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Effect of Aqueous and Ethanol Extract of *Acacia nilotica* L. Leaves on Seed Germination of V*igna radiata* L.

Nailul Rahmi Aulya[1a)], Supriyatin[2], Eka Putri Hartanti[3], Wiwit Asma Qothrun Nada[4], Luthfiah Achmad [5], Achmad Syahputa[6]
[1,2,3,4,5,6]Biology of Education, Universitas Negeri Jakarta, Jl. Rawamangun Muka, Jakarta Timur, Jakarta
e-mail: [a)]rahmiaulya@unj.ac.id

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ABSTRACT

This research aimed to tested the effect of extract on *Vigna radiata* L. germination. Tested the effect of crude extract of *A. nilotica* leaves on germination was conducted experimentally using Factorial Completely Randomized Design (FCRD). The first fact or was the type of crude extract and the second factor was levels of extract concentration. The result showed that the application of aqueous and ethanol extract enhanced all parameters of V. radiata L. germination (percentage of seed germination, rate of seed germination, radicle length, seedling fresh weight). Application of lower concentration (0,25%) of aqueous and ethanol extract increased speed of germination, radicle length, and fresh weight of seed. The application of 75% aqueous extract increased the percentage of germination to 100% than control and another treatment. However, further research is needed to confirm the effect of this plant extract on the vegetative growth of *Vigna radiata* L.

Keyword: *Acacia nilotica*, aqueous extract, ethanol extract, seed germination

INTRODUCTION

Acacia nilotica is one of the plants of the Mimosaceae family, which was scattered in several countries in Asia and Africa. In Indonesia, this plant was founded in the savanna area of Baluran National Park, East Java. *A. nilotica* can grow in high temperatures (-1 to 50°C) areas. A. nilotica is used for medicinal ingredients. The stem can be used to treat ambient disease (hemorrhages), colds, tuberculosis, and illness leprosy. The roots can be used as a stimulant lust (aphrodisiac) and flowers to treat syphilis.

Extract of A. nilotica leaf is reported to contain L-arabinose, catechol, galactan, galactoaraban, galactose, Nacetyldein kolic, N-acetyldein kolic acid, sulphoxides pentosan, saponins, and tannins. Seeds contain crude protein 18.6%, ether extract 4.4%, fiber 10.1%, free nitrogen extract 61.2%, ash 5.7%, silica 0.44%, phosphorus 0.29%, and calcium 0.9%. Based on that compound, the extract of *A. nilotica* leaves usually used as antibacterial because of the secondary metabolites compound of the extract (Ali et al., 2012).

The secondary metabolite of A. nilotica leaves extract could be a potential biostimulant in plant growth. Biostimulants are organic compounds that can stimulate and influence the physiological growth process of plants (Du Jardin, 2012). The source of biostimulant could be from a microbe, seaweed extract, and secondary metabolite of plant extract. The chemical compound from plant extract can be potential a biostimulant (Ertani et al., 2013). There was some research that reported the application of 75% and 100% extract of *Chromolaena odorata* L. leaf increased the germination of the mustard seed (Damayanti et al., 2013). The application of G*leicheinia linearis* leaves extracts increased the growth and productivity of maize (Aulya et al., 2018).

Therefore, the researcher wants to test the effect of secondary metabolite compounds of extract *A. nilotica* on seed germination of V. radiata L.

RESEARCH METHODS

A. nilotica leaves were collected at Baluran National Park, using purposive sampling. Then the research about the phytochemical test and seed germination was conducted at Laboratory of Plant Physiology Universitas Negeri Jakarta.

Phytochemical Test

Leaves of *A. nilotica* dried then crushed to powder form. Simplicia (10 g) was macerated with methanol and water for 24 hours. The macerate tested by phytochemical testing of Flavonoids, tannins, terpenoids, alkaloids, and terpenoids. Phytochemical test by using a color test followed by a standard laboratory method (Harborne, 1973)

Seed Germination Test

Design

The extract of *A. nilotica* leaves on seed germination was carried out experimentally using two factors of completely randomized design (CRD) with repetition three times. The type of extract (aqueous and ethanol) as a first and extract concentration (0%, 25%, 50%, and 75%) as the second factor.

Preparation of extract

The extract dissolved with 1 L of water according to the concentration used in the study (25%, 50%, 75%). Then extract poured on Petri dishes as media of germination.

