The Potential of Quercetin in *Psidium Guajava L.* Leaves Extract as Urease Bioinhibitor for Fertilizer Application

Nur Kamila Ramli, Nurldidia Mansor, Zahid Majeed, Anis Suhaila Shuib, Zakaria Man

Department of Chemical Engineering, Universiti Teknologi PETRONAS, 31750 Tronoh, Perak, Malaysia.

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**ABSTRACT**

This study was done to investigate the usage of natural products as one of the materials in fertilizer application. Urease inhibitors that are commonly used in agriculture are usually chemical based which affects the environment. Introducing natural products will ensure biodegradability of the material. *Psidium Guajava* L. (guava) has been reported to have properties such as antibacterial, anti-oxidant, anti-cancer, and anti ulcer for medical purposes. Guava leaves extract contains an active compound named quercetin that was successfully reported to exhibit significant urease inhibitory activities. Spectrophotometric method was used in this study with the theory of Beer's Law in order to measure the changes in ammonia concentration. Small reduction of ammonia (NH3) concentration with different about 0.1 mol/L was calculated and the releasing was almost equal till the end of incubation time. The guava leaves extract prepared showed the potential to reduce the release of NH3 concentration during urea application.

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**INTRODUCTION**

Plant extracts have been discovered to become one of important source as bioinhibitor due to their environmentally acceptable, readily available and renewable source for wide range of inhibitors. They contained many sources of ingredients that highly efficient in inhibitory process (Pandian et al., 2008). Enzyme inhibitor for agricultural as well as medical purposes is well developed and been commercialized in terms of chemical based products (Watson, 2005). In agriculture, high urease activity releases abnormally large amounts of ammonia into the atmosphere after urea application and causes significant environmental problems and economical loss (Khan et al., 2010). In order to overcome this problem, the usage of inhibitors to inhibit urease enzyme has been applied which it prevents the hydrolysis of urea over a certain period of time by slowing down the rate at which urea hydrolyzes in the soil, thus avoiding or reducing volatilization losses of ammonia to the atmosphere (Martin, 1997). N-N-(n-butyl) thiophosphoric triamide (NBPT) is one of the compound that has been found commonly in commercial urease inhibitor for agriculture. It has showed some visible effects which is on the first week of treatment, the leaf tips transforms from green color to yellow. Besides, plants treated with urea and NBPT were found to have higher urea content in their tissues and retard the plant growth (P.M Tejo et al., 2011). Based on Department of Health and Ageing (2010) draft report, exposure of NBPT for 15 days on rats cause decrease in total cholesterol, triglyceride, brain red blood cells and the target organ is the liver. Urine sample from tested animal was taken and been reported to contain NBPT. This compound also affect fertility in animals and NBPT proved to cause weight changes in reproductive organs in both males and females, as well as abnormalities in sperm assessments. Obviously, chemical based inhibitors have negative side effects, not safe and have low efficiency [Zaborska et al., 2009]. The research on bioinhibitor is due to world demanding on environmentally products from the natural sources. Due to this phenomenon as well as deeply concern about the environment, researchers try to discover on bioinhibitors. The search for green inhibitor is continuously and been confirmed by recent publications (Tarun et al., 2004).

*Guava (Psidium guajava L.*) has been demonstrated to have several biological activities such as antidiabetic (Oh et al, 2005) anticough, antibacterial (Jaiarj et al, 1999) and antispasmodic actions (Lozoya et al, 2002). Guava tree is member of myrtaceae family, all the parts of this tree widely*
use in curing many health problems. Extraction from guava leaves mostly essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C. Flavonoids particularly rich in quercetin, saponins, alkaloids, cardiac glycosides, phlobatannins and anthraquinones. Guava leaves extract that contains an active compound named quercetin was successfully reported to exhibit significant urease inhibitory activities with IC50 value of 47.5 ± 2.3 (Irshad et al., 2011). The aim of this study is to investigate the capability of guava leaves extract that contain quercetin to inhibit urease enzyme in order to prevent the ammonia released.

