the process of making new varieties with better technology. One technology for making varieties is done with the help of irradiation rays. Irradiation can make plants into mutants, so the expected varieties could be obtained. Irradiation is a faster method that can produce mutant plants that have certain advantages.

Efforts to obtain superior varieties are often made by crossing various existing varieties. This method is considered inefficient because it requires quite longer time. One of the technologies for making varieties is done with the help of irradiation rays. Haris et al. (3) states that, induction of plants with gamma rays can improve the quality and quantity of plants because they can change the nature of plants. Irradiation is an effective source for producing genetic diversity in plants (4). Mahendra et al. (5) states, gamma-ray irradiation has the benefit of combining the advantages of organic and inorganic materials. This study aimed to determine the differences in morphological

characteristics between the crossing Vanda sp. irradiation results.

Materials and Methods

The research was carried out in two places. The radiation was carried out at BATAN Jakarta, then the F1 hybrid from *Vanda celebica* and *Vanda dearei* were acclimatized in the greenhouse at the village of Plosorejo Matesih, Karanganyar, from July 2018 to January 2019.

The study was conducted by observing each individual in the control (0 Gy), 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy treatment plants. The data obtained during the observations were analyzed in the descriptive method by comparing each plant in the various doses of radiation treatment with the control treatment. Observation variables consisted of plant height, plant length, root length, number of roots, number of leaves, leaf width, and leaf color.

Results and Discussion

Plant height

The growth measurements of mutant orchid plants are showed in table 1. Mutant plants did not show any changes in height compared with control plants. Gamma-ray irradiation is thought to cause stunted root growth due to stress. Physiological changes can be an imbalance of the hormone auxin in plants (6). Auxin is a hormone that regulates various developmental processes such as stem extension, apical dominance, and root initiation (7). High-dose irradiation interferes with protein synthesis, hormone balance, leaf gas exchange, water exchange, and enzyme activity. High irradiation doses changed the ratio of auxin and cytokinin to phytohormones, thereby changing cell differentiation patterns (8).

The increase in plant height occurs in 10 and 30 Gy irradiation, with 9.84 cm and 9.25 cm respectively, then decreases were at 20, 40, and 50 Gy. Physiological damage caused by gamma irradiation can include cell death, inhibition of cell division, increased frequency of tissue formation, and changes in reproductive capacity. Increasing the dose of gamma-ray radiation can cause a decrease in plant height due to gamma rays that damage the plant chromosome structure, thus affecting plant growth (9).

Table 1**.** The average of plant height, leaf length, root length, number of roots, number of leaves, leaf width in various doses of gamma irradiation

Dose	Plant height (cm)	Leaf length (cm)	Root length (cm)	Number of roots	Number of leaves	Leaf width (cm)
0 Gy	8.80	35.90	38.05	7.75	12.00	1.98
10 Gy	9.84	33.76	32.60	6.60	13.60	2.14
20 Gy	8.88	31.00	35.18	6.00	12.83	2.11
30 Gy	9.25	34.97	39.22	6.75	14.00	2.03
40 Gy	8.37	31.50	23.63	5.33	14.33	1.97
50 Gy	8.00	31.30	25.64	5.80	13.40	1.86

Leaf length and width

Leaves are important parts of a plant. Leaf length can affect photosynthesis in plants. Gamma-ray irradiation at certain doses causes changes in plant phenotypes. Gamma rays can generate free radicals in cells, which can destroy essential components of plant cells and affect plant morphology, anatomy, biochemistry, and physiology differently depending on the level of irradiation (10). The most prominent and easily detectable parts of the plant after irradiation are the leaves (11). The leaf length of mutant plants is shorter than control plants. Table 1 showed that the average leaf length of the control plant about 35.90 cm. Mutant plants with the highest average leaf length was found in 30 Gy with 34.97 cm, and the shortest average leaf length was found in 20 Gy treatment with 31 cm.

The increasing width of the orchid leaves will affect the photosynthesis process and absorption of essential plant elements. The wider the leaf of the orchid, the easier it is for the plant to absorb water and nutrients. Based on Table 1, the leaf width of the mutant orchids is significantly differed than that of non-irradiated. The widest leaves of mutant orchids were in 10 Gy dose with an average of 2.14 cm. The optimal dose for leaf width was known at a dose of 10 Gy, and the size of the leaf width decreased along with the increase of irradiation dose. Each irradiated individual plant with gamma rays has a different sensitivity to

irradiation dose. Generally, the higher the irradiation dose caused stunted leaf growth.

Root length

The root is the place where plants grow. The increase in root length is expected to make plants stronger. Irradiation on orchids is expected to increase the length of orchid plants. Based on table 1, it is known that control plants had the highest root length compared to all irradiated plants. The root length of mutant plants incread along with the increase of irradiation doses until its peak at the dose of 30 Gy, then decreasing at dose 40 Gy. Gamma-ray irradiation at low doses can increase growth, whereas high doses showed slow growth (12). Gamma-ray irradiation can cause random mutations that can cause physiological damage in the metabolism of cell development so that its growth potential can be faster or slower (13). Mutant plants with the highest root length were found in the dose 30 Gy, and the lowest root length was found in 40 Gy.

Number of roots and leaves

Gamma-ray irradiation inhibits the number of roots. All mutant plants have fewer roots than non-irradiated plants. The same thing was found in Lestari et al. (14) study, that increasing the dose of irradiation caused the number of roots of *Dendrobium sylvanum* and *Phalaenopsis sp*. to decrease. The average number of roots of non-irradiated plants was 7.75. The highest number of roots was in irradiated plants with a dose of 30 Gy, while the lowest was in a dose of 40 Gy. The low number of roots due to the dose of gamma-ray irradiation causes chromosomal changes to affect the growth of the radicles.

Leaves are essential parts of plants in photosynthesis. The increasing number of leaves will affect the absorption of water and nutrients needed for plants. In contrast with the number of roots, mutant plants at all doses had more leaves than control. The number of leaves increased with the increase in irradiation dose, and the peak was at 40 Gy. Based on Table 1, the highest number of leaves were found in the dose 30 Gy and 40 Gy, 14 and 14.33, respectively. Changes in cells in plant organs caused by gamma-ray irradiation can interfere with plant physiological processes (15).

Leaf color

Gamma irradiation can cause cell change in certain organ plants. Leaf color changes can be identified using the Munsell Color Chart. The results of observations of changes in leaf color are shown in Table 2. Non-irradiated plants had light green leaves color. The same result was shown in mutant plants at 20 Gy, 30 Gy, 40 Gy, and 50 Gy. While mutant plants dose, 10 Gy showed yellowing on leaves. The 20-50 Gy dose gamma irradiation in this study did not cause discoloration of the orchid leaves, in contrast to Astutik (15) which showed that the color of the leaves darkened with increasing irradiation doses. Color changes occur in mutant orchid by irradiation of 10 Gy, which showed a yellowish color. Leaf discoloration of orchid plantlets occured due to damage in chlorophyll after irradiation (14).

Table 2. Color change of vanda leaf with various dose treatment of gamma irradiation

Dose	Leaf Color
0 Gy	7.5 Gy 6/6.5
10 Gy	2.5 Gy 6/6.5
20 Gy	7.5 Gy 6/6.5
30 Gy	7.5 Gy 6/6.5
40 Gy	7.5 Gy 6/6.5
50 Gy	7.5 Gy 6/6.5

Conclusion

The increased gamma-ray irradiation dose in *Vanda sp*. hybrid can increase the diversity of plant height, leaf length, root length, and the number of roots. A 10 Gy gamma-ray irradiation dose increased plant height and changed the color of the yellowing orchid leaves. Irradiation doses of 20 Gy, 30 Gy, 40 Gy, and 50 Gy resulted in the color of orchid leaves became light green.

Conflict of Interest

All authors declare no conflicts of interest in this section.

References

1. Hadi H, Razali SNS, Awadh AI. A comprehensive review of the cosmeceutical benefits of vanda species (Orchidaceae). Nat Prod Commun. 2015;10(8):1483–8.
2. Hartati S, Darsana L. Morphological characterization of natural orchids in the context of germplasm conservation. J Agron Indones. 2015;43(2):133.
3. Haris A, Abdullah, Bakhtiar, Subaedah, Aminah, Jusoff K. Gamma ray radiation mutant rice on local aged dwarf. Middle

East J Sci Res.2013;15(8):1160–4.
4. El-Degwy IS. Mutation Induced Genetic Variability in Rice (*Oryza sativa* L.). Int J Agric Crop Sci. 2013;5(23):2789–94.
5. Mahendra K, Nayak KK, Fernandes BJ, Udayashankar NK. Gamma irradiation effect on structural, optical and electrical properties of organometallic potassium hydrogen oxalate oxalic acid dihydrate single crystal. J Mater Sci Mater Electron. 2018;29(22):18905–12.
6. Rosmala A, Khumaida N, Sukma D. Morphological changes and growth of The Handeuleum (*Graptophyllum pictum* L. Griff) due to gamma ray irradiation. J Agron Indones. 2016;43(3):241.
7. Pimonrat P, Suraninpong P, Wuthisuthimethavee S. Acute effect of gamma radiation on stable characteristics of *Spathoglottis plicata* Blume. Acta Hortic. 2012;953:173–80.
8. Setiawan RB, Khumaida N, Dinarti D. Induction of wheat embryogenic callus mutation (*Triticum aestivum* L.) through gamma ray irradiation for high temperature tolerance. J Agron Indones. 2015;43(1):36.
9. Dewi SDN., Agustina PLP., Nuriadi I. Effect of gamma ray radiation on green bean plants (*Vigna radiata* L.) on saline conditions. Agroekoteknologi. 2013;1(2):94538.
10. Dehgahi R, Joniyas A. Review of research on dendrobium sonia-28, a hybrid from orchidacea family and mutation as somaclonal variation. Int J Biosci. 2017;10(06):29–47.
11. Srivastava D, Gayatri MC, Sarangi SK. In vitro mutagenesis and characterization of mutants through morphological and genetic analysis in orchid *Aerides crispa* Lindl. Indian J Exp Biol. 2018;56:385–94.
12. Kapare V, Satdive R, Fulzele DP, Malpathak N. Impact of gamma irradiation induced variation in cell growth and phytoecdysteroid production in *Sesuvium portulacastrum.* J Plant Growth Regul. 2017;36(4):919–30.
13. Rosyidah AY, Iis AS, Darusman LK. Induction of physical mutations by gamma ray irradiation in turmeric (*Curcuma domestica* Val.). J Hortik Indones. 2015;5(2):84.
14. Lestari EP, Yunus A, Sugiyarto. The morphology effect of *Dendrobium sylvanum* and *Phalaenopsis* sp. with gamma ray irradiation. In: Proceedings of the National Seminar of the Faculty of Agriculture, UNS. Surakarta: Faculty of Agriculture Universitas Sebelas Maret; 2018. p. 143–52.
15. Astutik. Diversity of phalaenopsis clons from gamma radiation: changes in phenotypes of vegetative growth phase. Buana Sains. 2012;12(1):37–42.

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Genetic of Salak Pondoh, Gading Varieties and Its Hybrids Based on RAPD Markers

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Abstract
A molecular marker of parent and offspring is used to find fast and accurate markers influenced by DNA isolation and amplification. This research aims to find the most suitable DNA isolation and DNA amplification methods. This study used four DNA isolation methods; namely IM01, IM02, IM03, and IM04. DNA amplification used ten protocols (AP01, AP02, AP03, AP04, AP05, AP06, AP07, AP08, AP09, and AP010). The results of the research showed that the most suitable DNA isolation method for salak was IM0, and the most suitable DNA amplification for salak was AP04 that produces the highest value of DNA bands.

Keywords: DNA isolation; DNA amplification; hybrids

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Introduction

Salak (*Salacca zalacca* (Gaertner) Voss) has excellent potential to be planted in Indonesia; the tropical climate supports the growth of salak. Thirty varieties of zalacca are distributed in Indonesia (1), including Ivory and Pondoh. Pondoh zalacca is the most famous zalacca compared to other zalacca varieties. Pondoh Salak has a sweet taste even though it is not yet ripe and dark brown or blackish-brown skin color. Salak Gading also consudered as a well-known zalacca variety, which is unique to its yellow skin and the flesh is thicker than Pondoh zalacca, but has a mild taste. This research aimed to develop salak, which has a sweet taste, yellow skin, and thick flesh.

Zalacca breeding through crossing is objected to increase zalacca potential (2). Pondoh and Ivory zalacca crossing is expected to produce offspring with sweet taste, yellow skin, and thick flesh. According to Anna Meyer (3), all aspects of how living things look, function, and behave are determined by DNA. DNA analysis was carried out in this study to obtain accurate data in a short time. There are two stages of DNA analysis, namely DNA isolation and amplification. According to Thomas and Dominic (2013), DNA isolation aims to separate DNA in the cell nucleus from materials and separate DNA in the cell nucleus from other materials and molecules. DNA amplification aims to double the target DNA

This research modifies the existing isolation method and DNA amplification protocol, then analyzes each difference in it.. The main objective is to find superior salak varieties through studying the best and most appropriate methods in DNA isolation and amplification.

Materials and Methods

This study was conducted from March to August 2017 in the Genetics and Plant Breeding Laboratory of Faculty of Biology, Universitas Gadjah Mada. This research was conducted in three stages. First, male Pondoh and female Ivory were crossed in the Salak Nusantara Collection Garden, Bangunkerto Turi Temple, Sleman, Yogyakarta. Second, the results of the crossing were planted on Pucang Sawit Street RT 3 RW 2 Jebres Surakarta.

Third, DNA analysis was carried out at the Genetics and Breeding Laboratory of the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

This study used 4 DNA isolation methods, namely IM01, IM02, IM03, and IM04. IM01 uses DNA Nandariyah isolation method (4). IM02 used the Doyle and Doyle (5) method modified by Borges et al. (6). IM03 used the DNA isolation method that has been listed in the manual of the GE Healthcare Nucleon Phytopure Genomic DNA Extraction Kits RPN 8511. IM04 used the Nandariyah DNA isolation method (4) with modification of the extraction buffer.

DNA amplification used ten different protocols (AP01, AP02, AP03, AP04, AP05, AP06, AP07, AP08, AP09, and AP010). The 10 DNA amplification protocols were distinguished by DNA concentration, primary type, primary concentration, cycle, temperature, and pre denaturation time to post elongation. The difference in migration of DNA bands in agarose gel was the difference in migration of individual allele (7). The samples used were male Pondoh (P), female Gading salak (G), results of crossing Pondoh and Gading salak (F), and other salak varieties (K). The number of repetitions was adjusted to the needs. The salak material for analysis used young leaves.

Results and Discussion

DNA isolation

DNA isolation aims to obtain DNA with high concentration and good purity. DNA isolation consists of three stages, first destruction of cell walls (lysis), second, separation of DNA from other solid materials, and finally, DNA purification (8). The method of DNA isolation used depends on the type of plant or plant tissue (9). According to Syafaruddin et al. (8), the DNA isolation method is needed in plants containing high secondary metabolite compounds to get good quantity and quality of DNA, such as in salak leaves. Differences in the composition, dosage, time, repetition, etc., affect the DNA produced. This study uses 4 DNA isolation methods (IM01, IM02, IM03, and IM04). The difference between the four methods lies in the addition of PVP, extraction buffer, chloroform, isoamyl alcohol, sodium acetate, incubation at a specific temperature, and washing of DNA pellets.

IM01, IM02, and IM04 protocols used PVP to facilitate the grinding of salacca leaves while also using extraction buffers with the composition of CTAB, NaCl, EDTA, Tris-HCl and β-mercaptoethanol. According to Syafaruddin et al. (8), the composition can eliminate polysaccharides and polyphenols. IM01 mixed the entire extraction buffer composition into one, while IM02 and IM04 separated β-mercaptoethanol from other ingredients. β-mercaptoethanol had an unpleasant odor that disrupts DNA isolation activity (10). IM01 and IM04 added sodium acetate to keep the pH stable during the DNA isolation process. IM01, IM03, and IM04 washed DNA pellets using 70% ethanol three times. Ethanol was easily lost by evaporating. IM02 washed DNA pellets using absolute alcohol and 70% alcohol. The absolute alcohol, 70% alcohol, and ethanol have almost the same properties.

A230, A260, A280 and A320

The absorbance value at 230 nm represented polysaccharide contaminants or other contaminants. Nucleic acids such as RNA, DNA, and free nucleotides have a strong absorbance at 260 nm. Protein had a strong absorbance at 280 nm (11). The absorbance value at 320 nm provided a general measurement of the turbidity of the sample (12).

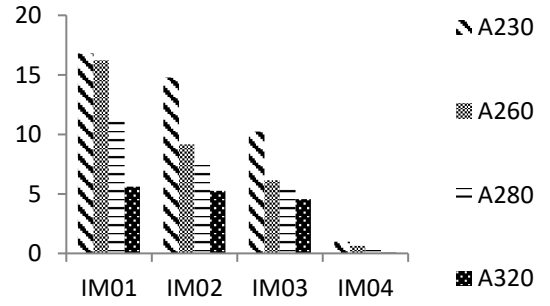


Figure 1. Average values of A230, A260, A280, and A320 of the DNA isolation method used

The results showed that the highest A230 was IM01 with a value of 16,800 and the lowest was IM04 with a value of 0.990. The highest A260 was IM01 with a value of 16.220 and the lowest was IM04 with a value of 0.623. The highest A280 was IM01 with a value of 11,300 and the lowest was IM04 with

a value of 0.336. The highest A320 was IM01 with a value of 5.600 and the lowest was IM04 with a value of 0.079 (Figure 1).

DNA purity

DNA purity compares pure DNA and impurities (protein) (13). A260 divided by A280 can be used to estimate the DNA purity. DNA is said to be pure if it has a purity value of 1.8-2.0. The value of DNA purity lower than 1.8 means that there was protein contamination; while a value higher than 2.0 means that there was a phenol contamination.

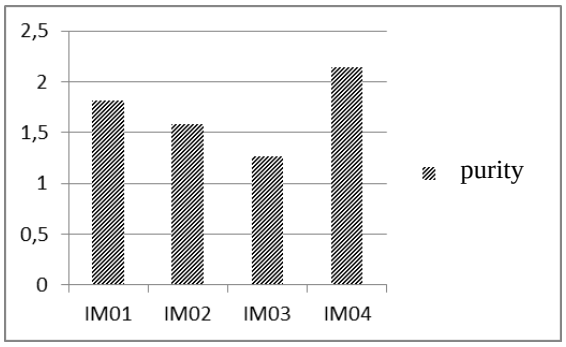


Figure 2. Average DNA purity value from the DNA isolation method used

Based on the results of the research, the purest DNA was IM01, with a value of 1,810. The DNA purity value of IM02 was 1.579, indicating that DNA was contaminated with a protein. The DNA purity value of IM03 was 1,261, indicating that DNA was contaminated with a protein. The DNA purity value of IM04 was 2,143, indicating that it was phenol contaminated (Figure 2).

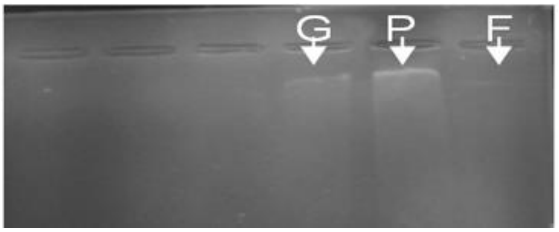
The comparison of chloroform and isoamyl alcohol used in IM01, IM02, and IM04 was 3: 1: 3, while IM03 only used chloroform without the addition of isoamyl alcohol according to the manufacturer's manual. Chloroform and isoamyl alcohol (CIA) are significant for binding proteins and cell membrane lipids and dissolve them to form deposits (14). The dose of chloroform and isoamyl alcohol IM01 was enough to produce DNA with good purity, while IM02 was not enough, so there were still protein contaminants.

