

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: - <a href="http://www.journalijar.com">www.journalijar.com</a></p> <h2 style="text-align: center;">INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p style="text-align: center;">Article DOI: 10.21474/IJAR01/9722 DOI URL: <a href="http://dx.doi.org/10.21474/IJAR01/9722">http://dx.doi.org/10.21474/IJAR01/9722</a></p>	
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### RESEARCH ARTICLE

#### EFFECT OF AZADIRACHTA INDICA A.JUSS. AND GYNURA PROCUMBENS (MERR.) LEAF EXTRACT COMBINATION TOWARDS FREE-RADICAL SCAVENGING ACTIVITY.

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#### Manuscript Info

##### Manuscript History

Received: 09 July 2019

Final Accepted: 12 August 2019

Published: September 2019

##### Key words:-

Azadirachta indica A.Juss., Gynura procumbens (Merr.), antioxidant, DPPH.

#### Abstract

**Introduction:** The use of traditional medicine in Indonesia, especially those of herbal origin, is growing in response to the increasing price of the conventional medicine and the limited purchasing ability of the society. Extracted leaves from plants such as *Azadirachta indica* A.Juss. and *Gynura procumbens* (Merr.) are examples of herbal medicine. The combination of both extract has the potential to be an antihyperglycemic agent through exploitation of its antioxidant properties.

**Aims & Objectives:** This study focuses on testing the antioxidant activity of a combination of ethanol extract *Azadirachta indica* A.Juss. and *Gynura procumbens* (Merr.) by in vitro.

**Material & Methods:** Antioxidant activity is tested using free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) reduction method in comparison to vitamin C. The resulting antioxidant activity level of strength is determined based on IC<sub>50</sub> values obtained.

**Results:** antioxidant activity test showed that *Azadirachta indica* A.Juss. and *Gynura procumbens* (Merr.) ethanol extract combination of 75:25 has the best IC<sub>50</sub> value of 67.407 µg/mL, while the 50:50 and 25:75 ethanol extract combination has IC<sub>50</sub> value of 68.864 µg/mL and 102.887 µg/mL respectively.

**Discussion:** The three combinations are weaker in comparison to the standard vitamin C antioxidant activity with IC<sub>50</sub> value of 3.546 mg/mL. Statistical analysis using one-way ANOVA concludes significant difference in antioxidant activity of all three comparing groups.

**Conclusions:** *Gynura procumbens* (Merr.) and *Gynura procumbens* (Merr.) extract combination of 75:25 has strong category antioxidant activity.

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#### Introduction:-

Free radicals are compounds or molecules that contain one or more unpaired electrons in their outer orbitals. The existence of unpaired electrons causes the compound to be very reactive looking for pairs by attacking the electrons of molecules around them (Pratama dkk., 2018). However, antioxidant compounds can inhibit the reactivity of free radicals by stabilizing it (Winarsih, 2007).

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Antioxidant intake is important for all age groups be it children or even adults. Consumption of antioxidants in sufficient amounts reportedly able to reduce degenerative disease occurrences (Winarsi, 2007). Leaf of both *Azadirachta indica* A. Juss. and *Gynura procumbens* (Merr) plants are widely reported to be the source of antioxidant.

Both plants are rich of phenolic compounds, polyphenols, flavonoids, coumarin, quercetin, routine, and tannins (Denardi et al., 2010; Biswas et al., 2002; Sudarsono et al. 2002). The main compounds responsible for antioxidant activity are tannins, alkaloids (Alfarabi et al., 2010), and phenolic components such as phenolic terpenes, phenolic acids, and flavonoids (Javanmardi et al., 2003).

Research conducted by Sunnarwidhi (2014) reports that ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) containing quercetin and routine have greater effect in reducing blood sugar level compare to that of each single extract. Antioxidant administering on patients with degenerative diseases such as diabetes mellitus inhibits the formation of free radicals, reducing oxidative stress and reducing the expression of TNF- $\alpha$  (Widowati, 2010).

Based on the aforementioned facts and supported by previous research on the presence of active compounds acted as antioxidants, it is necessary to conduct research to test the antioxidant activity in ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.). Furthermore, it deepens the information regarding the efficacy of this extract combination compare to that of each single extract.

### Aims & objectives:-

Understanding the combined activity of *Azadirachta indica* A. Juss (M) and *Gynura procumbens* (Merr.) ethanol extract as antioxidant through free-radical DPPH scavenging Method.