2. Methodology:

Preparation of materials:
Guava (Psidium guajava L.) leaves extract, urease enzyme (Jack bean urease), urea (50 mM), phosphate buffer solution (50 mM, pH 7.80) was prepared by adjusting the pH of phosphoric acid with sodium hydroxide (NaOH), 2 mM of ethylenediaminetetraacetic acid (EDTA). Equipment used was UV-VIS Spectrophotometer.

Preparation of guava leaves extract:
To prepare the extracts, 2 g powder of dry leaves were mixed with 200 ml of 0.9% NaCl (100°C, 5 min). The crude extract was filtered, centrifuged (1500 r/min, 5 min) to obtain the final extract. The supernatant was considered as the concentration at 1 g/ml of guava leaves (Abreu et al., 2006).

Results:
Beer’s Law equation was applied in this experiment to analyse the absorbance data obtained from UV-VIS spectrophotometer. The decreasing in value of ammonia concentration in this inhibition studies was calculated using the Beer’s Law, Eq. 1 and was illustrated in Figure 2.

Fig. 1: Standard calibration curve.

The standard calibration curve using ammonium stock solution was prepared before undergoing the inhibition and data was recorded at 640 nm. The slope value obtained was used as the value for molar absorptivity, ε.

4. Discussion:
Eq. 1 is used to determine the concentration of ammonia for each amount of enzyme-containing solution that has been added in the standard assay solution. The result is shown in Figure 2 which illustrates the effect of guava leaves extract in urea-guava leaves reaction on ammonia (NH3) release. Urea solution that was prepared as a blank showed small increasing of release of NH3 concentration starting from 0 minutes of incubation time until 80 minutes. Meanwhile, urea solution with addition of urease enzyme did show slightly higher of NH3 concentration release than the blank one. Otherwise, the urea solution with mixture of urease-guava leaves extract showed reduction in NH3 concentration release and fully stopped the release at 80 minute of incubation time. The inhibition happened with the smallest value of NH3 concentration release about 0.0081 mol/L. The obtained result showed that guava leaves extract have the potential to reduce NH3 concentration which is urease enzyme was conceptually inhibited by the

Studies of inhibition:
Two main mixtures and one blank were prepared to undergo the inhibition process which is standard assay mixture and the enzyme-containing solution. Two standard assay mixture that consisted of 50 mM urea, 50 mM phosphate buffer solution (pH 7.8) and 2 mM EDTA. Meanwhile, the enzyme-containing solution is consisted of 50 mM phosphate buffer solution (pH 7.8), 2 mM EDTA urease enzyme and the guava leaves extract. Then, the mixtures was incubated at room temperature (25°C). During the incubations, 1 ml aliquots of the enzyme-containing solution at different time intervals were immediately transferred into the one of the standard assay mixtures to determine the urease activity. The other standard assay was prepared as blank. The urease activity was determined by measuring ammonia concentration after 10 minutes of reaction time. Ammonia concentration was determined by the spectrophotometric, phenol–hypochlorite method where the absorbance was registered at 640 nm (Parson et al., 1984). The procedure is repeated and the trend of analysis was observed.
active compound, quercetin that posses the inhibitory properties as reported before. The ten minutes as time interval for each reaction was showed the importance of time-dependant in the whole inhibition process.

Table 1: Result of NH$_3$ concentration.

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Ammonia concentration (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2921</td>
</tr>
<tr>
<td>10</td>
<td>0.1593</td>
</tr>
<tr>
<td>20</td>
<td>0.1258</td>
</tr>
<tr>
<td>30</td>
<td>0.1085</td>
</tr>
<tr>
<td>40</td>
<td>0.0893</td>
</tr>
<tr>
<td>50</td>
<td>0.0598</td>
</tr>
<tr>
<td>60</td>
<td>0.0335</td>
</tr>
<tr>
<td>70</td>
<td>0.0162</td>
</tr>
<tr>
<td>80</td>
<td>0.0081</td>
</tr>
</tbody>
</table>

Fig. 2: NH$_3$ concentration against incubation time.

**Conclusion:**

The guava leaves extract prepared in this experiment was able to inhibit urease enzyme and showed reduction in NH$_3$ concentration release during urea application. Process was happened caused by the reaction of the quercetin compound that contained in guava leaves extract. The inhibition process successfully happened at 80 minute of incubation time with value of NH$_3$ concentration release about 0.0081 mol/L.

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**REFERENCES**