IM01 and IM04 used the same dose of chloroform and isoamyl alcohol but produced very different DNA purity, and this wasbecause the extraction buffer composition were different. IM01 extraction buffer composition was 2% CTAB 20 ml, 100 mM Tris pH 9.0 10 ml; 1.4 M NaCl 28 ml; 20 mM EDTA 4 ml; H2O 38 ml; and β-mercaptoethanol 0.1 ml; while the IM04 extraction buffer composition was CTAB 10 gr; 10 mM Tris-HCl 5 ml; 1.4 M 140 ml; 20 mM EDTA pH 8 20 ml; H2O 335 ml; and addition of 75 µ-mercaptoethanol separately. According to Maftuchah et al. (15), the composition of extraction buffer and pH is very important in the optimization strategy of DNA isolation, and extraction buffer is the most important compound to prevent DNA from being degraded. DNA and RNA have insoluble properties in organic solvents (8). The addition of chloroform will not lose DNA and RNA.

IM03 used GE Healthcare Nucleon Phytopure Genomic DNA Extraction Kits RPN 8511. According to manual from the factory, the extraction kit can isolate the DNA of Cocos nucifera, which in plain view has almost the same properties as the salacca leaves, but research on salak leaves yields less satisfactory purity. This can be caused by the salak leaves that have been used for too long.

DNA concentration

DNA concentration is used as a reference to improve the success of DNA amplification. DNA concentration was calculated by A260 times 50 times dilution factor. The higher the concentration of DNA, the easier it is for researchers because it can be diluted as needed and as a DNA stock



Note: G: Salak Gading; P: Salak Pondoh; F: Crossing of Salak Pondoh and Gading

Figure 3. DNA genome electrophoresis IM04

IM02 and IM03 did not produce DNA bands at all. Protein contaminants in IM02 and IM03 caused inaccurate estimates of DNA concentration. According to Hoy MA (16), chaotropic salt contaminants, RNA, and proteins such as EDTA and tris buffering lead to inaccurate estimates of DNA concentration. The improper storage of IM02 and IM03 caused DNA damage. DNA is readily damaged

at inappropriate temperatures. The quality of DNA will be maintained at low temperatures.

IM04 produces DNA bands that are thin, faint, and smear because of phenol contamination (Figure 3). The presence of phenolic compounds can affect DNA quality. The more phenolic compounds the DNA quality is getting worse (8). Genomic DNA electrophoresis produced from the four isolation methods, only two showed DNA bands, namely IM01 and IM04. There were differences between IM01 and IM04; IM01 had good DNA purity while IM04 was contaminated with phenols. Insufficient DNA purity may not necessarily produce genomic DNA bands.

DNA amplification

DNA amplification in this study used the PCR-RAPD method. RAPD (Random Amplified Polymorphic DNA) is a PCR-based DNA marker. RAPD is widely used in identifying interspecies and interspecies diversity (17). RAPD markers are widely used because they are faster, cheaper, more accessible, do not require special skills, do not use radioisotopes, do not require DNA sequence information, and DNA samples are needed (18). PCR optimization is essential to find the most suitable DNA amplification protocol. The trick is to try various DNA concentrations, primary types, temperatures, cycles, and pra denaturation times to post elongation (19).

DNA amplification protocols used in this study were AP01, AP02, AP03, AP04, AP05, AP06, AP07, AP08, AP09, and AP010. The ten DNA amplification protocols were distinguished by DNA concentration, primary type, primary concentration, cycle, temperature, and pre denaturation time to post elongation. Based on research, only AP04 and AP010 succeeded in producing DNA bands.

AP04 and AP10 have differences and similarities. The difference lies in the purity of DNA. AP04 uses IM01 DNA (good purity), while AP10 uses DNA IM04 (phenol contaminated). Maftuchah et al. (15) stated that PCR be done with moderate quality DNA, as in IM04. The AP04 and AP010 equations lied in the primers (OPA-16 [50]), pre denaturation (95.0^{o}C for 3 minutes), denaturation (95.0^{o}C for 15 seconds), annealing (36.0^{o}C, 37.6^{o}C, 38.5^{o}C and 40.0^{o}C for 15 seconds), extension (72.0^{o}C for 1 minute), post Elongation (72.0^{o}C for 5 minutes) and cycle (45 times).

Unsuccessful DNA amplification protocols (AP01, AP02, AP03, AP05, AP06, AP07, AP08, and AP09) used almost the same procedures have succeeded (AP04 and AP10), but there are some differences. AP01 used different annealing temperatures, annealing times, and PCR cycles. AP02 used annealing temperatures as well as different PCR cycles. AP03 and AP06 used different primary concentrations. AP05, AP07, and AP09 used damaged DNA. AP08 used denaturation temperature, denaturation time, annealing temperature, annealing time, extension time, and different PCR cycles. The quality of DNA used by AP08 also has declined due to improper storage.

The success of PCR is influenced by the concentration of printed DNA, primers, $MgCl_2$, and the number of cycles. A few that are not appropriate can affect the success of PCR (20). Unclear and faint DNA bands can be caused by the distribution of primary attachment sites to DNA, competition for primary attachment that causes specific fragments to be amplified in large quantities, and DNA concentrations that are too small (19). High DNA concentrations will increase the concentration of contaminants. Large concentrations of contaminants can inhibit the primary attachment to the printed DNA. Different brands of Thermocycler PCR devices can also affect whether DNA bands appear because different tools require different optimizations.

Genetic analysis

Efforts to increase the salak diversity can be made through the crossing. Crosses need different parental varieties to produce superior varieties (18). This study used male Pondoh salak and female Gading as parents. Pondoh Salak has sweet taste, brown skin, and medium size. Salak Gading has the properties of astringent taste, yellow skin, and large size. The expected phenotypes of the crossing are sweetness, yellow color, and large size. Crossovers between genotype can only be known after the plant is 4 years old when the plant bears fruits. The weakness can be overcomed by using molecular markers Random amplified polymorphic DNA.

The two parents are not pure strains, (even tend to be hybrid varieties) so they are considered to have heterozygous genes.

Mendel's Law 2 states that two heterozygous parents who are crossed will freely produced offspring with a combination of genes. Pondoh and Gading salak cross will produce offspring with a combination of random parents (dominant genes in superior traits cover recessive genes in traits that are not pure strains), so they are considered to have heterozygous genes. Pondoh and gading cross will produce offspring with a combination of random genes. One of the results of the crossing will inherit the superior traits of the two parents (dominant genes in superior traits cover recessive genes in traits that are not superior) to create a superior variety. These superior varieties are expected to be found in this study. The next stage, propagation, was done vegetatively in order to maintain its superior nature.

The results showed that F had a DNA band parallel to G, but none parallel to P. P gave DNA bands with sizes of 300, 450, and 700 base pairs. G raises DNA bands with sizes 300, 350, 400, 500, and 600 base pairs. F raises the DNA band with a size of 600 base pairs. F and G both gave DNA band on the size of 600 base pairs.

This study uses RAPD markers, so that homozygous dominant and heterozygous genes will both produce DNA bands. It cannot be distinguished which are homozygous dominant and which are heterozygous. Recessive homozygous genes do not produce DNA bands (Novalina and Sagala 2011). This can be interpreted that only dominant genes can produce DNA bands. Dominant genes usually describe superior traits, while recessive genes describe superior traits.

P and G are the parents of F, so P and G must have passed down their genes to F. Figure 6 shows the absence of DNA bands that are parallel between P and F. This indicates that P passed down the recessive gene to F, but P did not passed the dominant gene to F. Figure 6 shows the presence of DNA bands parallel to G and F. This indicates that G passed recessive genes or not to F.

Based on observations showing that F was not a superior variety as expected in this study. The expected superior varieties would get superior traits from both ancestors (get dominant genes from both ancestors). F only gets superior traits from G alone (F only gets dominant genes from G). It is assumed that the actual superior variety was one of the samples that has not been successfully observed in this study because the new recessive superiority might be seen in F2.

This research still has many limitations. It is hoped that at least it can contribute valuable ideas. Further research is needed to obtain superior varieties of salacca plants.

Conclusion

The most suitable method of DNA isolation for salak is IM0 that is the extraction buffer composition used of 2% CTAB 20 ml; 100 mM tris pH 9.0 10 ml; 1.4 M NaCl 28 ml; 20 mM EDTA 4 ml; β-mercaptoethanol 0.1 ml; and H2O 38 ml. Giving 500 µl CIA three times. Addition of sodium acetate 1:10 from reagent volume. Washing DNA pellets using 70% ethanol. The most suitable DNA amplification for salak was AP04 that was using OPA-16 primers [50]; pradenaturation 95.0ºC 3 minutes; denaturation of 95.0°C 15 seconds; annealing 38.5°C 15 seconds; 72.0ºC extension for 1 minute; post elongation 72.0ºC 5 minutes; and 45 cycles.

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Conflict of Interest

All authors declare no conflicts of interest in this section.

References

1. Lestari R. Growth and physiological responses of salak cultivars (*Salacca zalacca* (Gaertn.) Voss) to different growing media. J Agric Sci. 2011;3(4):261–71.
2. Budiyanti T, Hadiati S, Prihatini, Sobir S. Genetic diversity of Indonesian snake fruits as food diversification resources. J Int J Adv Sci Eng Inf Technol. 2015;5(3):41–4.
3. Meyer A. Hunting the double helix: how DNA is solving puzzlesof the past. Crows Nest: Allen & Unwin; 2005.
4. Nandariyah. Study genetic variabilitiy of cultivar salak based on morphology and RAPD. Yogyakarta: University Gadjah Mada; 2007.
5. Doyle J, Doyle J. A rapid DNA isolation procedure for small quantities of fresh leaf

tissue. Phytochem Bull. 1987;9:11–5.
6. Borges A, Rosa M, Recchia G, Queiroz-Silva J, Bressan E, Veasey E. CTAB methods for DNA extraction of sweetpotato for microsatellite analysis. Sci Agric. 2009;66(4):529–34.
7. Sinaga A, Putri L, Siregar L. Analisis Keragaman genetik andaliman (*Zanthoxylum acanthopodium* DC.) Sumatera Utara menggunakan marka RAPD (Random Amplified Polymorphic DNA). J Online Agroteknologi. 2015;3(1):350–8.
8. Syafaruddin, Randriani E, Santoso T. Efectivity and efisiency isolation and purification DNA jambu mete. J Bul Ristri. 2011;2(2):151–60.
9. Jose J, Usha R. Extraction of geminiviral DNA from a aighly mucilaginous plant (*Abelmoschus esculentus*). J Plant Mol Biol Rep. 2000;18:349–55.
10. Hermanson G. Bioconjugate techniques. Chennai: Academic Press; 1996.
11. Conn P. Laboratory methods in cell biology: biochemistry and cell culture. Chennai: Academic Press; 2012.
12. Mit Open Course Ware Massachusetts Institute of Technology. UV spectrophotometric measurement of DNA concentration and purity [Internet]. 2008. Available from: https://www.ocw.mit.edu
13. Thoyibi, Arifin M, Abraha K. Purity test DNA melon (*Cucumis melo* L.) cultivar “Gama Melon Basket" with Plasmon Resonance (SPR) based nanoparticle silver. Indones J Appl Phys. 2015;
14. Das S, Dash H. Microbial biotechnology a laboratory manual for bacterial systems. London: Springer; 2015.
15. Maftuchah, Winaya A, Zainudin A. Teknik Fundamental Analysis Technique of molecular Biology. Yogyakarta: Deepublish; 2015.
16. Hoy M, Yu F, Meyer J, Tarazona O, Jeyaprakash A, Wu K. Transcriptome sequencing and annotation of the predatory mite *Metaseiulus occidentalis* (Acari: *Phytoseiidae*): a cautionary tale about possible contamination by prey sequences. Exp Appl Acarol. 2013;59(3):283–96.
17. Siregar U, Olivia R. Genetic variability populasi sengon population (*Paraserianthes falcataria* (L) Nielsen) of community forest in Java based on RAPD. Bogor: Silviculture Department Faculty Forestry IPB; 2012.
18. Rosmaina, Zulfahmi, Handoyo D. Genetic relationship tanaman jambu bol (*Syzygium malaccense* (L.) Merr. & Perry) based on RAPD (Random Amplified Polymorphic DNA). J Agrotek Trop. 2013;2(1):6–10.
19. Langga I, Restu M, Kuswinanti T. Optimalisation temperatue and long inkubation in DNA extraction of bitti (*Vitex cofassus* Reinw) and genetic variability analysis by RAPD-PCR. J Sains Teknol. 2012;12(3):265–76.
20. Uslan, Pharmawati M. Optimation consentration DNA and MgCl2 reaction of Polymerase Chain Reaction Random Amplified Polymorphic DNA for analysis genetic diversity faloak (*Sterculia quadrifida* R.Br). J Bioslogos. 2015;5(1):26.

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Antibacterial Activity Test of Ethanol Extract and SAP of Betung Bamboo Shoot (*Dendrocalamus asper*) Against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Bacteria

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Abstract

Boiled water from bamboo shoots is often used for pneumonia and a cleanser for the wound. It is known that bamboo can be an antibacterial agent because it has saponin, alkaloid, and flavonoid. This research aims to study whether ethanol extract and sap from bamboo betung shoot (*Dendrocalamus asper*) has secondary metabolism and antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The extract was taken by maceration method using ethanol 96%, and the sap was taken from the water of bamboo shoot. Antibacterial activity test was conducted using disc diffusion method to measure the magnitude of inhibitory power at six concentration ratios of b/v solutions (10%; 20%; 30%; 40%; 50%; 60%). The result showed that ethanol extract and sap of bamboo betung shoot contained saponin, quercetin, and quinine which were supposed to have antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The largest inhibitory zone diameter was found in ethanol extract 60% with the category of moderate inhibition of 9.05 ± 0.12 mm against *Kliebsiella pneumoniae* and 5.07 ± 0.13 mm against *Pseudomonas aeruginosa*. While the sap with a concentration of 60% was included in the weak inhibitory category, which was 5.65 ± 0.05 mm in the *Kliebsiella pneumoniae* and 4.81 ± 0.22 mm in *Pseudomonas aeruginosa*.

Keywords: antibacterial; bamboo shoot; betung bamboo.

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Introduction

Klebsiella pneumoniae is a gram-negative opportunistic pathogenic bacteria that can cause respiratory infections, urinary tract infections, nosocomial infections, and even death by up to 10% in humans (1). *Pseudomonas aeruginosa* is a gram-negative bacteria that can cause infections in wounds and burns that cause bluish-green pus, urinary tract infections, respiratory infections that cause pneumonia, mild external muscularity in swimmers, and eye infections (2). Both of these bacteria can be found in most human environments (3).

The boiled water from bamboo shoots is used to treat coughing up blood (pneumonia) and is used as a wound cleanser (4). Bamboo shoots have excellent antioxidant, anti-free-radical, and anti-aging effects because of flavones and glycosides (5). Betung bamboo leaves (*Dendrocalamus asper*) have antibacterial activity against *E. coli* bacteria. All bacteria and fungi grow well in wet media and humid air. Water has a vital role in life because microorganisms can only take food from outside into the solution (holophytic). Bamboo shoots are used as an infection treatment, but bamboo shoots' water content is relatively high. Every 100 grams of fresh bamboo shoots has water content of 91% (6). The water content of the *Schizostachyum brachycladum Kurz* bamboo shoot ethanol extract was 9,12% (7). So far, there has been no test for the antibacterial activity of bamboo

shoots against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Material and Methods

This study was taken on Pharmacy Laboratorium and FMIPA Intergrated Laboratory. In this study, the extract was taken by the maceration method using 96% ethanol, and the sap was taken from the juice of bamboo shoots. Qualitative testing of the chemical content of bamboo betung using thin layer chromatography and tube testing. The antibacterial activity test used the disc diffusion method to measure the amount of inhibition at six concentration ratios of the w/v solution (10%;20%;30%;40%;50%;60%).

The tools used in this study include a set of maceration tools, blender, flannelette, stirring rod, rotary evaporator (Yamato RE-301-AW), UV light 254 nm, test tube (Pyrex), test tube rack, bunsen, sterile cotton swab, petri dish, measuring cup (Pyrex) 25 ml, ose needle, 10-100 µl series micropipettes (Master ptt®), bunsen, yellow tip, tweezers, analyte scale, Moisture Analyzer (Ohaous MB23), Laminar Air Flow (Biobase), autoclave (Sturdy®), oven, incubator (Incubator Hotcold- M), refrigerators, and calipers.

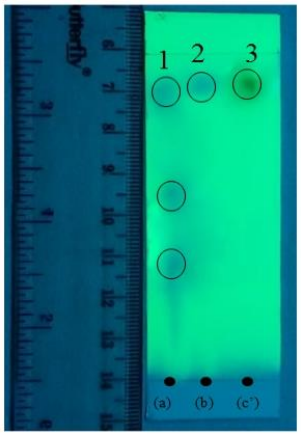


Figure 1. The elution identification of the flavonoid group at 254 nm UV light with a mobile phase of Butanol: Acetic Acid: Water (4: 1: 5). (a) Betung Bamboo Shoots Ethanol Extract (b) Betung Bamboo Shoots Sap (c) quercetin standard

The materials used in this study included bamboo shoots (*Dendrocalamus asper*), 96% alcohol, flannelette, aquadest, filter paper, silica gel 60 F254 (Merck), quercetin standards, quinine standards, butanol, acetic acid, disc paper (Oxioid), aluminum foil, MHA agar media, 0.5 Mac Farland standard, 0.9% sterile NaCl, Amoxicillin 25µg / disk antibiotic (Oxioid), Kliebsiella pneumoniae bacteria ATCC 13883, and Pseudomonas aeruginosa ATCC 27853.

Identification of ethanol extract compounds and bambu betung saps was done with TLC (Thin Layer Chromatography). The TLC method was used in this study because it has greater flexibility in selecting mobile phases (8). The stationary phase used was a 60 F254 silica gel plate. This plate is used because it can glow at 254 nm UV light to help visualize spots or stains on the TLC plate (9). While the mobile phase used is a mixture of Butanol: Acetic Acid: Water (4: 1: 5) (10) as shown in Figure 1.

The standard compound used to identify the flavonoid group is quercetin. Quercetin is a class of flavonoids that can be used as antibacterial because quercetin can bind to bacterial DNA gyrase, which plays a role in DNA replication. Quercetin interferes with the gyrase enzyme so that the DNA replication process stops (11). In this study, 6 series of different solution concentrations were made, both ethanol and sap extracts, namely 10% w / v, 20% w / v, 30% w / v, 40% w / v, 50% w / v, and 60%. b / v.

Results and Discussion

Characteristics of the extraction results obtained in this study were examined in organoleptic, yield calculations, and water content. The yield determination aims to quantify how much plant extract was obtained (12). A total of 150 grams of betung bamboo shoots macerated with 96% ethanol yielded 7 grams of the thick extract or around 4.67%. The extract obtained was relatively small because the main component of bamboo shoots was water. This is supported by research from Nofriyati and Ratima (6) that every 100 grams of fresh bamboo shoots contained 91% water. The characteristics of the ethanol extract of the Betung bamboo shoots can be seen in Table 1.

Table 1. Characteristics of tunas bambu betung ethanol extract

Examination		Result
Organoleptic	Consistency	Thick
	Color	Brown-yellow
	Aroma	Distinctive
Extract yield		4,67%
Water content		28,7 %

The water content requirement for an extract, according to Depkes RI (13), is less than 10%. The water content in the extract that was less than 10%; which aimed to avoid the rapid growth of the fungus in the extract (14). Based on the test results, it was found that the water content of the betung bamboo shoots ethanol extract was 28.7%, so that the resulting extract was not used for storage for a long time.