Preparation for germination

The seeds were soaked for 2 hours using aqua dest, then placed in 10 Petri dishes. Then it was placed in a dark room and observed every day until the radicles from the sprouts. The parameters measured were the percentage of germination, germination rate, radicle length, and germination fresh weight.

Data analysis

The germination parameters were analyzed with Two-Ways ANOVA using the SPSS program. If there is an influence between treatments, it continued with the Duncan Multiple Range Test (DMRT) ($p < 0.05$).

RESULTS AND DISCUSSION

Phytochemical Test of *Acacia nilotica* L.

The results of phytochemical testing with color test methods show that the leaves of *A. nilotica* L. contain terpenoids, flavonoid, tannins, alkaloids, and saponins. All of these secondary metabolites were found in both aqueous extracts and ethanol extracts except for terpenoids which were only found in ethanol extracts. The test results presented in Table 1.

Table 1. Phytochemical analysis of A. nilotica leaves

Chemical compound	Aqueous extract	Ethanol extract
Steroids	-	-
Terpenoids	-	+
Flavonoid	+	+
Tannins	+	+
Alkaloids	+	+
Saponins	+	+

Note: (+) Crude extract contains secondary metabolite compound
(-) The crude extract does not contain secondary metabolites

Based on Table 1. it can be seen that the water and ethanol extract of positive *A. nilotica* leaves contain flavonoids, alkaloids, saponins, and tannins. There are differences in the results of the isolation of metabolite compounds between aqueous and ethanol extract. In aqueous extracts, there were not terpenoids while in positive ethanol extracts they contained terpenoids. This is due to the nature of non-polar terpenoids and to isolate them must be with organic solvents namely chloroform or ethanol (Harborne, 1973). Therefore, the color test results of the leaves of *A. nilotica* L. are by following the results of the research of Djufri (2012) and Siegler (2003) that *A. nilotica* L. contains saponins, tannins, flavonoids, alkaloids, and terpenoids.

Seed germination test

The results effect of aqueous and ethanol extract with a different level concentration on germination testing showed in Table 2 and Table

Table 2. The analysis of variance (ANOVA) Effect of Aqueous and Ethanol extract with different level concentration on seed germination parameters (the percentage of germination, germination rate, radicle length and sedling fresh weight of *V. radiata* L.

Variance	The percentage of germination (%)		Germination rate		Radicle length (cm)		Seedling fresh weight (g)	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Type of extract	8.859	0.009 s	5.069	0.039 s	27.165	0.000 s	8.859	.009 s
Concentration (%)	0.050	0.984 ns	1.163	0.355 ns	1.068	0.391 ns	.050	.984 ns
Type of extract * concentration (interaction factor)	0.523	0.672 ns	0.665	0.586 ns	0.549	0.656 ns	0.523	.672 ns

Noted: ns : non signfikan; s : signifikan, $p < 0.05$ level

According to Table 2. F was significant at the $p < 0.005$ level, that first factor (the type of extract) significantly influenced all germination parameters. That treatment's means were continued to Duncan's New Multiple Range Test (DNMRT). the result of DNMRT was presented in Table 3.

Table 3. Effect of Aqueous and Ethanol extract with different level concentration on seed germination parameters (the percentage of germination, germination rate, radicle length and sedling fresh weight of *V. radiata* L.

Treatment		The percentage of germination (%)	Germination rate (Day After Sowed)	Radicle length (cm)	Seedling fresh weight (g)
Type of extract	Concentration (%)				
Aqueous	0,00 (Control)	79 ns	8.87 ns	2.5 ns	1.3 ns
	25	94	9.33	3.6	1.8
	50	88	9.17	2.8	1.5
	75	100	9	2.4	1.3
Ethanol	0,00 (Control)	29 ns	5.53 ns	0.9 ns	0.2 ns
	25	77	8.47	1.8	1.5
	50	65	8.1	1.1	0.7
	75	58	7.93	0.8	0.8

Noted: In a column an row, within treatment, same letter(s) indicate do not different significantly according to DNMRT ($p < 0.005$), ns : nonsignificant

Based on Table 3. Extract of *A.nilotica* leaves influenced to germination parameters of *V. radiata* seed. All the germination parameters such as the percentage of germination, germination rate, redicle length, and seedling fresh weight showed the highest result than control.