### Material & methods:-

The main tools used in this study namely: rotary evaporator (IKA® RV8), UV-Vis spectrophotometer (Genesys® 10S) and a micropipette (Eppendorf® Research plus). Materials used are leaf extracts of *Azadirachta indica* A. Juss. and *Gynura procumbens* (Merr) obtained from Moyudan Sleman, Yogyakarta, and also 70% ethanol (technical). Materials for the antioxidant activity test is a standard vitamin C (Sigma Chemicals Co.), DPPH (2,2-diphenyl-1-picrylhydrazyl) from Sigma Chemicals Co. and methanol pro analysis from E. Merck.

### Course of Study:

Test to examine the potency of ethanol extract combination toward free radical DPPH scavenging activity, carried out in three series of combination between *Azadirachta indica* A. Juss. and *Gynura procumbens* (Merr), respectively 25:75; 50:50; 75:25. Then, 1,000-ppm test liquid was diluted into concentrations of 300 ppm, 250 ppm, 200 ppm, 150 ppm, 100 ppm and 50 ppm using 5 mL volumetric. Vitamin C solutions of 5-ppm concentration; 10-ppm; 15-ppm; 20-ppm; 25-ppm and 30-ppm acted as positive control. Each solution was then reacted with DPPH and incubated for 30 minutes. Absorption reading is determined using a UV-Vis spectrophotometer at a maximum wavelength of 516 nm. Scavenging DPPH free radical activity by *Azadirachta indica* A. Juss. and *Gynura procumbens* (Merr) extract combination is calculated as %inhibition and then expressed in IC<sub>50</sub> value. The value indicates amount of concentration required for the test sample to capture 50% of DPPH free radical.

### Statistical Analysis:-

Statistical analysis using one-way ANOVA obtains significant different in antioxidant activity among all three comparison groups.

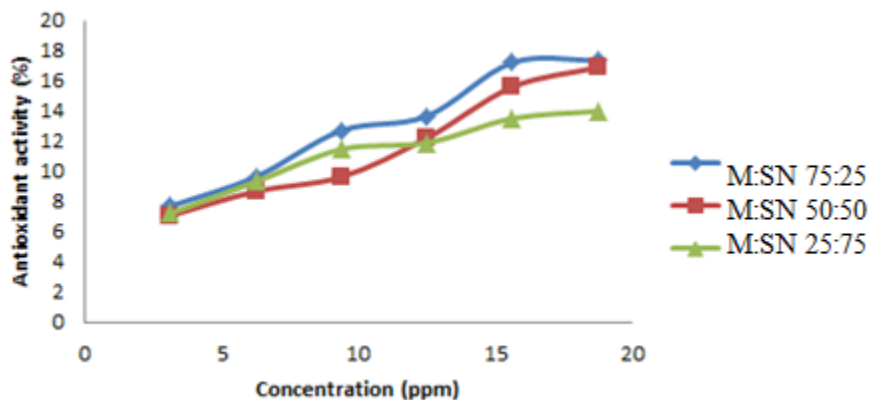
### Result:-

Organoleptic testing has an important role in applying quality of a product. Organoleptic testing can provide indications of decay, deterioration or damage to quality. The organoleptic extract results obtained shown in Table 1.

**Table 1:-**Extract Identity and Organoleptic Extract Test Results

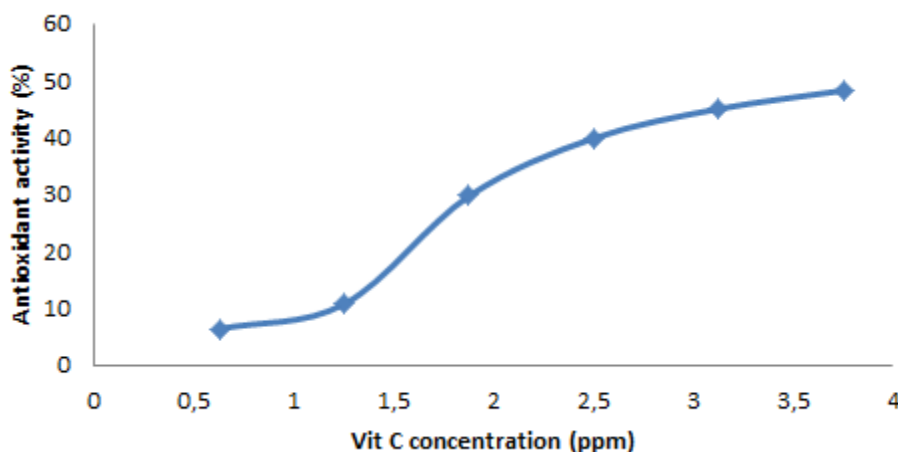
Parameter		
Extract Identity		
extrack	Ethanol extract of <i>Azadirachta</i>	Ethanol extract of <i>Gynura</i>