Identification of ethanol extract compounds and bambu betung saps

This identification test was conducted to qualitatively determine the content of secondary metabolites in the ethanol extract and sap of Betung bamboo shoots. According to Kalita et al (15), bamboo can have antibacterial activity because it has compounds of flavonoids, alkaloids, and saponins. From the results in Table 2, it can be seen that the ethanol extract and the sap of Betung bamboo shoots have quercetin compounds because the Rf value is the same (0.88). This result confirmed the research of Susilowati et al (10) that the Rf value of quercetin compounds with the mobile phase of Butanol: Acetic Acid: Water (4: 1: 5) is 0.88. Hence, it is confirmed that the ethanol extract and sap of Betung bamboo shoots positively have quercetin compounds.

Table 2. Results of the calculation of the rf value for the flavonoid group

Sample	Rf value
Tunas Bambu Betung Ethanol Extract	0.88
Betung Bamboo Shoots Sap	0.88
Quercetin standar	0.88

The identification of alkaloids is shown in Figure 2, the standard compound used was quinine. Quinine can be used as an antibacterial agent because it can interfere with peptidoglycan components in bacterial cells so that the cell wall layer is not formed entirely and causes cell death (16). The ethanol extract of betung bamboo shoots contained quinine compound because the Rf value was the same as the standard Rf value, which was 0.63 (Table 3). This confirmed the results of Susilawati et al. (10) that the Rf value of quinine compounds with the mobile phase of Butanol: Acetic Acid: Water (4: 1: 5) was 0.61. In contrast, the sap of bamboo shoots did not have quinine compounds because its Rf value was not the same as the Rf value of quinine.

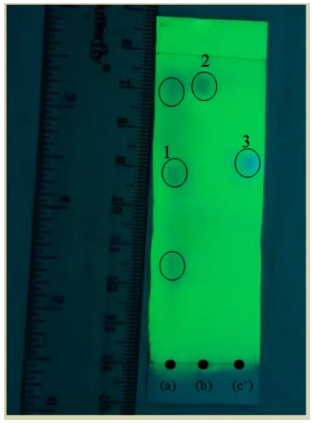
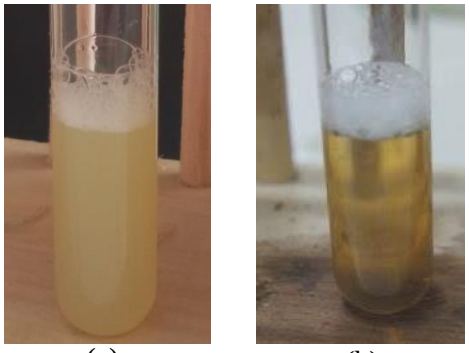


Figure 2. The results of the identification of alkaloids in UV light 254 nm with mobile phase Butanol: Acetic Acid: Water (4: 1: 5). (a) Betung Bamboo Shoots Ethanol Extract (b) Betung Bamboo Shoots Sap (c) quinine standard

Table 3. Results of the calculation of the rf value for the alkanoid group

Sample	Rf value
Betung Bamboo Shoots Ethanol Extract	0.60
Betung Bamboo Shoots Sap	0.88
Quinin standar	0.63



(a) (b)

Figure 3. The results of identification of saponin compounds in the extract and sap of bamboo shoots of Betung bamboo. (a) Betung Bamboo Shoots Ethanol Extract (b) Betung Bamboo Shoots Sap

Saponin identification test was carried out by shuffling. Saponin compounds can be detected because of their ability to form foam. Saponins can damage bacterial cytoplasmic membrane cells by increasing the permeability of bacterial cell membranes (17). Based on the study results shown in Figure 3, there was 1

cm foam after being shaken for 15 minutes. Hence, it can be seen that the ethanol extract and sap of Betung bamboo shoots were positive for saponins.

Antibacterial activity test of ethanol extract and saps of betung bamboo shoots

In this study, the antibacterial activity test used the Kirby Bauer or Disc Diffusion method. This method was used because it does not require special equipment. Its implementation was more accessible and more practical, suitable for liquid samples because of the saturation of the sample on disc paper (18).

The positive control used was the amoxicillin antibiotic because amoxicillin is a broad-spectrum antibiotic that works by preventing the synthesis of bacterial cell walls (19). The agar medium used was Mueller Hilton Agar (MHA) because this media was not a selective medium so that all types of bacteria can grow (20).

When viewed from the inhibition zone category according to Davis et al (20), the results of the inhibition zone diameter measurements of the ethanol extract of bamboo shoots against *Kliebsiella pneumoniae* were in the moderate category; while those in *Pseudomonas aeruginosa* were in the weak category. Betung bamboo shoot sap has weak inhibitory power against *Kliebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Based on the table 4, the largest diameter of the inhibition zone was found in a 60% concentration solution sample, both in the ethanol extract and sap of the bamboo shoots of Betung. In the 60% ethanol extract, the inhibition zone diameter was 9.05 ± 0.12 mm in *Kliebsiella pneumoniae* and 5.07 ± 0.13 mm in *Pseudomonas aeruginosa*. While the sap of Betung bamboo shoots with a concentration of 60% resulted in an inhibition zone diameter of 5.65 ± 0.05 mm in *Kliebsiella pneumoniae* and 4.81 ± 0.22 mm in *Pseudomonas aeruginosa*. Positive control resulted in an inhibition zone diameter of 6.09 ± 0.03 mm for *Kliebsiella pneumoniae* and 5.65 ± 0.05 mm for Pseudomonas aeruginosa. The negative control used, namely sterile aquadest, did not show antibacterial activity.

Table 4. Result of inhibition zone diameter measurement

Sample	Concentration (%) b/v	Average Inhibition Zone Diameter * (mm) ± SD	
		Kliebsiella pneumoniae	*Pseudomonas aeruginosa*
Betung Bamboo Shoots Ethanol Extract	10	5.43±0.40	-
	20	6.36±0.39	3.16±0.15
	30	6.74±0.18	3.63±0.46
	40	7.13±0.19	3.46±0.4
	50	8.10±0.13	4.21±0.21
	60	9.05±0.12	5.07±0.13
Betung Bamboo Shoots Sap	10	-	-
	20	-	-
	30	-	2.14±0.07
	40	3.51±0.32	2.68±0.31
	50	4.53±0.26	3.65±0.20
	60	5.65±0.05	4.81±0.22
Positive control (Amoxicillin 25µl/disk)	-	6.09±0.03	5.65±0.05
Negative control (Aquadest 25µl/disk)	-	0	0

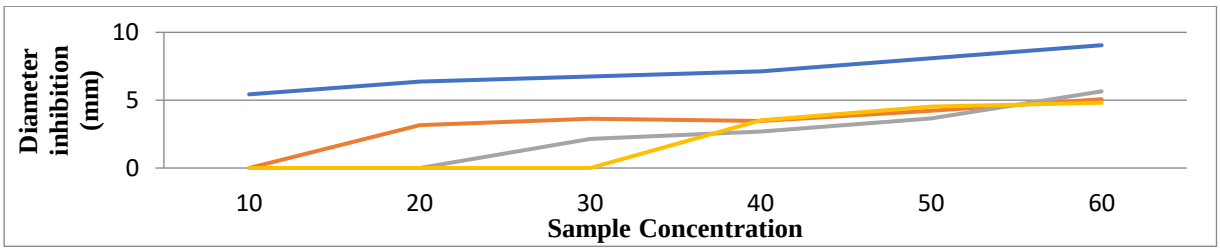


Figure 4. The graph between inhibition zone diameter and solution concentration. *Kliebsiella pneumoniae; Pseudomonas aeruginosa*

The diameter of the inhibition zone in the sap of the Betung bamboo shoots began to appear at a concentration of 30%, and the resulting average inhibition value was smaller than that of the ethanol extract. This can occur because the water content in the sap was higher than in the extract. The water content in the sample can affect the inhibition of bacteria because water is a medium for bacterial growth.

The greater the concentration, the greater the resulting inhibitory power because the more significant the concentration, the greater the active substance contained (21). Based on the Figure 4, it can be seen that the diameter of the inhibition zone is directly proportional to the total concentration of the solution. However, there may be a decrease in the area of the inhibition zone at a greater concentration. Research conducted by Ningtyas (21) stated that the diameter of the inhibition zone is not always directly proportional to the increase in antibacterial concentrations. This can occur due to differences in diffusion rates of antibacterial compounds on agar media and differences in the concentration of antibacterial compounds.

Conclusion

Qualitatively, the ethanol extract and sap of the bamboo shoots (*Dendrocalamus asper*) contain flavonoids, alkaloids, and saponins. The ethanol extract of bamboo betung has a moderate bacterial inhibitory power in *Klebsiella pneumoniae* and a weak Pseudomonas aeruginosa. Meanwhile, the sap of bamboo shoots has weak inhibition in the Klebsiella pneumoniae and *Pseudomonas aeruginosa* bacteria. It is necessary to research the activity test of the extract and sap of the shoots of bamboo betung against other bacteria, using other methods, and looking for other active compounds in the bamboo betung plant.

Conflict of Interest

All authors declare no conflicts of interest in this section.

Refference

1. Tarina N, Kusuma S. Deteksi Bakteri Klebsiella pneumonia. J Farmaka. 2017;15(2):119–26.
2. Mayasari E. *Pseudomonas aeruginosa*: karakteristik, infeksi dan penanganan. Medan: USU Repository; 2006.
3. Hidayat N, Masdiana C, Suhartini S. Mikrobiologi industri. Yogyakarta: CV Andi Offset; 2006.
4. Puri H. Rasayana: Ayurvedic herbs for longevity and rejuvenation. CRC Press; 2003.
5. Choudhury D, Sahu JK, Sharma GD. Value addition to bamboo shoots: A review. J Food Sci Technol. 2012;49(4):407–14.
6. Nofriati D, Ratima S. Kajian pascapanen dan manfaat rebung bagi kesehatan dalam menunjang keanekaragaman pangan yang berbasis pangan lokal. Jambi: Balai Pengkajian Teknologi Pertanian; 2019.
7. Alen Y, Agresa F, Yuliandra Y. Analisis kromatografi lapis tipis (KLT) dan aktivitas antihiperurisemia ekstrak rebung *Schizostachyum brachycladum* Kurz (Kurz) pada mencit putih jantan. J Sains Farm Klin. 2017;3(2):146–52.
8. Wulandari L. Kromatografi lapis tipis. 1st ed. Taman Kampus Presindo. Jember: PT. Taman Kampus Presindo; 2011. p. 20–26
9. Gandjar I, Rohman A. Kimia farmasi analisis. Yogyakarta: Pustaka Pelajar; 2007.
10. Susilowati S, Nuria M, Budiarti A. Identifikasi Kandungan Senyawa Kimia Ekstrak Etanol Herba Alfalfa *(Medicago sativa*, L). Media Farm Indones. 2014;9(2):732–42.
11. Kelly G. Quercetin. Altern Med Rev. 2011;16(2):172–94.
12. Widiasari N. Uji daya analgetik dan identifikasi kandungan kimia ekstrak etanol rimpang lempuyang wangi (*Zingiber aromaticum* Val.) secara Kromatografi Lapis Tipis. Surakarta: Universitas Sebelas Maret; 2015.
13. Depkes RI. Materia medika Indonesia jilid IV. Jakarta; 1995.
14. Soetarno S, Soediro I. Standarisasi mutu simplisia dan ekstrak bahan obat tradisional. In: Presidium Temu Ilmiah Nasional Bidang Farmasi. Depkes RI; 1997.
15. Kalita C, Ganguly M, Devi A. Evaluation of antioxidant capacity and antimicrobial properties of ethnic bambuseae species and identification of

the active components. Int J Pharm Biol Arch. 2016;7(1):61–71.
16. Santoso S, Soemardini, Nugroho P. Efektifitas ekstrak etanol seledri (*Apium graveolens*) sebagai antifungal terhadap Candida albicans secara in vitro. J Penelit. 2010;
17. Prayoga E. Perbandingan efek ekstrak daun sirih hijau (Piper betle L.) dengan metode difusi disk dan sumuran terhadap pertumbuhan bakteri *Staphylococcus aureus*. Jakarta: Universitas Islam Negeri Syarif Hidayatullah; 2013.
18. Katzung B. Farmakologi dasar dan klinik III. Jakarta: Salemba Medika; 2004.
19. Pratiwi S. Mikrobiologi farmasi. Yogyakarta: Erlangga; 2008.
20. Davis W, Stout T. Disc plate method of microbiological antibiotic assay. Appl Microbiol. 1971;22(4):666–70.
21. Ningtyas A. Perbedaan konsentrasi dan uji aktivitas antibakteri ekstrak etanolik batang pisang kluthuk (*Musa balbisiana* Colla) terhadap *Staphylococcus aureus* dan *Pseudomonas aeruginosa*. Surakarta Universitas Sebelas Maret; 2012.

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In Vitro Propagation of *Tribulus terrestris* with IAA and BAP Concentrations

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Abstract
Tribulus terrestris is conventionally propagated using seeds but has limited germination capacity. One approach that can be done for this plant multiplication is in vitro tissue culture. This study was conducted to determine the growth potential of *T. terrestris* in vitro by the addition of auxin (IAA) and cytokinin (BAP) as growth regulators at several concentrations. The study was conducted at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Universitas Sebelas Maret Surakarta from February to December 2017. The experiment employed factorial Completely Randomized Design (CRD) method with 2 factors, namely IAA concentration (0 ppm, 0.1 ppm, 0.2 ppm, 0.3 ppm), and BAP concentration (0 ppm, 0.3 ppm, 0.5 ppm, 0.7 ppm). The observed growth parameters were shoot emergence time, leaf emergence time, root emergence time, number of leaves, and number of roots. Data were analyzed using analysis of variance followed by DMRT with a 95% confidence level. The results showed that the addition of IAA only affects leaf emergence time, while the addition of BAP had a significant effect on the leaf emergence time and number of leaves. The interactions between IAA and BAP had a significant effect on leaf emergence time.

Keywords: explant; plant growth; tissue culture

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Introduction

Tribulus terrestris L. (Zygophyllaceae) is a plant that originates from the Mediterranean region and has been spread widely in warm climate regions such as Africa, Australia, and Asia (1). This plant is used in the practice of Chinese medicine for several diseases (2). Tribulus plant contains several active chemicals that are used as antibiotics and contain steroidal substances that function as an aphrodisiac. One of the main characteristics of this plant is the high amount of steroid chemicals contents. Steroid saponins, diosgenin, furostanol, spirostanol, tigogenin, ruscogenin, chlorogenin, and gitogenin are important chemical constituents which contents are very high in *T. terrestris* (3). The plant is rich in protein and calcium and also contains oil, peroxidase, diastase, resins, and even various glucosides in dried fruit from *T. terrestris*. These characteristics make *T. terrestris* a valuable plant for medicines and other uses such as stimulants and stamina enhancers (4).

Tribulus terrestris is conventionally propagated using seeds. Based on a study conducted on the multiplication of seeds, the germination rate capability by the seeds of this plant is very small (5). In Indonesia, the cultivation of this plant is quite difficult because it can only grow in certain conditions and times. In one year, cultivation can only be done one season in early May to August. The characteristics of seed dormancy and low germination from *Tribulus terrestris* makes this plant a suitable specimen for the study and development of plant propagation methods by in vitro tissue culture methods. The plant's tissue culture aims to provide information about the correct method in propagation by micropropagation for the purpose of seed

formation from cultured plantlets and other utilities. This also opens the pathway to medicinal substance production of *T. terrestris* secondary metabolite within the method of a bioreactor in vitro as the substitution for cultivation by means of a more sustainable method of agricultural production.

Modification of tissue culture media by regulating growth regulators needs to be done to increase the percentage of plant growth rate in tissue culture by means of propagation. There are two types of plant hormones (auxin and cytokinin) that are widely used in propagation in vitro. This experiment aims to observe whether the addition of IAA (*Indoleacetic Acid*) and BAP (*Benzyl Amino Purine*) at certain concentrations can promote *Tribulus terrestris* plant's grown in tissue culture.

Material and Methods

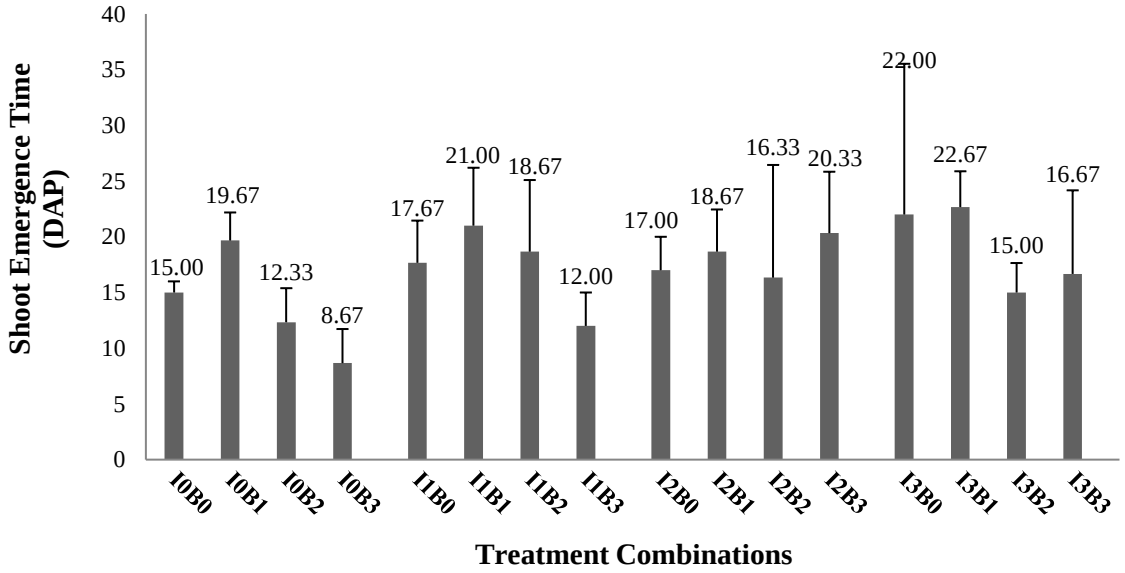
The research was conducted from February to December 2017 at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Universitas Sebelas Maret. The material used in this study were the seed sprouts of *Tribulus terrestris* plants as explants. The medium used was MS type, with aqua dest composition, sugar (80 g/L), macronutrient solution (50 ppm), micronutrient solution (5 ppm), vitamins (50 ppm), Fe-EDTA solution (50 ppm), IAA (I*ndoleacetic Acid*) and BAP (*Benzyl Amino Purine*), Kinetin (0.3 ppm), white agar (8 g/L), spiritus fuel, methanol, 70% alcohol, formalin, 5% chlorox, detergent, fungicide (Mankozeb) 0.1 g/100 mL, bactericidal (Agrept/Streptomycin) 0.1 g/100 mL, antibiotic solution (amoxycillin) 0.1 g/100 mL, ascorbic acid, and betadine 2 mL/100 mL. The tools used included autoclave, laminar airflow cabinet (LAFC), oven, bunsen lamp, hand sprayer, culture bottle, measuring cup, analytic scale, hot plate stirrer, tweezers, scalpel knife, pH meter, pipette, cutter, and petri dish. This research was carried out using a completely randomized design (CRD) with two factors. The first factor was the addition of auxin growth regulator of Indole-acetic Acid (IAA) (0; 0.1; 0.2; 0.3 ppm) and the second factor was the addition of cytokinin growth regulator of Benzyl Amino Purine (BAP) (0; 0.3; 0.5; 0.7 ppm). Research activities initialized by tools and materials preparation, explant sterilization, explant inoculation, and data observation. Data observation was done within 60 days after planting. The variables observed in this study were shoot emergence time (the day after planting/DAP), leaves emergence time (DAP), number of leaves, root emergence time (DAP), and number of roots. The obtained data were analyzed using analysis of variance followed by further DMRT (Duncan Multiple Range Test) with a confidence level of 95%.