Treatment with aqueous extract showed the highest percentage of germination was found in application with 75% aqueous extract (100%) while the control (79%). The highest germination rate (9.33 Day After Sowed/DAS) while the control (8.87 DAS). The highest radicle length and seedling fresh weight was found on application with 25% aqueous extract too (3.6 cm and 1.8 g), while control 2.5 cm and 1.3 g.

Treatment with ethanol extract showed the highest percentage of germination in (77%), germination rate (8.47 DAS), radicle length (1.8 cm), and seedling fresh weight (1.5 g) were found on application with 25%, compared with control and another concentration of ethanol extract.

The result showed aqueous extract could increase seed germination of *V. radiata* seed better than ethanol extract. For more details, the comparison of the germination parameters, treatment with aqueous extract and ethanol extract showed in graphic (Figure 1, 2,3 and 4). Based on graphic, all germination parameters treatment with aqueous extract showed higher than ethanol extract treatment.

The germination process, water has a role to stimulate the embryo to produce phytohormones especially gibberellic acid. Gibberelic acid (GAs) form tetracyclic diterpenoid. The function of GAs is to stimulate seed germination (Gupta and Chakrabarty, 2013). In the germination process, function water was stimulating the embryo to produce phytohormones, especially gibberellic acid. That the water extract is more effective increase germination parameters than the ethanol extract. Furthermore, the phytochemical compounds of the extract also affected the germination process. Based on this research, in extract *A. nilotica* leaves was found terpenoid. Terpenoid was a source of Gibberellic acid (GAs). The function of GAs is to stimulate seed germination (Gupta and Chakrabarty, 2013).

This research the lowest concentration of extract (25%) showed the best result on germination parameters. It is showed in Figure 1, 2, 3 and 4. Based on Figure 1. the water solvent with a concentration of 25% shows the most optimal results for the parameters of germination of V. radiata. Redicle length occurred by cell division and elongation. (Salisbury & Rose, 2005). The faster the hydrolysis process will accelerate the lengthening of the radicles. The longer the radicles owned by the sprouts will increase the wet weight of the sprouts.

Secondary metabolites that are isolated from plants can be used as biostimulants including triterpenoids, saponins, flavonoids, and alkaloids (Aulya et al., 2018). The terpenoid compounds present in the extract play a role in stimulating genes to synthesize the α-amylase enzyme (Taiz, & Zeiger, 2002). Culver et al. (2012) reported that application extract of plant as biostimulant with low concentration could be optimal on germination and plant growth. Because biostimulant in low concentrations that can stimulate the physiology process of plant growth (Du Jardi, 2012).

Effect of application 25% aqueous and ethanol extract on germination *V. radiata* seed showed at Figure 1.

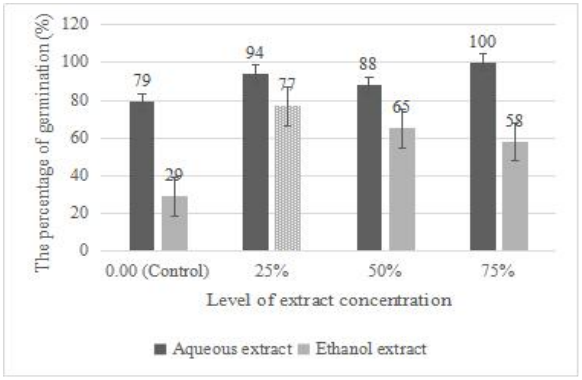

Figure 1. Effect of concentration aqueous and ethanol extract on percentage of germination *V. radiata*

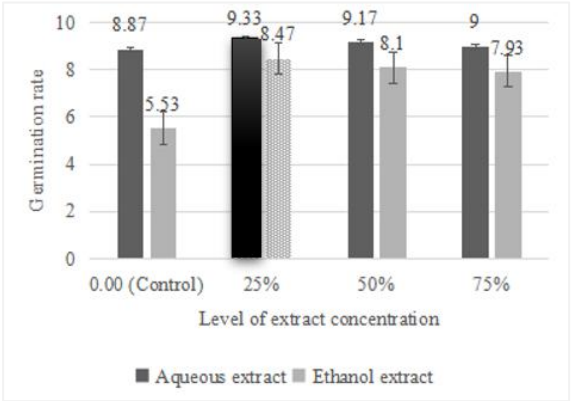

Figure 2. Effect of concentration aqueous and ethanol extract on germination rate *V. radiata*