Name of origin plant Parts used	<i>indica</i> A. Juss <i>Azadirachta indica</i> A. Juss Leaf	<i>procumbens</i> (Merr.) <i>Gynura procumbens</i> (Merr.) Leaf
<b>Organoleptic Extract Test</b>		
Color	Blackish green	Blackish green
Odor	Aromatic typical	Aromatic typical
consistency	Thick	Thick
Taste	bitter	bitter



**Figure 1:-**Relationship between the ethanol extract combination of *Azadirachta indica* A. Juss (M) and *Gynura procumbens* (Merr.) (SN) and Antioxidant Activity Percentage

The result of the antioxidant activity test for 75:25 combination between *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) extract has an  $IC_{50}$  value of 67.407  $\mu\text{g/mL}$ , while the combination of 50:50 has an  $IC_{50}$  value of 68.864  $\mu\text{g/mL}$ . These two combination ratios of 75:25 and 50:50 considered as powerful antioxidants. On the other hand, the 25:75 ratio combination had an  $IC_{50}$  value of 102,887  $\mu\text{g/mL}$  hence considered a moderate antioxidant. All three combination-groups had weaker antioxidant activity compared to standard vitamin C  $IC_{50}$  value of 3.546  $\mu\text{g/mL}$ .



**Figure 2:-**Graphical relation between Vitamin C Concentration and Antioxidant Activity Percentage

**Table 2:-** $IC_{50}$  Values of Vitamin C, M: SN (75:25) Combination Extract, M: SN (50:50) Combination Extract, and M: SN (25:75) Combination Extract

Sample	$IC_{50}$ ( $\mu\text{g/mL}$ )	The level of antioxidant activity with the DPPH method
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		Very Strong (IC <sub>50</sub> <50 µg/mL)	Strong (IC <sub>50</sub> 50- 100 µg/mL)	Moderate (IC <sub>50</sub> 101-150 µg/mL)	Weak (IC <sub>50</sub> >150 µg/mL)
Vit C	3,546	√			
M:SN 75:25	67,407		√		
M:SN 50:50	68,864		√		
M:SN 25:75	102,887			√	

### Discussions:-

The results of antioxidant activity test between ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) and the Vitamin C standard expressed in percent reduction using free radical DPPH scavenging method then plotted against the concentration to obtain linear regression equation. DPPH solution uptake is measured at maximum wavelength using a spectrophotometer. The maximum wavelength obtained is 516 nm. This wavelength corresponds to DPPH wavelengths ranging from 515 - 520 nm (Molyneux, P., 2004).

The result of the antioxidant activity test between ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) with a ratio of 75:25 has an IC<sub>50</sub> value of 67.407. Hence, it is included in the category of strong antioxidants. IC<sub>50</sub> 67.407 µg/mL indicates that the concentration required for 75:25 ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) to inhibit 50% of DPPH radical is 67.407 µg/mL. Result of the antioxidant activity test between ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) with a ratio of 50:50 has an IC<sub>50</sub> value of 68.864 µg/mL and also among the category of strong antioxidants. IC<sub>50</sub> 68.864 µg/mL indicates that the concentration required for 50:50 ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) to inhibit 50% of DPPH radical is 68.864 µg/mL. Result of the antioxidant activity test between ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) with a ratio of 25:75 has an IC<sub>50</sub> value of 102.887 µg/mL. It is considered moderate antioxidants. IC<sub>50</sub> 102,887 µg/mL indicates that the concentration required for 25:75 ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) to inhibit 50% of DPPH radicals is 102,887 µg/mL (Molyneux, P., 2004).

Based on the antioxidant activity test of the sample (Table 2), the 75:25 ethanol extract combination between *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) has a relatively stronger antioxidant activity compared to the 50:50 and 25:75 ratio combination. The 25:75 combination has the weakest antioxidant activity. All three combination-groups had weaker antioxidant activity compared to IC<sub>50</sub> standard vitamin C that has an IC<sub>50</sub> of 53,546 µg/mL.

SPSS statistical analysis test using one-way ANOVA with 95% confidence level indicating significant value ( $p < 0.05$ ). Hence, there is a significant difference in the antioxidant activity between each ethanol extract combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) with respective ratio of 75:25, 50:50, and 25:75. Combined antioxidant activity depends not only on concentration, but also on the structure of antioxidant compounds. The antioxidant activity of plant extracts is usually associated with phenol groups in these plants. Both *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) leaf has many flavonoids containing hydroxyl (OH) groups. Hydrogen atoms in both extracts can be detached and captured by DPPH radical. It then stabilize DPPH radical to be a non-radical.

### Conclusions:-

Ethanol extract combination between *Azadirachta indica* A. Juss and *Gynura procumbens* (Merr.) has the best antioxidant activity at a ratio of 75:25 with an IC<sub>50</sub> value of 67.407 µg/mL.

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