Results and Discussion

Shoot emergence time

The appearance of shoots is an indicator in tissue culture studies that plants can grow and develop with the given treatment. The growth and regeneration process is determined by three factors, which are cultivar selection of explant used with certain regeneration capability, explant source optimization, and media adaptation to the given genotype (6). Embryo culture has shoot growth characteristics that emerged between cotyledons, characterized by the appearance of plumula. Adaptation of explants in in-vitro media is determined by nutrient availability factors, microclimate conditions that are following ex-vitro conditions, and additional concentrations of growth promoter (7). The mean value of shoots emergence time with various IAA and BAP concentrations were shown in Figure 1.

The fastest shoot emergence time was shown by the treatment of 0 ppm IAA and 0.7 ppm BAP which was 8.67 DAP, while the latest shoot emergence time shown by 0.3 ppm IAA and 0.3 ppm BAP which was 22.67 DAP. Based on the average value of shoot emergence time from IAA treatment independently, the response of the fastest shoot time was shown in the treatment of 0 ppm IAA, which was about 13 DAP on average, while the longest shoot emergence time was shown at 0.3 ppm IAA which was 19 DAP in average. This showed that the use of BAP independently without the addition of IAA has been able to give better shoot emergence times than the addition of IAA. Despite this result, another study on *Cucumis melo* L. (8) showed that IAA and BAP independently did not significantly affect the regeneration and shoot induction, but the combination of both proved highly affecting differentiation, development, and elongation of new buds.



Description: I0B0: IAA 0 ppm + BAP 0 ppm; I0B1: IAA 0 ppm + BAP 0.3 ppm; I0B2: IAA 0 ppm + BAP 0.5 ppm; I0B3: IAA 0 ppm + BAP 0.7 ppm; I1B0: IAA 0.1 ppm + BAP 0 ppm; I1B1: IAA 0.1 ppm + BAP 0.3 ppm; I1B2: IAA 0.1 ppm + BAP 0.5 ppm; I1B3: IAA 0.1 ppm + BAP 0.7 ppm; I2B0: IAA 0.2 ppm + BAP 0 ppm; I2B1: IAA 0.2 ppm + BAP 0.3 ppm; I2B2: IAA 0.2 ppm + BAP 0.5 ppm; I2B3: IAA 0.2 ppm + BAP 0.7 ppm; I3B0: IAA 0.3 ppm + BAP 0 ppm; I3B1: IAA 0.3 ppm + BAP 0.3 ppm; I3B2: IAA 0.3 ppm + BAP 0.5 ppm; I3B3: IAA 0.3 ppm + BAP 0.7 ppm.

Figure 1. The average time of shoot emergence in various IAA and BAP concentrations

Leaf primordia have accumulated IAA in apical tissue (9). This endogenous IAA content provides the needs of explants to carry out the growth process. The addition of exogenous IAA made the IAA concentration used by explant tissue to be excessive and not optimal, which resulted in growth inhibition. This was indicated by the lower average value of shoot emergence time from the use of IAA concentrations when the concentration was increased. An increase in IAA concentration did not affect the growth of shoot biomass on tomato seeds (10). Auxin at lower concentrations compared to cytokinins is needed in the shoot induction process because the combination of the two with the appropriate concentration will accelerate the process of cell division (11).

Table 1. The effect of BAP treatment on average shoot emergence time (DAP)

	BAP Concentration (ppm)			
	0	0.3	0.5	0.7
Shoot emergence time	17.91^{ab}	20.5^{b}	15.58^{ab}	14.41^{a}

Description: Number followed by the same letters showed no significant difference (P<0.05).

Based on the results in Table 1, the fastest shoot emergence time was indicated by the treatment of 0.7 ppm BAP independently which was 14.41 DAP; while the slowest shoot emergence time was shown in the use of 0.3 ppm BAP which was 20.5 DAP. Table 1 showed that a higher concentration of BAP in the treatments brought a faster explant shoot emergence time. The addition of BAP to tissue culture can increase the growth rate of shoots, so BAP is often used in tissue culture due to this several superior properties compared to other types of cytokines (12, 13).

Table 2. The result of the analysis of the various uses of IAA and BAP concentrations on the leaves emergence time.

Treatments	Leaf Emergence Time
IAA	0.011*
BAP	0.000**
IAA * BAP	0.000**

Description: * has a significant effect (P<0.05), ** has a very significant effect (P<0.01)

Leaves emergence time

Leaf formation in explants has a function to carry out essential physiological processes such as photosynthesis which is used as a place energy generator for other physiological processes such as cell proliferation and organogenesis. Time of leaf emergence was positively correlated with leaf growth and biomass (14). The results of variance analysis

of leaves emergence time of *Tribulus terrestris* explants at several IAA and BAP concentrations are shown in Table 2.

Based on the results in Table 2, all treatments of IAA, BAP, and combination concentrations have a significant effect on the growth response of leaves emergence time. Changes in leaf growth, leaf area, and biomass were followed by changes in the content of cytokinin concentrations in the media and tissues (15). The effect of IAA and BAP concentrations combination on the response of leaves emergence time is shown in Table 3.

Table 3. Effect of IAA and BAP concentrations on leaves emergence time (DAP)

	BAP Concentration (ppm)			
IAA Concentration (ppm)	0	0.3	0.5	0.7
0	18.33^{abcd}	26.00^{def}	28.00^{ef}	16.67^{abc}
0.1	26.00^{def}	25.33^{def}	15.00^{ab}	16.00^{abc}
0.2	23.33^{cde}	24.00^{cde}	12.50^{a}	25.00^{def}
0.3	32.75^{f}	26.33^{def}	19.33^{abcd}	22.33^{bcde}

Description: Number followed by the same letters shows no significant difference in DMRT test of 5% level.

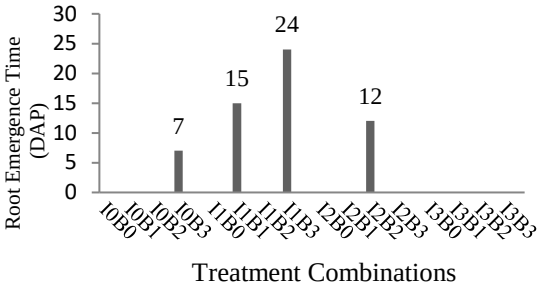
Table 3 showed the fastest response time for leaf emergence was indicated by of the 0.2 ppm IAA and 0.5 ppm BAP treatments, which were 12.5 DAP; while the latest leaves emergence time was shown by 0.3 ppm IAA and 0 ppm BAP which was 32.75 DAP. The use of 0.2 ppm IAA concentration and 0.5 ppm BAP was able to increase the response rate of leaf emergence by 160% faster than the use of 0.3 ppm IAA and 0 ppm BAP with a very significant difference.

Based on the average value of the IAA treatment, the fastest response time of leaves appearance time was shown in 0.1 ppm IAA, which was 20.58 DAP, while the highest average value of leaves emergence time was shown by 0.3 ppm IAA which was 25.5 DAP. This showde that the use of IAA at low concentrations in this study gave better results in the growth response of leaf organogenesis compared to high IAA concentrations. The growth rate of shoots and the average time of shoot emergence decreased with increasing concentration of auxin in the media, which indicates that excess exogenous auxin actually reduces the rate of organogenesis (16). Depending on concentration and tissue, auxin stimulates or inhibits cell elongation. Thus, differential auxin is stimulated and distributed to all organs, such as roots or stems, leading to differential growth which results in organ formation (17).

The average time of leaf emergence on the use of BAP concentration independently showed the fastest value in the treatment of BAP 0.5 ppm which was 18.83 DAP. The average time of leaf emergence was indicated by the use of BAP with a concentration of 0 and 0.1 ppm with a value of 25.54 DAP on average. This data showed the different effects of BAP concentration on the growth response of shoots emergence time, with the fastest shoot emergence time indicated by BAP 0.7 ppm. Cytokinins have an important role in bud and root formation, regulate seed development, abiotic stress, and plant senescence (18, 19, 20, 21, 22). The role of BAP as the most stable type of cytokinin is to encourage cell proliferation, tissue lengthening, and tissue enlargement. Hamad and Taha (23) showed that a positive correlation between the number of shoots, total weight, total length, biomass, and propagule from the results of the tissue cell proliferation process was obtained from media added with the concentration of BAP.

Root emergence time

Roots emergence indicates good explant growth. Root induction is influenced predominantly by auxin activity in explants. Research about in vitro culture of Pecan plants by Zhang et al. (24) showed that exogenous auxin had an effective influence on root growth. IAA content in meristematic tissues can trigger root growth as apical meristem tissue. IAA as phytohormone can regulate many aspects of plant growth and development, such as branching in shoots and roots, as well as differentiation of the vascular vessel system (25, 26). Apart from playing a role in organogenesis in the apical meristem, IAA also determines the regulation of phyllotaxis (27). The effect of the use of IAA and BAP growth regulators on the root emergence time in *Tribulus terrestris* explants is presented in Figure 2.



Description: I0B0: IAA 0 ppm + BAP 0 ppm; I0B1: IAA 0 ppm + BAP 0.3 ppm; I0B2: IAA 0 ppm + BAP 0.5 ppm; I0B3: IAA 0 ppm + BAP 0.7 ppm; I1B0: IAA 0.1 ppm + BAP 0 ppm; I1B1: IAA 0.1 ppm + BAP 0.3 ppm; I1B2: IAA 0.1 ppm + BAP 0.5 ppm; I1B3: IAA 0.1 ppm + BAP 0.7 ppm; I2B0: IAA 0.2 ppm + BAP 0 ppm; I2B1: IAA 0.2 ppm + BAP 0.3 ppm; I2B2: IAA 0.2 ppm + BAP 0.5 ppm; I2B3: IAA 0.2 ppm + BAP 0.7 ppm; I3B0: IAA 0.3 ppm + BAP 0 ppm; I3B1: IAA 0.3 ppm + BAP 0.3 ppm; I3B2: IAA 0.3 ppm + BAP 0.5 ppm; I3B3: IAA 0.3 ppm + BAP 0.7 ppm.

Figure 2. The root emergence time of *Tribulus terrestris* explant with various concentrations of IAA and BAP

Figure 2 showed that not all samples were able to grow roots. The root can only grow in 4 treatments, which were 0 ppm IAA and 0.7 ppm BAP; IAA 0.1 ppm and BAP 0.3 ppm; IAA 0.1 ppm and BAP 0.7 ppm; 0.2 ppm IAA and 0.5 ppm BAP. Roots emergence time ranged from 7-24 DAP with the fastest response root appearance time on the treatment of 0 ppm IAA and 0.7 ppm BAP which is 7 DAP and the latest root emergence time in the treatment of 0.1 ppm IAA and 0.7 ppm BAP.

The absence of roots in most of the samples was suspected because the condition of the radicular tissue in the newly planted explants was not in good condition. This was shown in the visual observations that most of the roots grow from newly grown sprouts are transparent and withered in color media, indicate that the tissue that has been died caused by the sterilization process. The root induction in some samples comes from the buds of the new roots from the root base, not the lateral root shoots of the radicular development. Besides being due to the dead radicular tissue conditions, the influence of auxin was also thought to play a role in the growth response. This was showed by the control treatment without external IAA addition which can still grow roots. IAA used in the media is thought to have not been in optimal condition since the sterilization process in the autoclave. IAA's ability as a plant growth regulator will experience a reduction in enzymatic work at high temperatures (28).

Number of leaves

Leaves are one of the organs of plants that are very important especially for photosynthesis which is used as a food production factory and supports optimum growth (29). The number of leaves is one of the important indicators in determining tissue potential which is regulated by genetic factors and growth hormone. The analysis result of the effects of IAA and BAP growth regulators on the response of leaf growth in *Tribulus terrestris* are shown in Table 4.

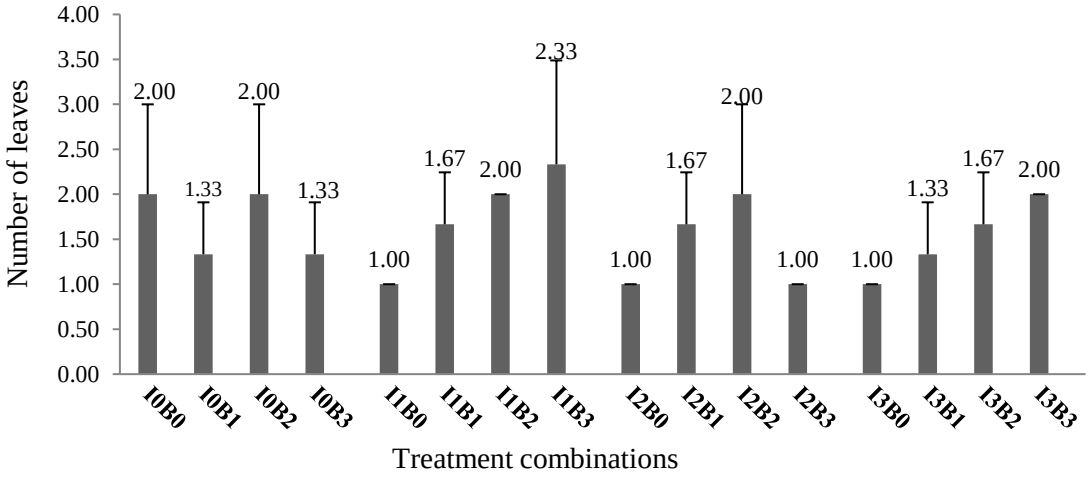
Table 4. Analysis result of various IAA and BAP concentrations effect on the number of leaves

Treatment	Number of Leaves
IAA	0.518^{ns}
BAP	0.050^{*}
IAA * BAP	0.238^{ns}

Description: * has a significant effect, ^{ns} has no significant effect.

Table 4 showed that the addition of BAP independently has a significant influence on the number of leaves ($P<0.05$). The effect of IAA addition and the interaction between IAA and BAP on the number of leaves did not occur. Akhiriana et al. (30), suggested that the addition of IAA was independently able to affect the process of leaves formation, especially in a low concentration of 0.15 ppm. The previous research showed that significant changes in leaf growth, leaf area, and biomass were followed by changes in the content of cytokinin concentrations in the media and tissues (15). The average number of leaves from the treatment given by various IAA and BAP concentrations were shown in Figure 3.

Figure 3 showed the highest average response value of the number of leaves which were indicated by the treatment of 0.1 ppm IAA and 0.7 ppm BAP with 2.33 strands; while the least average number of leaves was 1 piece which obtained by 4 different treatments: IAA 0.1 ppm and BAP 0 ppm; 0.2 ppm IAA and 0 ppm BAP; 0.2 ppm IAA and 0.7 ppm BAP; and IAA 0.3 ppm and BAP 0 ppm. One of the roles of auxin in leaf growth is to help the development of meristem tissue of leaf induction (29). Cytokinin such as BAP in leaf formation is used for morphogenesis and leaves enlargement and the result will be used for growth and increasing the number of leaves (31).



Description: I0B0: IAA 0 ppm + BAP 0 ppm; I0B1: IAA 0 ppm + BAP 0.3 ppm; I0B2: IAA 0 ppm + BAP 0.5 ppm; I0B3: IAA 0 ppm + BAP 0.7 ppm; I1B0: IAA 0.1 ppm + BAP 0 ppm; I1B1: IAA 0.1 ppm + BAP 0.3 ppm; I1B2: IAA 0.1 ppm + BAP 0.5 ppm; I1B3: IAA 0.1 ppm + BAP 0.7 ppm; I2B0: IAA 0.2 ppm + BAP 0 ppm; I2B1: IAA 0.2 ppm + BAP 0.3 ppm; I2B2: IAA 0.2 ppm + BAP 0.5 ppm; I2B3: IAA 0.2 ppm + BAP 0.7 ppm; I3B0: IAA 0.3 ppm + BAP 0 ppm; I3B1: IAA 0.3 ppm + BAP 0.3 ppm; I3B2: IAA 0.3 ppm + BAP 0.5 ppm; I3B3: IAA 0.3 ppm + BAP 0.7 ppm.

Figure 3. The average number of leaves of *T. terrestris* explant with various concentrations of IAA and BAP

Table 5. The effect of BAP concentrations on the number of leaves

	BAP Concentration Treatment (ppm)			
	0	0.3	0.5	0.7
Number of Leaves	1.25[a]	1.33[a]	1.97[b]	1.67[ab]

Description: Number followed by the same letters shows no significant difference in DMRT test of 5% level.

The use of 0.5 ppm BAP concentration independently showed a significantly different and greater effect compared to the concentration of BAP 0 ppm and 0.3 ppm. The concentration of BAP of 0.7 ppm gave a relatively lower average number of leaves compared to the number of leaves from the treatment of a 0.5 ppm BAP concentration. Thus, the concentration of BAP of 0.5 ppm was assumed as the best concentration in this study with regard to the number of leaves. Table 5 shows that the number of leaves tends to increase in line with the increase in BAP concentration. This followed the previous research by Kristina (31) which stated that cytokines such as BAP in leaf formation have a role in morphogenesis and leaves enlargement which is used for supporting the growth and increase the number of additional leaves. The role of BAP in leaf formation is more dominant than the effect of IAA.

Higher number of leaves formed will give more energy for explant tissue to grow. The increasing number of leaves and leaf area width will support the growth and yield of plants, due to the increasing number of leaves that allow more sunlight to be received which improve photosynthesis rate, produce many photosynthates which then stored as carbohydrates (32).

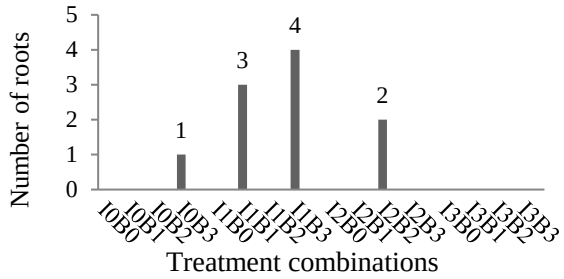
Number of roots

High quantity, large, and long-sized roots are very useful for the absorption of nutrients from the media because the field of absorption of nutrients from the media will be wide. Roots can be formed optimally on the media if supported by the appropriate concentration of growth regulator, especially from the auxin group. The number of roots is an indicator of the ability of total tissue potential. Although in embryo culture the explants have formed roots, the supplementation of auxin and cytokinin will trigger explants to form additional roots. The effect of the use of IAA and BAP growth regulators on the number of *Tribulus terrestris* roots is shown in Figure 4.

Figure 5 showed that not all explant samples observed can grow roots. Root only appears in 4 treatments, which were 0 ppm IAA and 0.7 ppm BAP; 0.1 ppm IAA and 0.3 ppm BAP; 0.1 ppm IAA and 0.7 ppm BAP; 0.2 ppm IAA and 0.5 ppm BAP. The highest number of roots was produced by explants with the treatment of 0.1 ppm IAA and 0.7 ppm BAP which was 4 pieces, while the smallest number

of roots was shown by explants from 0 ppm IAA treatment and 0.7 ppm BAP.

