



ANTIFEEDANT ACTIVITY FROM NEEM LEAF EXTRACT (*Azadirachta indica* A Juss)

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Abstract. Antifeedant activity of neem leaf (*A. indica* A. Juss) has been identified by against *Tenebrio molitor* bio-indicator. The highest activity was obtained on ethyl acetate extract at 0.5% concentration having Antifeedant Index (AI) of 51.53% and most active at 10% concentration of 82.05%. The method used to test the antifeedant activity is the no choice leaf disk method. Secondary metabolites contained in neem leaf extract (*A. indica* A. juss) include terpenoids, steroids, flavonoids, saponins and phenolics.

Keywords: Neem leaf (*Azadirachta Indica* A.Juss), No choice leaf disk method, Antifeedant Activity

I INTRODUCTION

Neem (*Azadirachta Indica* A. Juss) is a plant in the Meliaceae family. *A. indica* A. Juss can easily grow in the tropical area with rainfall of 450 - 750 mm/year above the sea level. It is mainly found in India and its leaf, flower, fruit, seed and root have been used as medicine [1]. The plant of this family contains secondary metabolites including alkaloids, flavonoids, quinones, and terpenoids [2]. *A. indica* A. Juss pharmaceutically carries high anti-oxidant, anti-diabetes, anti-inflammatory, anti-malaria, anti-bacterial, and anti-fungal activity [3]. Based on the previous research, beside its benefits in pharmaceutical field, *A. indica* A. Juss has been widely used as pesticide for various species of pests. There are four important components contained in neem, those are azadirachtin, salanine, meliantriol and nimbin which are tetranortriterpenoid beneficial for insect barrier, repellent, and antifeedant [4]. A study on antifeedant activity reported that the neem seed extract yields antifeedant index (AI) of 98.19 % against *plutella xylostella* bioindicator [5]. Its extract against *hymenia recurvalis* bioindicator gave higher AI of 52.70 % compared to the result against *psara basali* bio-indicator which gives AI of 49.85% [6]. Other bio-indicators which were used for antifeedant activity test are *Helicoverna armigera* and *Spodeptera litura*. They were used on ethyl acetate extract of *Pergularia daemia* leaf and showed AI of 70.3% and 71.82% respectively [7]. *Spodoptera frugiperda* and *Drosophlia melanogaster* bio-indicator used on ethyl acetate extract of *Calceolaria talcana* shoot gave AI of 89.7% and 75.1% respectively [8]. Meanwhile, testing antifeedant activity by using *Tenebrio molitor* bioindicator has not yet been studied.

II METHODOLOGY

In this research, we used laboratory glassware, analytical balance of brand CPA 6235 SARTORIUS, tube rack, vacuum rotary evaporator of Hidolph Laborota 4004 Contro brand, distillation apparatus, drop pipette, micro pipette. The materials which were needed in this research are n-hexane technical, ethyl acetate technical, methanol technical, reagent Liberman-Bourchard (acetate acid glacial-H₂SO₄(P)), reagent Mayer (potassium tetraiodomercurate), reagent Dragendorf (Bi(NO₃)₃) and reagent Wagner (I₂ in KI), silica gel 60 (0.2 – 0.5 mm). *T. molitor* bio-indicator and spinach leaf as test media. The plant material in this research is neem leaf (*Azadirachta indica* A Juss) from Darussalam, Syiah Kuala district, Banda Aceh city. Sample voucher was identified by Saida Rasnovi and stored in herbarium taxonomy in Biology Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

Extraction of *A. indica* A. Juss' leaf

As for the first step before the extracting process, phytochemical test was needed to be conducted. It consisted of phenolic, flavonoid, terpenoid, steroid and saponin tests. This process aimed to predict the class of compound contained in the *A. indica* A. Juss leaf sample. Furthermore, 1.5 kg of dried samples were roughly pounded and macerated in n-hexane solvent for 2 x 24 h. Thereafter, it was filtered to separate the extract and the residue. The residue was remacerated in the ethyl acetate solvent for another 2 x 24 h. Filtration was then carried out to separate the residue from the ethyl acetate macerate. The final residue was macerated again in the methanol solvent. Each macerate was evaporated by using rotary

evaporator such that rough extracts of n-hexane, ethyl acetate, and methanol were obtained. The phytochemicals and antifeedant activity in the extracts were then tested.