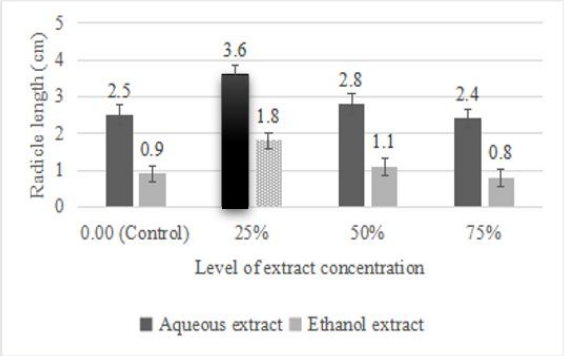

Figure 3. Effect of concentration aqueous and ethanol extract on redicle length of *V. radiata*

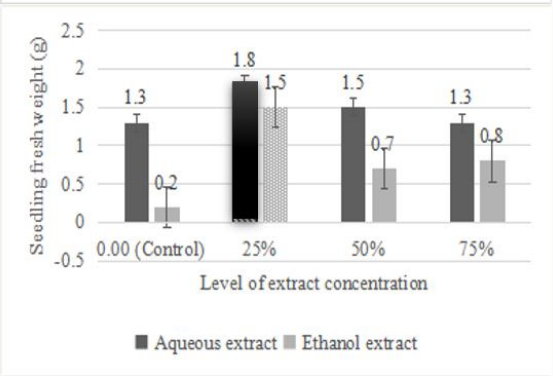

Figure 4. Effect of concentration aqueous and ethanol extract on seedling fresh weight of *V. radiata*

Based on Figure 1. The water solvent with a concentration of 25% shows the most optimal results for the growth of mung bean sprouts. This is seen in the parameters of germination rate, radicle length, and germination wet weight. Redicle length occured by cell division and elongation. (Salisbury & Rose, 2005). The faster the hydrolysis process will accelerate the lengthening of the radicles. The longer the radicles owned by the seedling will increase the wet weight of the sprouts.

Figure 1 showed the lowest germination rate, radicle length, and seedling fresh weight was found in application aqueous and ethanol extract with high concentration. That could be high concentration inhibited the germination of seed. This result could be related to the seedling condition in the last day measured germination parameters (Figure 5).

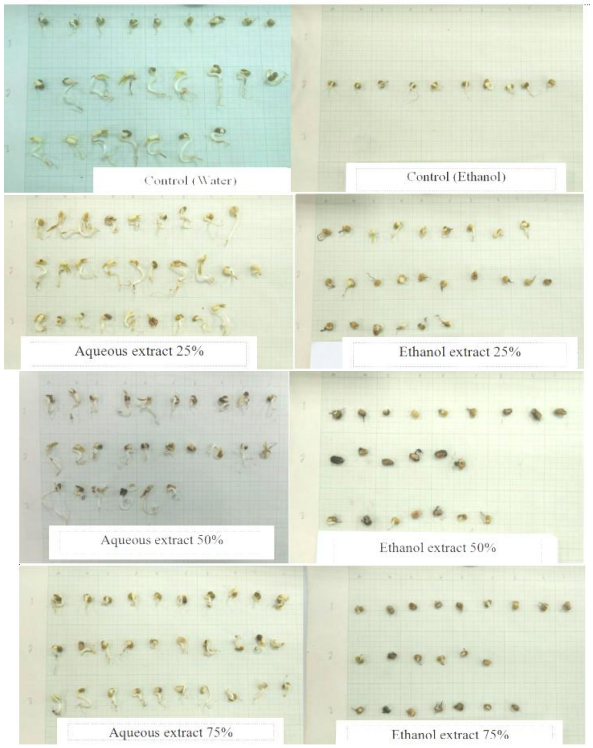

Figure 5. Seedling of *V. radiata* L. treatments with aqueous and ethanol extract of *A. nilotica* leaves in several concentration level.

CONCLUSION

Aqueous extract of *A. nilotica* leaves 75% effective in influencing the percentage of germination up to 100%, followed by treatment with 25% aqueous extract affecting the germination rate, radicle length, and wet weight of the sprouts. Compared with the control treatment and treatment with ethanol extract. Based on this research aqueous extract of *A. nilotica* leaves potentially as biostimulant on seed germination. However, research needs further research to know the effect of *A. nilotica* leaf extract on plant growth and yield.

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