Figure 4. *T. terretris* explant with 0.1 ppm IAA and 0.3 ppm BAP with 3 strands of leaves



Description: I0B0: IAA 0 ppm + BAP 0 ppm; I0B1: IAA 0 ppm + BAP 0.3 ppm; I0B2: IAA 0 ppm + BAP 0.5 ppm; I0B3: IAA 0 ppm + BAP 0.7 ppm; I1B0: IAA 0.1 ppm + BAP 0 ppm; I1B1: IAA 0.1 ppm + BAP 0.3 ppm; I1B2: IAA 0.1 ppm + BAP 0.5 ppm; I1B3: IAA 0.1 ppm + BAP 0.7 ppm; I2B0: IAA 0.2 ppm + BAP 0 ppm; I2B1: IAA 0.2 ppm + BAP 0.3 ppm; I2B2: IAA 0.2 ppm + BAP 0.5 ppm; I2B3: IAA 0.2 ppm + BAP 0.7 ppm; I3B0: IAA 0.3 ppm + BAP 0 ppm; I3B1: IAA 0.3 ppm + BAP 0.3 ppm; I3B2: IAA 0.3 ppm + BAP 0.5 ppm; I3B3: IAA 0.3 ppm + BAP 0.7 ppm.

Figure 5. The number of root from various IAA and BAP concentrations

From the data, we cannot yet conclude the influence of each type of growth regulator treatment on the growth response of root growth. The addition of IAA can increase the formation of root numbers but this does not mean that without the external IAA, explants can't grow roots. Root induction as meristem tissue is dominantly affected by the function of endogen auxin such as IAA (33). It was because explants used in this research already have an endogenous growth regulator, so that the content of endogenous IAA might have beed sufficient to grow roots. Root cells generally contain enough or almost enough auxin to elongate normally. Therefore, the addition of hormones is expected to stimulate faster and denser rootd growth.

The number of roots has a positive effect on explant growth. From all four explants that produce roots, their growth response by the number of leaves and the life span of the media which were relatively larger than the treatments that does not give rise to roots. This was due to the high number of roots increased the amount of nutrient uptake by explants. The inability of other explants in inducing roots was thought to be because the content of endogenous auxin was not able to stimulate the organogenesis process, especially the formation of lateral roots so that the addition of auxin concentration is needed.

Conclusion

The addition of IAA independently did not affect all explant growth variables observed, whereas the addition of BAP affects the growth response on leaves emergence time and the number of leaves. The combination treatment of 0.2 ppm IAA and 0.5 ppm BAP was a growth regulator concentration with the greatest effect on the leaves emergence time response, which was 12.5 DAP.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

References

1. Sharifi S. Enhanced callus induction and high-efficiency plant regeneration in *Tribulus terrestris* L., an important medicinal plant. Journal of Medicinal Plants Research, 2012;6(27):4401-4408. https://doi.org/10.5897/jmpr12.260.

2. Austin DF.. Handbook of medicinal herbs. Second Edition. In Economic Botany, 2006;57(2):290.https://doi.org/10.1663/0013-0001(2003)057[0290:homhse]2.0.co;2

3. Xiong W, Luo G, Zhou L, Zeng Y, Yang W. In vitro and in vivo antitumor effects of acetylshikonin isolated from *Arnebia euchroma* (Royle) Johnst (Ruanzicao) cell

suspension cultures. Chinese Medicine, 2009;4:1-7. https://doi.org/10.1186/1749-8546-4-14

4. Jiji MJ, Visalakshi S, Meenakshi P, Rathi MA, Thirumoorthi L, Chinna GD, Gopalakrishnan VK. Antilipidemic activity of *Cissus quadrangularis* and *Tribulus terrestris* on obesity in high fat fed rats. Pharmacologyonline, 2009;2: 1250-1258.

5. Raghu AV, Geetha SP, Martin G, Balachandran I, Mohanan KV. Micropropagation of *Tribulus terrestris* Linn. Indian Journal of Natural Products and Resources, 2010;1(2): 232-235.

6. Armas I, Pogrebnyak N, Raskin I. A rapid and efficient in vitro regeneration system for lettuce (*Lactuca sativa* L.). Plant Methods, 2017;13(1):1-9.

7. Pinhal HF, Araruna E da C, Carneiro PAP, Asmar SA, Melo B de, Luz JMQ. Concentration of MS medium and cutting of seeds on in vitro establishment of baruzeiro (*Dipteryx alata* Vog.). Bioscience Journal, 2017;33(2):306-313.

8. Valdez M, Gatica-AAM. Effect of BAP and IAA on shoot regeneration in cotyledonary explants of Costa Rican melon genotypes. Agronomia Costarricense, 2009;33(1): 125-131.

9. Aloni R, Schwalm K, Langhans M, Ullrich CI. Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in Arabidopsis. Planta, 2003:(5):841-853.

10. Gravel V, Antoun H, Tweddell RJ. Effect of indole-acetic acid (IAA) on the development of symptoms caused by *Pythium ultimum* on tomato plants. European Journal of Plant Pathology, 2007;119(4):457-462.

11. Peixe A, Raposo A, Lourenço R, Cardoso H, Macedo E. Coconut water and BAP successfully replaced zeatin in olive (*Olea europaea* L.) micropropagation. Scientia Horticulturae, 2007;113(1): 1-7.

12. Antony CS, Lenin MS, Bhargav PK, Karthigan M, Ignacimuthu S. Highly efficient shoot regeneration of *Bacopa monnieri* (L.) using a two-stage culture procedure and assessment of genetic integrity of micropropagated plants by RAPD. Acta Physiologiae Plantarum, 2010;32(3): 443-452.

13. Mazri MA. Role of cytokinins and physical state of the culture medium to improve in vitro shoot multiplication, rooting and acclimatization of date palm (*Phoenix dactylifera* L.) cv. Boufeggous. Journal of Plant Biochemistry and Biotechnology, 2015;24(3): 268-275.

14. Mathieu RA, Cournède PH, de Reffye P. A dynamical model of plant growth with full retroaction between organogenesis and photosynthesis. Arima, 2006;4: 101-107.

15. Juvany M, Müller M, Munné-Bosch S. Plant age-related changes in cytokinins, leaf growth and pigment accumulation in juvenile mastic trees. *Environmental and* Experimental Botany, 2013;87:10-18. https://doi.org/10.1016/j.envexpbot.2012.09.007

16. Nakhooda M, Watt MP, & Mycock D. Auxin stability and accumulation during in vitro shoot morphogenesis influences subsequent root induction and development in Eucalyptus grandis. Plant Growth Regulation, 2011;65(2): 263-271. https://doi.org/10.1007/s10725-011-9597-7

17. Vanneste S, Friml J. Auxin: a trigger for change in plant development. Cell, 2009;136(6): 1005-1016.

18. Kim HJ, Ryu H, Hong SH, Woo HR, Lim, PO, Lee IC., Hwang I. Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in Arabidopsis. Proceedings of the National Academy of Sciences, 2006;103(3): 814-819.

19. Kuderová A, Urbánková I, Válková M, Malbeck J, Brzobohatý B, Némethová D, & Hejátko J. Effects of conditional IPT-dependent cytokinin overproduction on root architecture of arabidopsis seedlings. Plant and Cell Physiology, 2008;49(4): 570-582.

20. Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Kyozuka, J. Direct control of shoot meristem activity by a cytokinin-activating enzyme. Nature, 2007;445(7128): 652-

655. https://doi.org/10.1038/nature05504

21. Riefler M, Novak O, Strnad M, Schmülling T. Hospital uses EMR to improve handoff process and create electronic “Hall Pass". The Plant Cell, 2006;18: 40-54.

22. Tran LP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-shinozaki K.. Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in Arabidopsis. PNAS, 2007;104(51): 3-8. https://doi.org/10.1073/pnas.0706547105

23. Hamad AM, Taha RM. Effect of benzylaminopurine (BAP) on in vitro proliferation and growth of pineapple (*Ananas comosus* L. Merr.) cv. smooth cayenne. Journal of Applied Sciences, 2008;8(22): 4180-4185.

24. Zhang JY, Guo ZR, Zhang R, Li YR, Cao L, Liang YW, Huang LBin. Auxin type, auxin concentration, and air and substrate temperature difference play key: Roles in the rooting of juvenile hardwood pecan cuttings. HortTechnology, 2015; 25(2): 209-213.

25. Leyser O. Dynamic integration of auxin transport and signalling. Current Biology, 2006; 16(11): 424-433.

26. Reinhardt D, Mandel T, & Kuhlemeier C. Auxin regulates the initiation and radial position of plant lateral organs. *The Plant* Cell, 2007; 12(4): 507-518.

27. Sassi M, Vernoux T. Auxin and self-organization at the shoot apical meristem. *Journal of Experimental Botany*, 2013;*64*(9): 2579-2592.

28. Karcz W, Burdach Z. Effect of temperature on growth, proton extrusion and membrane potential in maize (*Zea mays* L.) coleoptile segments. Plant Growth Regulation, 2007;52(2): 141-150. https://doi.org/10.1007/s10725-007-9184-0

29. Arimarsetiowati R, Ardiyani F. Pengaruh penambahan auxin terhadap pertunasan dan perakaran kopi arabika perbanyakan Somatik Embriogenesis. Pelita Perkebunan, 2012:28(2):82-90.

30. Akhiriana E, Samanhudi, Yunus A. Coconut water and Iaa effect on the in vitro growth of *Tribulus terrestris* L. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 2019;*67*(1): 9-18. https://doi.org/10.11118/actaun201967010009

31. Kristina NNSFS. Pengaruh air kelapa muda terhadap multiplikasi tunas in vitro, produksi rimpang, dan kandungan xanthorrhizol temulawak di lapangan. Jurnal Litrri, 2012;18, 126-134.

32. Rahmawati F, Wicaksono KP, Aini N. Pengaruh fermentasi urin kelinci dan paitan terhadap pertumbuhan dan hasil tanaman paprika (*Capsicum annuum* var Grossum) dengan sistem hidroponik subtrate. Jurnal Produksi Tanaman, 2018;6(2), 194-202.

33. Prabowo H, Samanhudi, Yuniastuti E, Yunus A. Effects of media combination with concentration of ab-mix nutrient on growth of banana shoots on in vitro. Bulgarian Journal of Agricultural Science, 2018;24(3): 404-410.

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Vitamin E and Antioxidants Content of *Portulaca oleracea* L. Form Various Altitudes in East Java, Indonesia

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Abstract
This study aims to obtain purslane plants as a source of local food and a quality bioactive component from various altitudes in East Java, Indonesia. Purslane planting materials were collected from the lowlands <200 m asl in Surabaya, medium plains 200-800 m asl in Malang DAU, and highlands> 800 m asl in Batu, Malang. Analysis of vitamin E was as total tocopherol. The results of the analysis of the content of *Portulaca oleraceae* L. of vitamin E were as followed: in the highlands 0.1056%, medium lands 0.1253%, and lowlands 1.162%. The analysis showed that *Portulaca oleraceae* L. could be a source of high quality local food and bioactive components because it contains vitamin E and antioxidants.

Keywords: bioactive components; local food; lowland

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Introduction

Public demands for natural food ingredients that have a higher health function, along with the many degenerative diseases, such as cancer, coronary heart disease, diabetes mellitus, liver, kidney failure, etc. One of the plants that have multiple benefits, both as a food with high nutritional value and medicinal properties (functional food), is purslane (*Portulaca oleracea* L.).

Purslane contain many components of active compounds. Some compounds include organic acids (oxalic acid, caffeine acid, malic acid, and citric acid), alkaloids, coumarin, flavonoids, cardiac glycosides, anthraquinone glycosides, alanine, catecholamines, saponins, and tannins. There are five types of flavonoids in purslane namely kaempferol, apigenin, myricetin, quercetin, and luteolin. Purslane also contains urea, calcium, iron, phosphorus, manganese, copper, and fatty acids, especially omega-3 fatty acids (1). Omega-3 fatty acids are an important chemical component that the body cannot produce. The seeds contain β-sitosterol. All parts of this plant contain l-norepinephrine, carbohydrates, fructose, vitamin A, vitamin B1, vitamin B2, vitamin E and are rich in ascorbic acid (2) and beta carotene (3,4).

Purslane is a weed that has nutritional value (5). The purslane is used as a source of food that has very good benefits. Purslane has several advantages by looking at its nutritional content, including as a local food resource, easily obtained and cultivated, and affordable. In addition to being a food source, it also has medicinal properties. The quality of purslane plants as quality food requires proper handling during its cultivation, harvest, and post-harvest to maintain good nutritional content. Among the efforts to maintain the quality of secondary metabolite contents is by knowing the effect of altitude on antioxidant activity, phenolic content and flavonoids. This research aimed to obtain purslane plants as a source of quality

local food and bioactive components from various altitudes in East Java, Indonesia.

Material and Methods

The study was conducted by taking purslane planting materials from the lowlands <200 m above sea level in Surabaya, medium plains 200-800 m above sea level DAU in Malang, and highlands> 800 m above sea level in Batu, Malang.

The analysis of vitamin E was conducted in the Center for Food and Nutrition Studies laboratory at Gadjah Mada University, Jogjakarta, Indonesia.

Analysis of vitamin E as total tocoperol

Methods: Analysis of vitamin E conducted with a sample weighing of 1 g, dissolved in 10 ml Hexane; then take 1 ml of stock solution, heated in a water bath until only oil remainined. Add 3.5 ml of 2.2 Bipyridine 0.07%, and add 0.5 ml of FeCl3 0.02%, then dilute to 10 ml using 96% ethanol, Tera at λ 520 NM (AOAC, 1995).

$$\%\ \text{Vit E content} = \frac{\text{Xn Dilution factor}}{\text{Weight sample (mg)}} \times 100\%$$

$$X = \frac{y-\alpha}{b}$$

Examination of vitamin E content in Purslane Plants (code samples 5-23, 5-26 and 5-29)

Purslane samples are washed and drained. After draining, roasted at 60 ° C to dry, then the dried Simplicia is blended and sieved. Simplisia powder weighed as much as 1 gram, put into a 100 ml measuring flask. Add methanol p.a. right up to the mark. Ultrasonication for 30 minutes. Shake with hands for 1 minute then let it settle. Take the methanol extract and filter it with a 0.45-micron membrane filter. The filtrate analyzed by HPLC. Instrument = Agilent 1100 Series HPLC with autosampler and PDA detector. Column = Merck LiChrosper 100 RP-18, 4 × 250 mm, 5 um. Eluent = 100% methanol 1 ml / minute. Column temperature = 30 ° C. Duration of observation = 284 nm. Injection volume of 100 Ul.

Results and Discussion

Table 1. Analysis of the content of *Portulaca oleracea* L.

Analysis	Upland (>800 m asl)	Mediumland (200 m – 800 m asl)	Lowland (< 200 m asl)
Vitamin E (%)	0.1056	0.1253	1.162

Vitamin E

Antioxidants are substances that can slow down the oxidation processes that harm the body, such as damaged cells, thus accelerating premature aging of the skin, causing cancer, heart disease, etc. Antioxidants found in plants are used to ward off free radicals. Plants that are used as antioxidants usually contain carotenoid compounds, flavonoids, polyphenols, and allyl sulfide. These antioxidants are found in fruits, vegetables, and seeds. The colors of fruits and vegetables are useful pigments as antioxidants.

Antioxidants can protect the skin from the negative effects of free radicals that can cause skin disorders. Types of antioxidants that can benefit the skin are vitamin A, vitamin E, carotenoids, beta-carotene, lycopene, polyphenols, flavonoids, and lutein (6). Vitamin E is a fat-soluble vitamin that is very useful in addition to being an antioxidant and protects the body from polyunsaturated fatty acids (PUFAs), such as oleic acid, linoleic linolenic acid, and arachidonic acid. Besides, vitamin E in the body is an antidote to free radicals and oxygen molecules important in preventing the peroxidation of unsaturated fatty acid membranes (7).

Vitamin E is an efficient stopping reaction for the cause of free radicals in the fat membrane because the form of free radicals is stabilized by resonance. Therefore the vitamin E radical has a small tendency to extract a hydrogen atom from another compound and spread the reaction. Vitamin E radicals can also regenerate in the presence of vitamin C or glutathione (8). As an antioxidant, vitamin E functions as a hydrogen ion donor capable of changing peroxyl radicals (lipid peroxide yields) into less reactive tocopherol radicals, so it cannot damage the fatty acid chain (9).

The antioxidant mechanisms of tocopherol include the transfer of one hydrogen atom from the 6-hydroxyl group to the chroman ring and the inactivation of oxygen singlets and other reactive species. The phytopillol tocopherol chain is bound to the bilayer cell membrane, while the active chroman ring is located on the cell surface. This unique structure causes tocopherol to work effectively as an antioxidant and can be regenerated through reactions with other antioxidants such as ascorbic acid (10). The analysis of vitamin E based on altitude were 0.1056% in the highlands, 0.1253% in the middle plains, and 1.162% in the lowlands.

Conclusion

Based on the results of the study, the analysis of the content of *Portulaca oleraceae* L. Analysis of vitamin E at highland 0.1056%, medium plain 0.1253%, and lowland 1.162%. This shows that Portulaca oleraceae L. can be a source of quality local food and bioactive components because it contains vitamin E and antioxidants.

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All the authors contributed conducted the experiment and prepared the paper.

Conflict of Interest

All authors declare" no conflicts of interest" inthis section.

References

1. Palaniswamy UR, McAvoy RJ, Bible BB. Omega-3 fatty acid concentration in purslane (*Portulaca oleraceae*) is altered by photosynthetic photon flux. J Am Soc Hortic Sci. 2001;126(5):537–43.
2. Hariana H. Tumbuhan obat dan khasiatnya seri 3 agrisehat. Seri 2. Jakarta: Swadaya; 2005. 14 p.
3. Liu L, Howe P, Zhou YF, Xu ZQ, Hocart C, Zhang R. Fatty acids and β-carotene in Australian purslane (*Portulaca oleracea*) varieties. J Chromatogr A. 2000;893(1):207–13.
4. Barbosa-Filho JM, Alencar AA, Nunes XP, Tomaz ACA, Sena-Filho JG, Athayde-Filho PF, Silva MS, Souza MFV, Da-Cunha EVL. Sources of alpha, beta, gamma, delta and epsilon-carotenes: A twentieth century review. Rev Bras Farmacogn. 2008;18(1):135–54.
5. Sudhakar D, Kishore RK, Parthasarathy PR. *Portulaca oleracea* L. extract ameliorates the cisplatin-induced toxicity in chick embryonic liver. Indian J Biochem Biophys. 2010;47(3):185–9.
6. Widyastuti W, Kusuma AE, Nurlaili N, Sukmawati F. Antioxidant and Sunscreen Activities of Ethanol Extract of Strawberry Leaves (*Fragaria x ananassa A.N. Duchesne*). J Sains Farm Klin. 2016;3(1):19–24.
7. Burke J, Fayaz S, Graham K, Matthew R, Field M. Peer-assisted learning in the acquisition of clinical skills: A supplementary approach to musculoskeletal system training. Med Teach. 2007;29(6):577–82.
8. Berdanier C, Dwyer J, Feldman E. Handbook of nutrition and food. 2nd ed. USA: CRC Press; 2008. 221–226 p.
9. Winarsi H. Antioksidan alami dan radikal. Yogyakarta: Kanisius; 2007. 189–190 p.
10. Salonen, JT, Nyysso¨ nen K, Salonen R, Porkkala-Sarataho E, Tuomainen TP, Diczfalusy U. Lipoprotein Oxidation and Progression of Carotid Atherosclerosis. Circulation. 1997;95(4):840–5.

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The Effect of Concentration and Time Interval of Kinetin Application on The Growth of Daun Duduk (*Desmodium triquetrum* L.) Seeds

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Abstract
Daun duduk (*Desmodium triquetrum* L.) is a medicinal plant that has the power to treat hemorrhoids, but has not been widely cultivated. This research was conducted to obtain the right concentration and time interval of kinetin application to increase the growth of Daun duduk seedlings. This research was conducted from November 2019 until March 2020 at the Screen House, Faculty of Agriculture, UNS. The factors of the experiment were concentration of kinetin (0 ppm, 10 ppm, 20 ppm and 30 ppm) and the time interval of application (once every three months, once every 1.5 months and once a month). The parameters observed in this study were plant height, stem diameter, number of leaves, number of branches, number of roots, root length, fresh stover and dry stover. The results showed that the single factor of kinetin concentration could increase plant height, stem diameter, number of leaves, number of branches and dry stover. Meanwhile, the time interval for application and the interaction between the two factors did not have any significant effect. The most effective kinetin concentration in increasing the growth of Daun duduk was 30 ppm.