Bio-indicator

Bio-indicator which was used in this study was *Tenebrio molitor* instar III.

Antifeedant Activity

Antifeedant activity was tested by employing no choice leaf disk method [6]. The rough extract of *A. indica* A. Juss leaf was made into four concentrations which were 0.5, 1, 5 and 10%. Fresh spinach leaves were used as the test media. The spinach leaves were dipped in to the extracts for 1 min, dried for 15 min, and weighed to record their masses (X1). The leaves were placed in test containers and added in 5T. molitor bio-indicators. The containers then were covered with gauze. Observation was conducted after 1 x 24 h of the application. The spinach leaves were weighed to record their shrunk masses. The difference of X2 and X1 indicated the leaves' masses scraped by the bio-indicators (treatment) while the fresh spinach leaves dipped in the solvents were the controls (Eq. (1)).

$$\% AI = \frac{\text{control} - \text{treatment}}{\text{control} + \text{treatment}} \times 100\% \quad (1)$$

III RESULTS AND DISCUSSION

Extraction of *A. indica* A. Juss' Leaf

Preparing the *A. indica* A. Juss leaf sample by drying it was done to blot out the containing water in the sample. The dried sample was refined to widen its surface such that the solvent can penetrate through the cell wall and accelerate the extraction process of the secondary metabolite compound. Maceration in the solvent could draw out the compound contained in the plant. n-hexane extract drew out non-polar compound, ethyl acetate drew out semi-polar compound, while methanol drew out polar compound. The results of *A. indica* A. Juss leaf extraction are presented in Table 1.

Table 1 Results of neem leaf (*A. indica* A. Juss) extraction.

Extract samples	Dried extracts (g)	Yields (%)
n-hexane	18.83	1.25
Ethyl acetate	82.10	5.47
Methanol	93.09	6.21

Based on Table 1, the methanol extract gives highest percentage of yield. It is because methanol is a solvent which can draw out the polar and non-polar compound. In the previous study, it was reported that the yield of *A. indica* A. Juss leaf is higher in the dried area (which was 36.65%) compared to the one in semi-dried area (which was 25.24%). The yield is

also affected by the soil acidity. *A. indica* A. Juss which grows on alkali soil with pH 8.05 will have higher yield, which is 27.5% [13]. Semi-polar solvent generally can draw out compounds such as tannin, polyphenol, poliacetylene, flavonoid, terpenoid, steroid and alkaloid [10].

Phytochemical test

Fresh *A. indica* A. Juss leaf was phytochemically analyzed in order to determine the presence of secondary metabolites content in it. The tests which were conducted were terpenoid, steroid, flavonoid, phenolic and saponin tests. The phytochemical test's results are presented in Table 2.

Table 2 Results of phytochemical test of the fresh leaf and the extract of *A. indica* A. Juss

Secondary metabolite	n-hexane extract	Ethyl acetate extract	Methanol extract
Alkaloid			
Mayer	-	-	-
Dragendorff	-	-	-
Wagner	-	-	-
Steroid	+	+	+
Terpenoid	+	+	+
Saponin	-	-	+
Flavonoid	-	+	-
Phenolic	-	+	-

Note: (+) shows positive result and (-) shows negative result

Based on Table 2, the phytochemical test's results explain that the neem *A. indica* A. Juss leaf extract contains secondary metabolites such as steroid, terpenoid, flavonoid, phenolic and saponin, while the alkaloid component may exist in very small amount or zero amount. Phytochemical test on steroid and terpenoid content can be performed by using Liebermann-Buchard reactor. In the neem leaf, there exists steroid content which is indicated by color change from green to blue. Moreover, there is also color change to red which indicates the terpenoid's existence. The presence of phenolic compound in the ethyl acetate extract is shown by color change to blackish green through the FeCl₃ reactor. The presence of terpenoid together with steroid is commonly found in plants because steroid compound is biosynthetically derived from terpenoid compound [9]. In the *A. indica* A. Juss leaf, there are terpenoid contents which are 17-(5-methoxy-2-oxoferrin-3-yl)-28-deoxonimbolide and 2 α ,4 α -dihydroksi-pregn-5-en-16-one-3 α -O-D-glucopyranoside (steroid saponin) [10]. Saponin can decrease the food absorption in the insect's intestine [14]. Therefore it can also inhibit feeding by *Spilosoma oblique* and *S. litura* bio-indicators [15]. Moreover, *A. indica* A. Juss leaf also contains azadirachtin, salanine, meliantriol and nimbin which are tetranortriterpenoid which can act as insect barrier, repellent, and antifeedant [4]. In the previous research, it was