Keywords: growth regulators; herbal medicines; plant height.

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Introduction

Indonesia is a country that has a tropical climate, so that the level of biodiversity is very high, one of which is medicinal plants. Nowadays, many people are returning to using herbal medicine as an alternative treatment. According to Ismail (1), the factor that encourages people to look for alternatives in reducing chemical drugs is that herbal medicines are considered more economical and have fewer side effects.

Central Bureau of Statistics (BPS) data (2) states that the export value of medicinal plants from Indonesia in 2018 reached USD 601.2 million; which makes Indonesia potential in medicinal plants development. However, not many people have cultivated medicinal plants. According to Salim and Ernawati (3), medicinal plants have not been widely cultivated. One of the reasons is that it is difficult to maintain, especially those from the forests. Wild plants grow well in ther habitats because of the optimum environmental condition.

Daun duduk is a medicinal plant that grows and develops in Indonesia. Daun duduk is efficacious as a hemorrhoid medicine. Besides that, they can also be used to cure several diseases, including dysentery, fever, prostate, obesity and diabetes. Daun duduk can be used, especially the leaves, as herbal medicine. Secondary metabolites produced by Daun duduk are saponins, flavonoids, glycosides, trigonelline, tannins, phenolics, alkaloids and terpenoids.

Daun duduk has not been widely cultivated in general, and this is due to a lack of information about the properties and good cultivation practice of this plant. Daun duduk

still comes from their natural habitat; and the harvest must be limited to maintain forest sustainability; hence, Daun duduk cultivation is necessary. Karmawati et al. (1996) in Mirza et al. (4) stated that the cultivation of medicinal plants in Indonesia faces obstacles, namely fluctuations in production caused by not implementing good cultivation. Propagation of Daun duduk plants is done generatively, namely using seeds. One of the growth regulators that can affect plant growth is kinetin. Kinetin is a cytokinin group that functions to regulate cell division and morphogenesis (5). The purpose of this study was to determine the kinetin concentration and the right time to increase the growth of Daun duduk seedlings. In addition, this research is also intended to add information about the excellent cultivation of Daun duduk (1).

Material and Methods

This expertiment was conducted from November 2019 to March 2020 at the Screen House, Faculty of Agriculture, Sebelas Maret University. The materials used in this study, the seeds of Daun duduk, were obtained from the Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) Tawangmangu, E. Merck trademark kinetin (98% kinetin) (0 ppm. 10 ppm. 20 pp. 30 ppm), andosol soil, roasted husks and cow manure.

This research used a factorial Completely Randomized Design (CRD), which consisted of two factors. The first factor was the kinetin concentration consisting of 4 levels, namely K0 (0 ppm), K1 (10 ppm), K2 (20 ppm) and K3 (30 ppm). The second factor was the time interval of administration consisting of 3 levels, namely W1 (once every three months), W2 (once every 1.5 months) and W3 (once a month) for three months of observation. The variables observed in this study were plant height, stem diameter, number of leaves, number of branches, number of roots, length of roots, fresh and dry stover.

Results and Discussion

General conditions of the research

The research location was in the Screen House, Faculty of Agriculture, Sebelas Maret University, Surakarta. The geographic location of the research location is 7°33'39.5 "South Latitude and 110° 51'31.4' East Longitude is at an altitude of 95 meters above sea level and has an average temperature of 26.4°C-37.8°C with air humidity 37% -70%.

This research used andosol soil type growing media. Andosol soil came from volcanic materials found in areas around volcanoes and are known as fertile soils compared to other soil types. Andosol soil has the characteristics of a dark brownish color, especially in humus horizons, has high levels of organic matter, looks loose, has crumbly soil structure, and feels slippery when held (6). The pests found at the study sites were grasshoppers (*Atractomorpha crenulata*) and urethens (*Lepidiota stigma*).

Plant height

Height is one of the parameters in plant growth, where the plant cell divides so that the plant will increase in length. Following Hakim et al. (1986) in Haryadi et al. (7), the event of cell division and extension dominated by the tip of the plant shoot will cause an increase in the size of a plant.

Table 1. Effect of kinetin concentration and time interval on the growth of daun duduk

Treatment	Height	Stem diameter	Number of leaves	Number of branches	Dry stover
K0 (control)	36.08[a]	4.04[a]	32.89[a]	9.89[a]	5.30[a]
K1 (10 ppm)	38.58[a,b]	4.06[a]	36.11[a]	12.67[a]	6.62[a]
K2 (20 ppm)	38.50[a,b]	4.27[a,b]	38.67[a,b]	12.67[a]	7.45[a,b]
K3 (30 ppm)	43.29[b]	4.61[b]	44.56[b]	16.44[b]	9.78[b]
W1 (once every three months)	39.27[a]	4.25[a]	38.67[a]	13.17[a]	7.38[a]
W2 (once every 1.5 months)	40.32[a]	4.33[a]	38.75[a]	12.83[a]	8.10[a]
W3 (once a month)	37.73[a]	4.15[a]	36.75[a]	12.75[a]	6.38[a]

[a,b] The numbers followed by the same letter in each treatment show no significant effect (P<5%)

The results of the ANOVA analysis test with a level of 5% indicated that a single treatment of kinetin concentration has a significant effect on the plant height of Daun duduk. In contrast, the treatment interval between kinetin administration and the

interaction between the two treatment factors did not significantly affect plant height parameters. Table 1 shows that the K3 treatment (30 ppm) was the best in this study after the 5% DMRT difference test was carried out; an average of 43.29 was obtained. K3 treatment (30 ppm) was significantly different compared to K0 treatment (control), which was 36.08 on average. This is presumably because the kinetin concentration given was sufficient to stimulate the height growth of Daun duduk. Kinetin can play a role in shoot growth, when shoot growth is good, it will affect the size of the plant. Cytokinins play a positive regulatory role in shoot development and a negative regulatory role in root development. Cytokinins move acropetally through the xylem and systemically through the phloem. Following the research results of Bakar et al. (8), the use of kinetin with a concentration of 3 ppm showed a significant effect on plant height *Dendrobium*. The average plant height at a concentration of 3 ppm was 1.510, then the control treatment of 1.043. Treatment K1 (10 ppm) and K2 (20 ppm) did not show significantly different results compared to treatment K0 (control). This was presumably because the treatment of K1 (10 ppm) and K2 (20 ppm) were still inaccurate so that it did not give any natural effect. Growth regulators can positively influence growth and development, influenced by several factors, one of which is the concentration (9).

Stem diameter

Stem diameter width that is balanced with the height of a plant will make the plant stand firm to support the growth and development of the plant.

The results of the ANOVA analysis test with a level of 5% can be seen that the time interval for giving kinetin and the interaction between the concentration and the time interval application of kinetin did not have a significant effect on the stem diameter of Daun duduk. However, in a single treatment, the kinetin concentration significantly affected the stem diameter of Daun duduk, then continued with the DMRT difference test of 5%. Table 2 showed that the best treatment was found in K3 treatment (30 ppm) with an average of 4.61. The results of K3 treatment (30 ppm) were significantly different compared to K0 treatment (control), with on average of 4.04. This was presumably because the K3 treatment (30 ppm) was considered to have met the right concentration in stimulating cell division so that it can stimulate plant diameter enlargement. Trisna et al. (10) explained that growth regulators quickly diffuse into the plant body and strengthen and enlarge the stems. Following the research results of Fatima and Bano (1998) in Naeem et al. (5), an expansion in rod diameter was seen after kinetin application. Treatment K1 (10 ppm) obtained an average of 4.06, significantly different when compared to treatment K0 (control). Treatment of K2 (20 ppm) obtained an average of 4.27 and did not show significantly different results compared to treatment of K0 (control) and K3 (30 ppm). Growth regulators in the right concentration will work well, but on the other hand, if given in the wrong concentration, it will inhibit the growth of stem diameter. Each type of plant has a different response to the provision of growth regulators in type and concentration (11).

Number of leaves

The number of leaves are counted to determine the vegetative growth of the plant. The number of leaves can be counted when the leaves are fully opened. Leaves are plant organs used to synthesize food.

The results of the ANOVA test analysis at a 5% level in a single treatment, namely the kinetin concentration, gave significant results on the parameter of the number of leaves. This was presumably because the function of cytokinins, among others, is to stimulate the growth of lateral shoots and stimulate morphogenesis. In contrast, the treatment interval of kinetin application and the interaction between the two treatments did not have a significant effect. Sawan et al. (12) explained that the application of exogenous growth regulators in plants could work actively depending on the application speed, application time and the stage of plant development at the time of application. After the DMRT difference test at 5% level was carried out in table 3, the best results were obtained on the leaf number parameter, was the K3 treatment (30 ppm), the average result was 44.56. It was significantly different when compared to the K0 treatment (control) with an average of 32,89. Following Naeem et al. (5)'s research, the application of kinetin increased the number of dark green leaves. This increase may be due to cell division and increased meristem activity promoted by growth hormone. Mukherjee and Kumar (13) added that there were significant increases after kinetin application in the number of leaves, number of branches, and leaf area in

previous studies. During the cell expansion phase, cytokinins help stimulate cell expansion and differentiation. As a result, the addition of cytokinins can produce an increase in leaf and rosette size, which is triggered by a higher rate of cell expansion, which results in higher biomass. Treatment K1 (10 ppm) obtained an average result of 36.11, and treatment K2 (20 ppm) obtained an average result of 38.67. The two treatments were not significantly different when compared to the K0 treatment (control). The indirect effect of kinetin was thought to be due to inaccurate kinetin concentrations. Yunus et al. (14) stated that several factors could determine the success of the application of growth regulators, including the concentration used, the method of administration, the time of administration and the combination of growth regulators used.

Number of branches

Observation of the number of branches in this study is an essential variable because flowers will form on plant branches. The appearance of flowers is significant for propagation, especially in plants that are usually propagated sexually, such as Daun duduk.

The 5% ANOVA analysis test results showed that a single treatment kinetin concentration significantly affects the growth of Daun duduk branches, then the DMRT difference test was carried out at the 5% level. Table 4 showed that the treatment with the highest results, namely the K3 treatment (30 ppm), obtained an average of 16.44, significantly different compared to treatment K0 (control) which obtained an average of 9.89. This was presumably because growth regulators of cytokinins influence the growth of plant branches. Lakit (1996) in Darmanti et al. (15) stated that cytokinin hormones have an essential role in branch formation because the cytokinins found at the root tips will be transported through the xylem to the top of the plant. Cytokinins play a role in promoting shoot branching by activating the axillary buds. Sari and Tatik (16) added that in previous research, treatment of 30 ppm kinetin concentration could increase the number of chrysanthemum mother plant shoots compared to treatment without kinetin administration. Kieber and Eric (17) stated that cytokines' characteristics play a role in cell division and control of meristem activity, then the cells that divide will develop into shoots, branches and leaves. The results of observations on K1 (10 ppm) and K2 (20 ppm) treatments obtained the same average values of 12.67, indicating that it was not significantly different when compared to K0 treatment (control). When compared to K3 treatment (30 ppm), however, it showed significantly different results. The response of plants to growth regulators depends on the type, concentration, plant genotype and phases of plant physiology (1).

Dry stover

Dry stover is a growth parameter that can be used to measure plant growth along with all the events it has experienced. Dry weight is obtained by reducing water content, and the metabolic process has stopped until it reaches a constant weight. Solichatun et al. (18) stated that the size of growth is net weight. About 90% of the dry matter content of plants is the accumulation of material resulting from the photosynthesis process.

The results of the ANOVA test analysis at a 5% level showed that the kinetin concentration treatment has a significant effect on the dry stover parameter of Daun duduk, then continued with 5% DMRT difference test. Table 5 showed that the treatment with the highest yield of dry stover parameters, was the K3 treatment (30 ppm), with an average of 9.78, which was significantly different from the K0 treatment (control) with an average of 5.30. This was because the treatment of 30 ppm kinetin concentration in most of the previous parameters was the treatment with the best growth results and undoubtedly contributed to plant weight. According to Larcher (1975) in Suntoro et al. (19), plant dry weight was obtained from an accumulation of coassimilation$_2$ during plant growth and development. The accumulation of dry matter and increase in wet weight are considered as the growth of a plant. So, the better the plant growth, the more dry weight will increase. Harjadi (1993) and Hopskin (1990) in Sartika and Djoko (20) added that the increase in plant size or dry weight reflects the increase in protoplasm that occurs due to the increased of size and number of cells. Moringa leaf extract and cytokinins were applied to the leaves, and both treatments significantly increased the biomass of tomato plant shoots. K2 treatment (20 ppm) obtained an average of 7.45, where the treatment was not significantly different when compared with K0 treatment (control) and K3 treatment (30 ppm).

Meanwhile, K1 treatment (10 ppm) obtained an average of 6.62, which was not significantly different from K0 (control). This was presumably because the application of growth regulators with inappropriate concentrations will not have a natural effect and can even inhibit the growth and development. The K0 treatment (control) gave the lowest yield on dry stover parameters, presumably because there was no addition of growth regulators to the treatment, so the growth was not as good as the other treatments. Following the statement of Trisna et al. (10), that some growth regulators can function to stimulate plant growth and development under normal conditions, while plants that do not use growth regulators will have a slower growth rate.

Number of roots

Roots are vegetative organs that support plant growth which is very important in obtaining food. According to Irwan (2015) in Putri et al. (9), roots function as a means of absorption or absorption of nutrients from the soil and as a place to store food reserves.

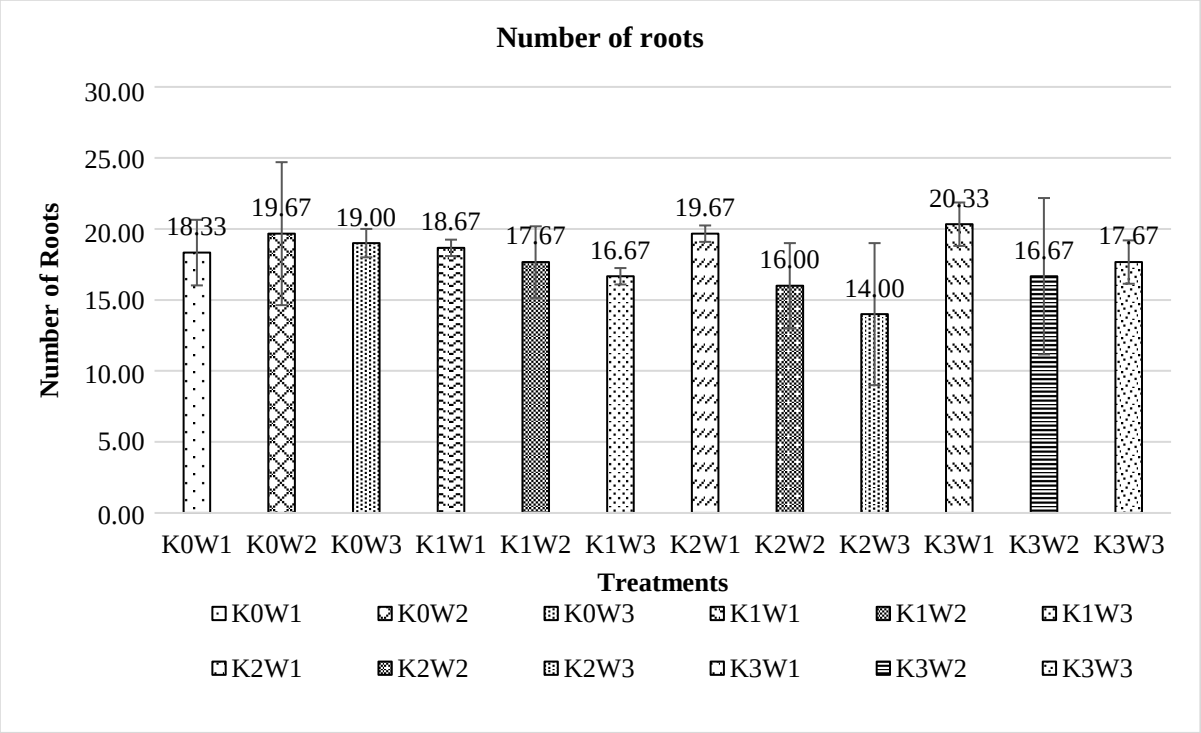

Figure 1. Effect of Concentration and Time Interval of Kinetin on the Daun Duduk Number of Roots. K0 (control, K1 (10 ppm, K2 (20 ppm), K3 (30 ppm), W1 (once every three months), W2 (once every 1.5 months) and W3 (once a month)

The results of the ANOVA analysis at a 5% level showed that all treatments, both kinetin concentration, kinetin administration time interval and the interaction between the two, did not have a significant effect on the growth of the number of sitting leaf roots. The difference in kinetin concentration does not affect the increase in the number of roots. This is presumably because the growth regulators of cytokinins are more focused on stimulating the vegetative growth of the canopy, not on root growth. According to Wybouw and Bert (6), cytokinins harm root initiation. Lateral and auxin accumulation. Fastest callus initiation speed, namely on media with the addition of a growth regulator 2,4-D 1 ppm and kinetin 0.1 ppm, where the kinetin concentration is lower than the 2,4-D concentration. This is because 2,4-D is a type of auxin that can cause root growth, while kinetin can stimulate cell division in plant tissue.

The number of roots was not consistent among all treatments in this study, and this was presumably because the endogenous auxin contained in the plant was sufficient to support root growth. Without the provision of growth regulators, each plant naturally produces growth regulators in its body, such as auxins. Asra et al. (21) stated that auxins could be found in meristematic areas such as stem tips, root tips, flower buds (at the time of flower formation) and seed embryos. Then auxin will be translocated towards the bottom of the stem to stimulate plant

root growth. According to Wattimena (1991) in Indriani et al. (11), root growth only requires auxins without cytokinins or cytokinins in low concentrations. Putriana et al. (22) added that kinetin is a growth regulator for the cytokinin group, which generally has an organogenetic effect on inducing shoot growth so that the response to root growth is not affected.

Roots length

Roots are planted vegetative organs that can grow and develop if growth-supporting factors such as sunlight, water, space to grow and nutrient needs are met. Ai and Patricia (23) explained that long roots would be able to absorb more water.

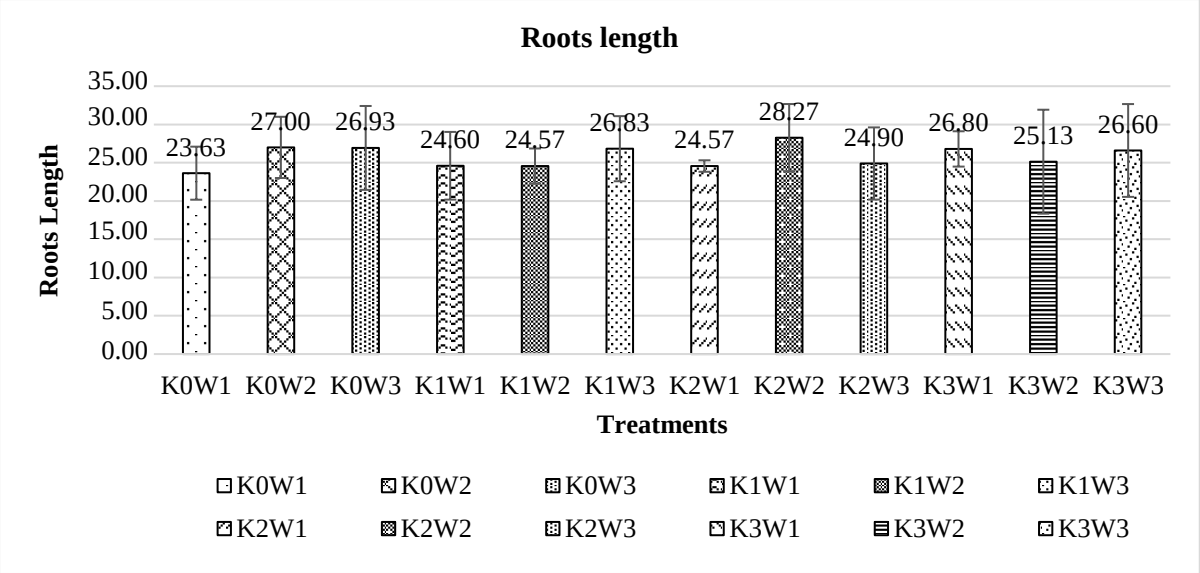

Figure 2. Effect of Concentration and Time Interval of Kinetin on the Daun Duduk Length of Roots. K0 (control, K1 (10 ppm, K2 (20 ppm), K3 (30 ppm), W1 (once every three months), W2 (once every 1.5 months) and W3 (once a month)

The results of the ANOVA analysis at a 5% level showed that all treatments in this study, both kinetin concentration, kinetin administration time interval and the combination of the two, did not have a significant effect on Daun duduk roots length. This is thought to be because, according to Angelina et al. (24), cytokinins are not growth regulators that focus on affecting root growth but affect various physiological processes in plants, especially in promoting cell division. Root length is influenced by the media planting media, such as soil density in the growing medium and limited root space. The higher the soil density and the lower the volume of the planting medium, the longer roots will be less. This is because the plant roots will continue to look for water and nutrients in the soil. Root length growth was almost uniform across all treatments because this study using polybags of the same size. Marzukoh et al. (25), in their research, said that planting tomatoes in polybags will cause the space to grow roots to be more limited, so that root growth and development are also limited. The volume of soil in polybags also causes the volume of available water to be limited, so root growth is not optimal.

Endogenous auxins play a role in root length parameters. Auxins that are synthesized near the shoot meristem will be translocated to the bottom of the stem. Asra et al. (21) explained that when translocated to all parts of the plant, each part gets a different amount of auxin levels. This causes the response to plant growth, such as root length, to vary from plant to plant. Gunawan (1992) in Aprilyani (26) added that the balance between exogenous and endogenous growth regulators can spur growth and development. Suryanto (2005), in Wijayanto and Iftitah (27), stateed that the physiological concept of root growth is based on the morphogenetic balance between roots and plant canopy.

Fresh stover

Fresh stover was obtained by weighing the plants at the end of the observation. According to Sitompul and Guritno (1995) in Sartika and Djoko (20), fresh stover is influenced by water in cells, metabolism, and moisture conditions of a plant.

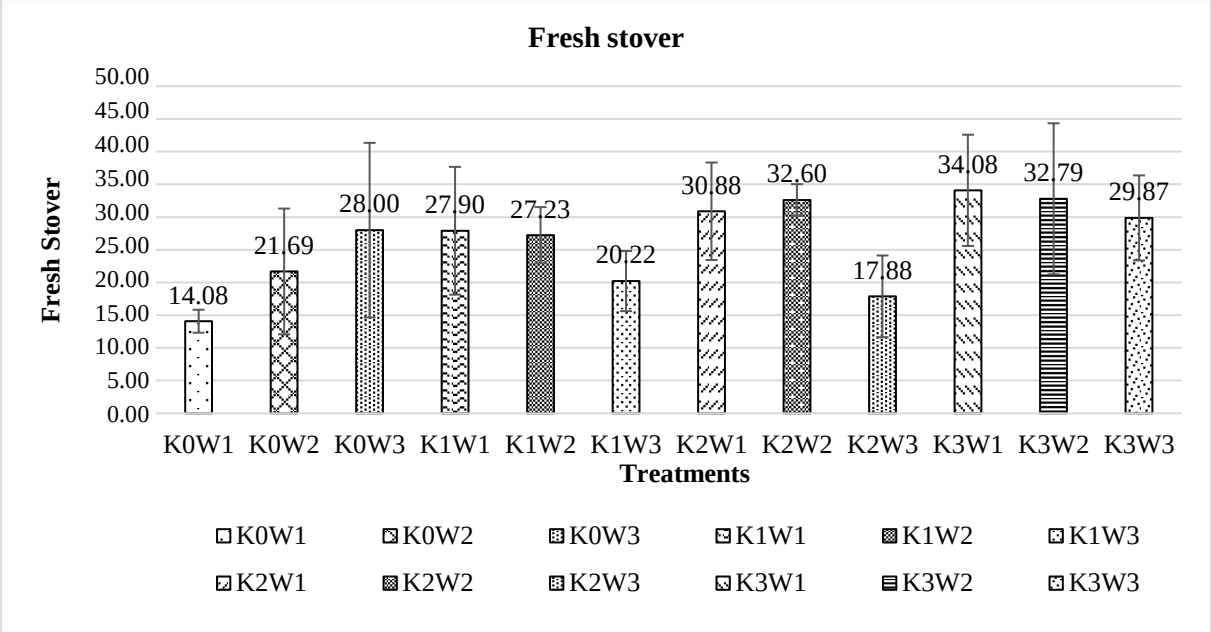

Figure 3. Effect of Concentration and Time Interval of Kinetin on the Daun Duduk Fresh Stover. K0 (control, K1 (10 ppm, K2 (20 ppm), K3 (30 ppm), W1 (once every three months), W2 (once every 1.5 months) and W3 (once a month)

The results of the ANOVA test analysis at a 5% level showed that all treatments did not have a significant effect on the fresh stover of Daun duduk. This was suspected because, according to Sitompul and Guritno (1995) in Sartika and Djoko (20), fresh stover is still influenced by the presence of water in the cells, metabolism, and moisture conditions of a plant. Fresh stover accumulates photosynthate weight in the form of biomass and water content in plants, especially in the leaves. Biomass is an accumulation of photosynthate products in the form of carbohydrates, proteins and lipids. Metabolic processes in plants will run well if the plants have a heavier weight. Likewise, if the biomass of a plant is low, it indicates an obstacle in the plant's metabolic process. Yunus et al. (14) said that growth regulators used in the right concentration could increase yield, whereas they can inhibit growth, poison, and even kill the plant at high concentrations.

Conclusion

1. The concentration of 30 ppm kinetin alone can increase the growth of Daun duduk on plant height parameters obtained by an average of 43.29, and stem diameter obtained an average of 4.61. The number of leaves obtained an average of 44.56, the number of branches obtained an average 16.44 and dried stover obtained an average of 9.78. In contrast, the time interval of offering kinetin alone did not stimulate the growth of sitting leaves in all experimental parameters.
2. The most effective kinetin concentration in increasing the growth of Daun duduk was 30 ppm, while the time interval for offering kinetin has not got the right time.
3. The interaction between the concentration and the time interval of kinetin administration did not affect all the experimental parameters.

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Conflict of Interest

All authors declare no conflicts of interest in this section.

References

1. Ismail I. Faktor Yang Mempengaruhi Keputusan Masyarakat Memilih Obat Tradisional Di Gampong Lam Ujong. Idea Nurs J. 2015;6(1):7–14.
2. Badan Pusat Statistik. Ekspor tanaman obat, aromatik, dan rempah-rempah menurut negara tujuan utama 2012-2019. Badan Pusat Statistik. Jakarta; 2019.
3. Salim Z, Ernawati M. Info komoditi

tanaman obat. Jakarta; 2017.
4. Mirza M, Amanah S, Sadono D. Tingkat kedinamisan kelompok wanita tani dalam mendukung keberlanjutan usaha tanaman obat keluarga di Kabupaten Bogor, Jawa Barat. J Penyul. 2017;13(2):181–93.
5. Naeem M, Bhatti I, Ahmad RH, Ashraf MY. Effect of some growth hormones (GA3, IAA and kinetin) on the morphology and early or delayed initiation of bud of lentil (Lens culinaris medik). Pakistan J Bot. 2004;36(4):801–9.
6. Wybouw B, De Rybel B. Cytokinin – A developing story. Trends Plant Sci. 2019;24(2):177–85.
7. Haryadi D, Yetti H, Yoseva S. Pengaruh pemberian beberapa jenis pupuk terhadap pertumbuhan dan produksi tanaman kailan (*Brassica alboglabra* L.). J Online Mhs Fak Pertan Univ Riau. 2015;2(2):1–10.
8. Bakar M, Jeany M, Deanne K, Sofia D. Penggunaan bap dan kinetin pada induksi tunas dari protocorm anggrek dendrobium (*Dendrobium* sp.) pada kultur in vitro. Cocos. 2016;7(4).
9. Putri BF, Fakhrurrozi Y, Rahayu S. Pengaruh perbedaan jenis media tanam terhadap pertumbuhan setek hoya coronaria berbunga kuning dari kawasan hutan kerangas air anyir, Bangka. Ekotonia J Penelit Biol Bot Zool dan Mikrobiol. 2018;3(1):20–8.
10. Trisna N, Umar H, Irmasari. Pengaruh berbagai jenis zat pengatur tumbuh terhadap pertumbuhan stump jati (*Tectona grandis* L.F). War Rimba. 2013;1(1):1–6.
11. Indriani M, Manuhara S, Utami E. Pengaruh zat pengatur tumbuh 2,4-D, kinetin dan bap terhadap pertumbuhan dan perkembangan ekstrak daun sambung nyawa (*Gynura procumbens* Merr.). Surabaya: Universitas Airlangga; 2016.
12. Sawan Z, Mohamed A, Sakr R, Tarrad A. Effect of kinetin concentration and methods of application on seed germination, yield components, yield and fiber properties of the Egyptian cotton (*Gossypium barbadense*). Environ Exp Bot. 2000;44(1):59–68.
13. Mukherjee D, Kumar R. Kinetin regulates plant growth and biochemical changes during maturation and senescence of leaves, flowers, and pods of Cajanus cajan L. Biol Plant. 2007;51(1):80–5.
14. Yunus A, Muji R, Samanhudi, Pujiasmanto B, Himawan J. Respon kunir putih (*Kaempferia rotunda*) terhadap pemberian iba dan bap pada kultur in vitro. Agrosains. 2016;18(2):44–9.
15. Darmanti S, Setiari N, Romawati TD. Perlakuan defoliasi untuk meningkatkan pembentukan dan pertumbuhan cabang lateral jarak pagar (*Jatropha curcas*). Anat Fisiol. 2008;16(2):13–9.
16. Sari A, Wardiyati T. Pengaruh pemberian kinetin dan pgpr (plant growth promoting rhizobacteria) terhadap pertumbuhan mother plant krisan (Chrysanthemum sp.). J Produksi Tanam. 2018;6(3):488–94.
17. Kieber JJ, Eric Schaller G. The perception of cytokinin: A Story 50 years in the making. Plant Physiol. 2010;154(2):487–92.
18. Solichatun E, Mudyantini W. Pengaruh ketersediaan air terhadap pertumbuhan dan kandungan bahan aktif saponin tanaman ginseng jawa (*Talinum paniculatum* Gaertn.). Biofarmasi. 2005;3(2):47–51.
19. Suntoro, Syamsiyah J, Rahina W. Ketersediaan dan serapan Ca pada kacang tanah di tanah alfisols yang diberi abu vulkanik kelud dan pupuk kandang. Agrosains J Penelit Agron. 2017;19(2):51–7.
20. Sartika D, Djoko S. Pengaruh kombinasi zat pengatur tumbuh (2,4 D dan kinetin) terhadap pertumbuhan dan kandungan metabolit sekunder pada kalus *Phaleria macrocarpa* (Scheff.) Boerl.). J Tumbuh Obat Indones. 2012;5(1):53–62.
21. Asra R, Ririn A, Mariana S. Hormon tumbuhan. Jakarta: UKI Press; 2020.
22. Putriana, Gusmiaty, Restu M, Musriati, Aida N. Respon kinetin dan tipe eksplan jabon merah (*Antocephalus macrophyllus* (Roxb.) Havil) secara in vitro. J Biol Makassar. 2019;4(1):48–57.
23. Ai N, Torey P. Karakter morfologi akar sebagai indikator kekurangan air pada tanaman (Root morphological characters as water-deficit indicators in plants). J Bios Logos. 2013;3(1):31–9.
24. Angelina N, Luthfi A, Lollie A. Pengaruh zat pengatur tumbuh terhadap induksi akar (*Rhizogenesis*) pada tanaman bangun-bangun (*Plectranthus amboinicus*

(Lour.) Spreng) secara in vitro. J Agroekoteknologi FP USU. 2017;82:644–9.
25. Marzukoh RU, Sakya AT, Rahayu M. Pengaruh volume pemberian air terhadap pertumbuhan tiga varietas tomat (*Lycopersicum esculentum* Mill). J Agrosains. 2013;15(1):12–6.
26. Apriliyani N, Mukarlina, Rizalinda. Pertumbuhan stek batang sirih merah (*Piper crocatum* Ruiz.) setelah perendaman dengan ekstrak bawang merah (*Allium cepa* L.) dan air kelapa (Cocos nucifera L.). J Protobiont. 2018;7(3):54–61.
27. Wijayanto N, Rhahmi I. Panjang dan kedalaman akar lateral jabon (*Anthocephalus cadamba* (Roxb.) Miq.) di Desa Cibening, Kecamatan Pamijahan, Kabupaten Bogor, Jawa Barat. J Silvikultur Trop. 2013;4(1):23–9.

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Relationship of Personal Hygiene and Nutritional Status to Intestinal Parasitic Infection in Simo, Boyolali

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Abstract

Intestinal parasitic infections are caused by protozoa, STH and non-STH worms. Immunity, which can be determined by measuring nutritional status, and personal hygiene can affect the occurrence of intestinal parasite infections. This study aims to determine the relationship between personal hygiene and nutritional status against intestinal parasite infections in elementary school students in Simo Boyolali. This type of analytic observational research with a cross sectional approach. Total sampling was taken at elementary school of Wates, Talakbroto 1, and Kedunglengkong 1 Simo, Boyolali. There were 11 students with worms infections, 16 with protozoa infections, and 4 with worms and protozoa infection. There was a relationship between washing hands before eating (p = 0.004), after eating (0.027), after defecating (p = 0.04), biting nails (p = 0.008), wearing footwear when leaving the house (p = 0.008) , removing shoes while playing during school breaks (p = 0.001), and nutritional status (p = 0.002) with intestinal parasite infection. There was no relationship between nail clipping once a week (p = 0.118) and the availability of a latrine (p = 0.416) with intestinal parasitic infections. So, there is a relationship between personal hygiene (washing hands before eating, after eating, after defecating, biting nails, wearing footwear when leaving the house, removing shoes when playing during school breaks, and nutritional status) and intestinal parasitic infections.

Keywords: elementary school; protozoa; worms

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Introduction

Intestinal parasitic infections mostly occur in areas with tropical and subtropical climates, especially in developing countries (1). Indonesia is one of the endemic countries for intestinal parasitic infections, mainly caused by protozoa (most commonly *Entamoeba histolytica, Giardia lamblia,* and *Blastocystis hominis*), worms that are classified as Soil Transmitted Helminths (STH) (*Ascaris lumbricoides, Trichuris trichiura, Necator americanus, Ancylostoma duodenale and Strongyloodenale. stercoralis*) and non-STH worms, namely *Enterobius vermicularis and Taenia sp.* (2,3). One of the factors that influence the occurrence of intestinal parasitic infection is the individual factor consisting of immunity and personal hygiene. Immunity is influenced by the intake of nutrients that enter the body where the intake of these nutrients can be determined by measuring nutritional status. Decreased nutritional status indicates decreased immunity so that the body is susceptible to intestinal parasitic infections. Personal hygiene includes washing hands, nail hygiene, defecating, and wearing footwear. Intestinal parasite transmission through faecal oral and intestinal parasite contamination in food and water, so that personal hygiene is the most priority in preventing the occurrence of this intestinal parasitic infection (4, 5, 6, 7).

As much as one third of the world's population has intestinal parasitic infections (2). The highest prevalence is in 270 million pre-school children and 600 million school-age children (7). This infection in children occurs because the child does not maintain personal hygiene and is less aware of clean and healthy

living, and in children who have poor nutritional status can also affect the occurrence of this intestinal parasitic infection (1, 4). The prevalence of intestinal parasitic infection in Boyolali, Central Java, cannot be found equally, but it has been found in several locations in Boyolali, where 35 children (47.3%) had STH infection in Teras District, Boyolali Regency (8). In addition, to determine the possibility of intestinal parasitic infection, it can be seen from the proportion of risk factors for intestinal parasitic infection, namely personal hygiene, including washing hands properly (56.75%) and defecating behavior (89.42%) at ages 5-9. years and 89.36% at the age of 10-14 years can indicate that the behavior of washing hands and defecating is not correct, so that intestinal parasitic infections can occur (9, 10). Geographically, the soil structure of Boyolali Regency, especially in the northeast area around the sub-district Karanggede and Simo consist of clay / clay so that the possibility of intestinal parasitic infection can still occur. The nutritional status of children aged 5-12 years in Boyolali Regency is 31.43%, not included in the normal group where the nutritional status is not normal can cause the body to be susceptible to intestinal parasitic infections so that it can strengthen the possibility of developing intestinal parasitic infections (9). The purpose of this study was to determine the relationship of personal hygiene to intestinal parasitic infection of elementary school students in Simo, Boyolali and to determine the relationship of nutritional status to intestinal parasitic infection of elementary school students in Simo, Boyolali.

Material and Methods

This type of research is analytic observational with a cross sectional approach. This research was conducted in 2 stages, namely data collection was carried out at elementary school of Wates, Talakbroto 1, and Kedunglengkong 1 which are located in Simo District, Boyolali Regency, Central Java from February to August 2020 by providing a sheet of willingness to be the subject of research to student guardians and Guardians who agree were given a personal hygiene questionnaire to fill out, then the BMI of the students were measured and stool samples taken. The second stage was sample examination which was carried out at the Parasitology Laboratory of the Faculty of Medicine, Sebelas Maret University, Surakarta City, Central Java from February to August 2020 using the Kato Katz method and Trikrom staining.

The population of this study were 68 students of Wates elementary school, 53 of Talakbroto 1 elementary school, and 74 of Kedunglengkong 1 elementary school, so that the total population of the three elementary schools was 195 people. The sample of this study used a non-probability sample, namely total sampling technique or saturated sampling technique, in which all members of the population become the research sample (11). The data obtained from this study were analyzed statistically using SPSS, by performing univariate analysis, bivariate analysis using chi-square test, and multivariate analysis using logistic regression test.

Results and Discussion

Description of research location

Simo District is one of the sub-districts in Boyolali Regency and is located in the middle of the western part and directly adjacent to Semarang Regency. The area of Simo District is 4,804,0275 Ha (more than 44% of rice fields) and a population density of 1,026 people per km^2. Simo sub-district consists of 13 villages, namely Pelem, Bendungan, Temon, Teter, Simo, Walen, Pentur, Gunung, Talakbroto, Kedunglengkong, Blagung, Sumber, and Wates (12). This research was conducted in 3 villages, namely Talakbroto village at Talakbroto 1 elementary school, Kedunglengkong village at Kedunglengkong 1 elementary school, and Wates village at Wates elementary school. Simo sub-district is located at an altitude of 150,325 m above sea level with a moderate climate, which is around 27 ° C. The rainfall in Simo District reaches 1,938 mm. Soil structure in Simo sub-district is clay soil with the association soil of dark gray grumosol and dark brown mediterranean soil (13).