explained that the result of phytochemical test on ethyl acetate extract of the fruit and seed showed the presence of secondary metabolites in terpenoid and steroid class [7]. All parts of neem tree including leaf, stem's skin, flower, seed and sap have been used as antifeedant, anti-allergy, anti-bacteria, insecticide, and larvacide [12].

Antifeedant activity of neem leaf (*A. indica* A. Juss) extract

The antifeedant extract test of *A. indica* A. Juss leaf by applying no choice leaf disk method was conducted by creating variation on the extract's concentration, which are 0.5, 1, 5 and 10 %. The results can be observed in Table 3.

Table 3 Antifeedant Index (AI) of *A. indica* A. Juss leaf extract.

Sample	Concentration (%)	AI (%)
n-hexane extract	0.5	10.45
	1	15.56
	5	41.41
	10	57.51
Ethyl acetate extract	0.5	51.53
	1	62.67
	5	71.90
	10	82.05
Methanol extract	0.5	32.10
	1	57.13
	5	65.70
	10	70.96

According to Table 3, all of the three *A. indica* A. Juss leaf extracts contain antifeedant activity which can inhibit feeding of *T. molitor* bio-indicator. The ethyl acetate extract has antifeedant activity greater than methanol extract, while the one in methanol extract is greater than the one in n-hexane. Antifeedant activity can be determined by antifeedant index (AI). If AI is higher than 50%, it indicates active antifeedant. Antifeedant is the most active if the AI is 80 – 100% [11]. Ethyl acetate extract has the most active antifeedant compared to the methanol and n-hexane. On the ethyl acetate extract with the lowest concentration 0.5%, its AI index is greater than 50%, which is 51.53%, and the antifeedant is the most active on the 10% concentration, which is 82.05%. Methanol extract has active antifeedant at 1% concentration, which is 57.13%, while n-hexane has active antifeedant at 10% concentration, which is 57.51%. On the study of antifeedant activity in the *Calceolaria talcana* extract against *S. frugiperda* and *D. melanogaster* bioindicators, it was obtained that the highest AI was founded in the ethyl acetate extract (AI = 89.7%) compared to the one in methanol extract (AI = 71.30%) and n-hexane extract (AI = 33.2%) when it was measured at extract's concentration of 25 g/ml [8]. The similar result was also explained in the study on *P. daemia* extract with *Helicoverpa*

armigera and *S. litura* bio-indicator, the highest AI was found in the ethyl acetate extract (AI = 70,3%) compared to chloroform (AI = 54,04%) and n-hexane (AI = 27,84%) [7].

Antifeedant's work mechanism happens by producing feeding inhibition stimulant such that it disturbs the stimulation perception for eating and affects the growth and the development of hormonal system (neuroendokrin). Moreover, it also acts as ecdysone bloker that insects fail to moult their skin (moulting inhibition) such that it causes anatomical abnormality and death [15]. The difference in the antifeedant indices is caused by the different concentrations of the chemical compounds contained in the each extract, which affects the behavior of the insects. The higher the concentration is, the higher the antifeedant activity is.

CONCLUSION

The present study shows that neem leaf (*A. indica* A. Juss) extract contains antifeedant activity to the bio-indicator *T. molitor*. Ethyl acetate extract of neem (*A. indica* A. Juss) leaf is active antifeedant at the concentration of 0.05% which gives AI for 51.53% and its most active antifeedant is at the concentration of 10% which gives AI of 82.05%. Secondary metabolite contained in the neem (*A. indica* A. Juss) leaf are terpenoid, steroid, flavonoid, saponin and phenolic.

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