Subject characteristics

The age characteristics of the respondents in table 1 showed that the average age of the respondents was 9.7 years, with the youngest age being 6 years old as many as 4 students and the oldest age being 13 years as many as 2 students. Age 11 years was the age of the most respondents in this study, as many as 30 students. The gender characteristics of the respondents in table 2 indicate that there were

more female respondents than male respondents.

Table 1. Subject characteristics by age

Mean	Mod	SD	Min	Max
9.7	11	1.652	6	13

Table 2. Subject characteristics by gender

Gender	Frequency (n)	(%)
Male	59	44.7
Female	73	55.3
Total	140	100

Intestinal parasitic infection

Table 3. Frequency distribution of intestinal parasitic infection

Intestinal Parasitic Infection	Frequency (people)	(%)
Positive	31	23.5
Worms		
A. lumbricoides	4	3
Hookworm	1	0.8
T. trichiura	5	3.8
A. lumbricoides and *T. trichiura*	1	0.8
Protozoa		
B. hominis	13	9.8
E. coli	1	0.8
E. histolytica	1	0.8
B. hominis and *E. coli*	1	0.8
Worms dan Protozoa		
B. hominis and *A. lumbricoides*	4	3
Negative	101	76.5
Total	132	100

Table 3 above showeds that there were 31 respondents experiencing intestinal parasitic infections, so no more than 50% of the total respondents experienced intestinal parasitic infections. Of the 31 respondents, there were 15 people who only experienced infection of one protozoan species, this shows that protozoa infection was the most common infection in this study. Respondents were 1 person infected with two species of protozoa, namely Blastocystis hominis and Entamoeba coli. There were 10 respondents who were infected with one species of worm. Respondents with infection of two species of worms namely Ascaris lumbricoides and Trichuris trichiura were 1 person. Respondents with concurrent worm and protozoa infections, namely Blastocystis hominis and Ascaris lumbricoides infection were 4 people. Blastocystis hominis infection occurs most frequently in this study because Blastocystis hominis infection occurs because there is an association with poor personal hygiene, exposure to animals, consumption of air or food contaminated with this parasite, and in immunocompromised people (14). The age of children can also be one of the reasons for this infection because the age of children with personal hygiene and toilet training is still low and children with low nutritional status can cause low body immunity so that the body is susceptible to infection with these parasites. STH worm infections including Ascaris lumbricoides, Trichuris trichiura, and Hookworm are only a few infected, this can be due to personal hygiene, especially the use of good footwear so that there are fewer infections.

Analysis of the relationship between the habit of washing hands and parasitic intestinal infection

The relationship between the habit of washing hands before eating, after eating, and after defecating with intestinal parasitic infections in this study showed a relationship between the two, this is in line with other studies which state that there is a relationship between the habit of washing hands and the incidence of asymptomatic giardiasis (15). Another study also states that there is a relationship between the habit of washing hands before eating and after defecating with the incidence of worms (16). The results of this study also showed that students who had done the habit of washing their hands before eating, after eating, and after defecating were still infected with intestinal parasites due to improper or incorrect hand washing, personal hygiene apart from bad hand washing, not washing hands before eating snacks, food hygiene, food processing, and poor food equipment, unavailability of hand washing facilities or inadequate facilities such as unavailability of soap or clean running water, lack of knowledge about the importance of washing hands in preventing disease transmission , and other parties such as health workers, families, and teachers who are lacking in doing basic health promotion such as hand

washing habits (16,17). Students who do not have the habit of washing their hands both before and after eating and after defecating but not infected with intestinal parasites can occur because personal hygiene besides washing hands is good and is supported by a good environment.

Table 4. Analysis of the relationship between the habit of washing hands and parasitic intestinal infection

		Intestinal Parasitic Infection		
		Positive	Negative	P-value
before eating	Yes	24	97	0/004
	No	7	4	
after eating	Yes	27	99	0/027
	No	4	2	
after defecating	Yes	28	100	0.04
	No	3	1	

Note: *Fisher test

Analysis of the relationship between nail hygiene and Intestinal parasitic infection

The results of this study indicate that there was no relationship between the habit of cutting nails once a week with intestinal parasitic infections, but there was an association between nail biting habits and intestinal parasitic infections. Another study which is in line with the study stated that there was no association between nail hygiene and STH infection (18). The absence of this relationship was due to the many factors, both personal hygiene and the student's environment that affect intestinal parasitic infections. Although though one did not cut their nails once a week, one still conducted other personal hygiene procedure such as washing hands to prevent the occurrence of intestinal parasitic infections. Different nail growth between individuals can cause differences in the frequency of cutting nails in that individual. As many as 9 students in this study were not infected even though they did not have the habit of cutting nails once a week where the number was more than students who were negative for intestinal parasitic infections even though they had practiced the habit of cutting nails once a week. This could also indicate that there was no relationship between the these two variables. Students who did not bite their nails but are still infected can be caused by other poor personal hygiene, as well as in children whom were not infected, although nail biting can be caused by other factors that cause intestinal parasitic infection.

Table 5. Analysis of the relationship between nail hygiene and intestinal parasitic infection

		Intestinal Parasitic Infection		
		Positive	Negative	P-value
The habit of cutting nails once a week	Yes	25	92	0.118
	No	6	9	
Nail biting habit	Yes	8	7	0.008
	No	23	94	

Note: *Fisher test

Analysis of the relationship between Footwear and intestinal parasitic infections

Table 6. Analysis of the Relationship between Footwear and Intestinal Parasitic Infection

		Intestinal Parasitic Infection		
		Positive	Negative	P-value
The habit of wearing footwear when leaving the house*	Yes	23	94	0.008
	No	8	7	
Habit of Taking Off Shoes While Playing School Break**	Yes	18	26	0.001
	No	13	75	

Note: *Fisher test; *Chi-Square test

The results of this study indicated that there was a relationship between the habit of wearing footwear when leaving the house and the habit of removing shoes when playing at school breaks with intestinal parasitic infections. This is in accordance with other studies which stated that there is a relationship between the habit of wearing footwear and worm infections (17); as well as other studies which stated that not using good footwear has 14 times greater risk of experiencing worm infections than using good footwear (19). Students whom were not infected even though they did not use footwear can occur because they practice good personal hygiene procedures. Whereas children whom were infected even though they already have the habit of wearing footwear can be caused by other factors that cause intestinal parasitic infection.

Analysis of the relationship between latrine availability at home and parasitic intestinal infection

The results of this study indicated that there was no relationship between the

availability of latrines at home with intestinal parasitic infections. This is in line with other studies which stated that there is no relationship between the availability of latrines for intestinal parasite infections (20). The absence of this relationship might be due to the various factors regarding latrine availability described above which were not examined in this study. Students who already have a latrine but were still infected with intestinal parasites can be caused by the latrine that was not in accordance with healthy latrine standards. Requirements for a healthy latrine are as follows: not polluting water sources (minimum distance from water sources 10-15 meters), odorless, not polluting the surrounding environment, water and soap available, cleaning tools available, watertight floors and adequate space, adequate lighting and ventilation, available, protective walls and roofs, untouched dirt, easy to clean and safe (21).

Table 7. Analysis of the relationship between latrine availability at home and parasitic intestinal infection

		Intestinal Parasitic Infection		
		Positive	Negative	P-value
Latrines at home	Yes	30	100	0.416
	No	1	1	

Note: *Fisher test

Analysis of the relationship between nutritional status and parasitic intestinal infection

Table 8. Analysis of the Relationship Between Nutritional Status and Parasitic Intestinal Infection

		Intestinal Parasitic Infection		
		Positive	Negative	P-value
Nutritional status	Normal	20	91	0.00
	Below Normal	11	10	2

Note: *Fisher test

Nutritional status in this study became an independent variable against intestinal parasitic infection, so that nutritional status was one of the causes of intestinal parasitic infection. The results of this study indicated that there was a relationship between nutritional status and intestinal parasitic infection. This indicated that a BMI below normal, which is below the SD-2 line affected the occurrence of intestinal parasitic infections, which means that poor nutritional status can decrease immunity in response to parasitic infections. Another study stated that poor nutritional status will lead to a weak immune system so that the body will be susceptible to infection, one of which is intestinal parasitic infection (22). Students who have normal nutrition but were infected with intestinal parasites can occur due to various factors that affect intestinal parasitic infections, one of which is personal hygiene. Hence, if personal hygiene is not good but the nutritional status is good, intestinal parasitic infections can occur.

Personal hygiene, nutritional status, and intestinal parasitic infection

Table 9. Hosmer and Lemeshow Test

Chi-square	df	P-value
2,929	5	0.711

Note: *Regression Logistic test

Table 9 shows that the p-value was 0.711, where the value was greater than the significance level of 0.05 so that the logistic model was appropriate, this means that this logistic model can predict the occurrence of intestinal parasitic infections based on personal hygiene and nutritional status.

Table 10. Omnibus Test of Model Coefficients

	Chi- square	Df	P-value
Step 1 Step	45,801	9	0.000
Block	45,801	9	0.000
Model	45,801	9	0.000

Note: *Regression Logistic test

Table 10 above shows that the p-value was 0.000, which was less than the significance value of 0.005, so it was found that there was at least one independent variable in this study, namely each question points on personal hygiene and nutritional status of respondents, that affect the dependent variable of this study, namely intestinal parasitic infection.

Table 11. Model summary

-2Log likelihood	*Cox & Snell R Square*	*Nagelkerke R Square*
98.097	0.293	0.442

Note: *Regression Logistic test

Table 11 showed the Nagelkerke R Square value of 0.442, this means that the independent variables in this study were able to

explain 44.2% of the dependent variable and the remaining 55.8% were explained by other variables outside of this study.

Table 12. Variable in the Equation

Variable	Exp(B)
The habit of washing hands before eating	8.675
The habit of washing hands after eating	2.077
The habit of washing hands after defecating	5.119
The habit of cutting nails once a week	3.385
Nail biting habit	0.275
The habit of wearing footwear when leaving the house	6.508
The habit of removing shoes when playing during school breaks	0.170
Availability of latrines at home	0.002
Nutritional status	0.138

Note: *Regression Logistic test

Table 12showed the value of exp (B), which is the odd ratio value, so that the following interpretation is obtained.

a. People with the habit of not washing their hands before eating are at 8 675 times the risk of developing intestinal parasitic infections.
b. People who do not wash their hands after eating have a 2 077 times risk of developing intestinal parasitic infections.
c. People with a habit of not washing their hands after having a bowel movement have a 5 119 risks of experiencing intestinal parasitic infections.
d. People who do not cut their nails once a week are at 3 385 times the risk of developing intestinal parasitic infections.
e. People with a habit of biting their nails are 0.275 times more likely to develop intestinal parasitic infections.
f. People with the habit of not wearing footwear when leaving the house are 6.508 times more likely to experience intestinal parasitic infections.
g. People with the habit of removing their shoes while playing during school breaks are 0.17 times more likely to develop intestinal parasitic infections.
h. Unavailability of toilets at home has 0.002 times the risk of developing intestinal parasitic infections.
i. People with below normal nutritional status have 0.138 times the risk of experiencing intestinal parasitic infections.

Multivariate analysis in this study showed that the logistic model of this study can predict the occurrence of intestinal parasitic infections according to personal hygiene and nutritional status. There was a relationship between the habit of washing hands before eating, after eating, after defecating, the habit of biting nails, the habit of using footwear when leaving the house, the habit of removing shoes while playing during school breaks, and nutritional status with intestinal parasitic infections. In addition, it was also found that 44.2% of the independent variables in this study were able to explain the dependent variable, namely intestinal parasitic infection. The value of each odd ratio of personal hygiene and nutritional status with intestinal parasitic infection can assess the risk of each independent variable on the dependent variable. The indicated odds ratio showed that latrine unavailability had the greatest risk of experiencing intestinal parasitic infection compared to other independent variables. Although there was no relationship between the two but it can be seen from the discussion that the absence of a latrine can cause intestinal parasitic infection due to the transmission of intestinal parasites through human feces more often occurs.

Conclusion

This study showed that there was a relationship between personal hygiene which includes the habit of washing hands before eating, after eating, after defecating, biting nails, wearing footwear when leaving the house, and removing shoes when playing during school breaks with intestinal parasitic infections. Other personal hygiene practices, namely cutting the nails once a week and the availability of latrines were not associated with intestinal parasitic infections. Nutritional status was associated with intestinal parasitic infections.

Suggestions for future research are to conduct further research related to factors outside of this study that affect intestinal parasitic infections and to use research instruments as accurate and detailed as possible.

Suggestions for the public are to be able to provide knowledge and awareness about the importance of personal hygiene and nutrition to

determine nutritional status as a defense for the body's immunity in preventing intestinal parasitic infections.

Suggestions for health service agencies are to routinely check intestinal parasitic infections to prevent transmission of intestinal parasitic infections. Meanwhile, the government should always improve adequate health facilities to minimize the transmission of intestinal parasitic infections.

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Conflict of Interest

The limitation of this study is that this study does not perform quantitative fecal microscopic examination so that it cannot determine the severity of infection in respondents, variables outside this study can affect the results of the study because these variables are not tested and are not controlled, and the personal hygiene questionnaire is filled in. by the parents of the students so that there may be bias or not really in accordance with the condition of the respondent.

References

1. Rosyidah, H. N. and Prasetyo, H. Prevalence of intestinal helminthiasis in children at North Keputran Surabaya at 2017. Journal of Vocational Health Studies, 2018;01:117–120.
2. Amer, O. H., Ashankyty, I. M., and Haouas, N. A. S.. Prevalance of intestinal parasite infections among patients in local public hospitals of hail, Northwestern Saudi Arabia. Asian Pacific Journal of Tropical Medicine. Elsevier (Singapore) Pte Ltd, 2015;1–5. doi: 10.1016/j.apjtm.2015.12.009.
3. Lee, J. and Ryu, J. Current Status of Parasite infections in Indonesia : A literature Review. Korean J Parasitol, 2019;57(4), pp. 329–339.
4. Fransisca, R. O., Iriani, A. D., Mutiksa, F. A., Izati, S., dan Utami, R. K.. Hubungan infeksi parasit usus dengan pengetahuan perilaku hidup bersih sehat pada anak SD Bekasi, 2012; 2–6.
5. Sungkar, S., Pohan, A. P. N., Ramadani, A., Albar, N., Azizah., Nugraha, A. R. A., and Wiria, A. E.. Heavy burden of intestinal parasite infections in Kalena Rongo village, a rural area in South West Sumba, eastern part of Indonesia : a cross sectional study. BMC Public Health. BMC Public Health, 2015; 1–6. doi: 10.1186/s12889-015-2619-z.
6. Saki, J., Khademvatan, S., Rad, M. F., and Gharibzadeh, M.. Prevalence of intestinal parasitic infections in Haftkel County, Southwest of Iran. Int J Infect, 2017;4(4):0–5.
7. Sitotaw, B., Mekuriaw, H., and Damtie, D. Prevalence of intestinal parasitic infections and associated risk factors among Jawi primary school children, Jawi town, north. BMC Infectious Diseases, 2019; 1–10.
8. Nadhiasari, A., Sakiman, B. S., dan Dirgahayu, P.. Hubungan antara infeksi Soil Transmitted Helminths (STH) dengan kadar eosinofil darah tepi pada siswa SD Barengan di Kecamatan Teras, Boyolali [Skripsi] Universitas Sebelas Maret : Digilib UNS; 2014.
9. Dinas Kesehatan Kabupaten Boyolali. Profil kesehatan Kabupaten Boyolali tahun 2018. Boyolali: Dinas Kesehatan Boyolali; 2018.
10. Riskesdas. Laporan provinsi Jawa Tengah riskesdas 2018. Jakarta; 2018b
11. Masturoh, I. dan Anggita T, N. Metodologi penelitian kesehatan. Jakarta: Kementerian Kesehatan RI; 2018.
12. BPS Kabupaten Boyolali, Kecamatan Simo dalam Angka 2019. Boyolali: Badan Pusat Statistik Kabupaten Boyolali, 2019b.
13. BPS Kabupaten Boyolali, Kabupaten Boyolali dalam Angka 2019. Boyolali: Badan Pusat Statistik Kabupaten Boyolali, 2019a.
14. Pramestuti, Nova dan Dewi Saroh. *Blastocystis hominis*: protozoa usus potensial penyebab diare. SEL Jurnal Penelitian Kesehatan, 2017;4(1):1-12.

15. Artika, Mentari, Nurhayati, dan Yustini Alioes. Hubungan kebiasaan mencuci tangan dan memotong kuku dengan kejadian giardiasis asimtomatik. Jurnal Kesehatan Andalas, 2017;6(1):70-75.
16. Erna, Aida dan Mukono J. Hubungan karakteristik santri dan kebiasaan mencuci tangan dengan kejadian kecacingan di Pondok Pesantren Kabupaten Blitar. Jurnal Kesehatan Lingkungan, 2015;8(1):14-24.
17. Waqiah, U. Hubungan hygiene perorangan dengan kejadian infeksi kecacingan pada Pemulung Anak Usia Sekolah Dasar di TPA Antang Makassar [Skripsi] Makassar: Universitas Islam Negeri Alauddin, 2010.
18. Dewi, Ratna, N L G D dan Laksmi, D A A S. Hubungan perilaku higienitas diri dan sanitasi sekolah dengan infeksi soil transmitted helminths pada siswa kelas III-VI Sekolah Dasar Negeri No. 5 Delod Peken Tabanan Tahun 2014. E-Jurnal Medika, 2017;6(5):1-4.
19. Pradinata, Bayu, K T, Sudarmaja, I M dan Ariwati, N L. Perilaku siswa SDN 4 antiga kelod karangasem terhdap infeksi soil transmitted helminth. Intisari Sains Medis, 2019;10(3):811-816.
20. Hardiyanti, L. T. dan Umniyati, S. R. Kualitas air, perilaku dan lingkungan pada infeksi parasit usus anak sekolah dasar di tepi sungai Batanghari. Berita Kedokteran Masyarakat, 2017;33.
21. Sari, Rita Kartika, Ratnawati, dan Livana PH. Gambaran faktor-faktor yang berpengaruh terhadap cakupan kepemilikan jamban sehat. Jurnal Ilmiah Permas: Jurnal Ilmiah STIKES Kendal, 2018;8(1):56-62.
22. Siddiq, M. Nuzul Azhim Ash. Penyakit infeksi dan pola makan dengan kejadian status gizi kurang berdasarkan BB/U pada balita usia 6-24 bulan di wilayah kerja puskesmas tanah sepenggal [Skripsi] Jambi : Universitas Adiwangsa, 2014.

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Acknowledgments

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Proceeding:
3. Cronje PB. Gut health, osmoregulation and resilience to heat stress in poultry. In: 19th Australian Poultry Science Symposium. Sydney: The Poultry Research Foundation, University of Sydney & World's Poultry Science Association, Australian Branch; 2007. p. 9–13.
4. Prastowo S, Widyas N, Ratriyanto A. Target gene prediction and pathway analysis of miRNA targeted AMPK involved in lipid accumulation of bovine granulosa cell luteinization. IOP Conf Ser Earth Environ Sci. 2019;387(1).

Book and Book Chapter:
5. Ott RL, Longnecker M. Introduction to statistical methods and data analysis. 5th ed. USA: Duxbury; 2001.
6. Patel VB, Mehta K. Betaine in context. In: Preedy VR, editor. Betaine: Chemistry, analysis, function and effects. Cambridge: Royal Society of Chemistry; 2015. p. 3–8.

Thesis/Dissertation:
7. Kpodo KR. Dietary Supplementation of choline and betaine in heat stressed broilers. The University of Tennese, Knoxville; 2015.

Online content:
8. R Core Team. R: A Languange and environment for statistical computing [Internet]. Vienna, Austria; 2019. Available from: https://www.r-project.org/

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