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# Toxicity and Alpha Glucosidase Inhibitory Activity of Roasted Kedawung Seed (*Parkia timoriana*)

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***ABSTRACT***

***Introduction**: Kedawung (P. timoriana) seeds are empirically utilised as a traditional medicine; it also contains various phytochemical compounds, antioxidant activities as well as other beneficial pharmacological effects. In some regions in Indonesia, the utilisation of kedawung seeds is usually done by involving the roasting process. **Aims**: This study aims to investigate the effect of roasting on phytochemical compounds, toxicity and alpha glucosidase inhibitory effects. **Method**: Kedawung seeds were roasted at 150 ºC (15 minutes); then separated from the skin, powdered and macerated in ethanol. **Result**: The extract was phytochemically screened with the results obtained showing positive for saponins, flavonoids, alkaloids, and terpenoids. The following test is toxicity test using BSLT (Brine Shrimp Lethality Test) method as well as inhibitory activity on alpha glucosidase enzyme. The test results showed that kedawung extract had an $LC_{50}$ of 78.18 ppm in the BSLT test, as well as inhibition of alpha glucosidase enzyme at 265.24 ppm. **Conclusion**: The results obtained show that the ethanol extract of roasted kedawung seeds still has phytochemical metabolite compounds, but is moderately toxic and has weak inhibition on alpha glucosidase.*

***KEYWORDS**: Roasted, P. timoriana, toxicity, alpha glucosidase, BSLT.*

## INTRODUCTION

Kedawung plant (*Parkia timoriana*) is one of the four parkia species that are widely used as traditional medicine for generations in several regions in Asia. Its medical uses cover a wide range of diseases such as diarrhoea, spasms, fever, bloating and treatment of infections and leprosy (Angami et al., 2018). Investigations into the pharmacological effects of this plant have been carried out extensively on several parts of this plant. Ethanol extract of kedawung leaves is known to have antibacterial effects against *Escherichia coli*, *Vibrio cholerae*, *S. aureus* and *B. cereus* (Angami et al., 2018; Zuhud et al., 2010). The pod part of kedawung was able to significantly reduce blood sugar levels at a dose of 10 mg/KgBW in streptozosin-induced rats (Sheikh et al.,

2016). The seed; the most widely utilised part of traditional medicine is known to have antioxidant activity with an IC50 of 37.56 ppm (distilled water extraction sample). In addition, this part is also known to have broad antibacterial and antifungal activities, as well as alpha amylase and alpha glucosidase inhibitory abilities (Angami et al., 2018; Sathya & Siddhuraju, 2015; Suryanti et al., 2022)

The various pharmacological activities of kedawung seeds are inseparable from its active metabolite content. GC-MS analysis of kedawung seeds showed at least 20 compounds consisting of terpenoids and unsaturated fatty acids. Literature searches of these compounds show evidence of pharmacological activities of anti-inflammatory, antioxidant, anticancer, antibacterial, antifungal, vasodilator, and various other pharmacological effects (Ralte et al., 2022). The utilisation of kedawung seeds is usually done with various processing methods consisting of fermentation, roasting and boiling. One of the purposes of the processing process is to prolong storage. The roasting method is one method of preserving simplisia (generally the seeds) by reducing the water content by utilising high temperatures (Ajatta et al., 2019; Sathya & Siddhuraju, 2015; Wijaya, 2019).

Until now, there are still few researches related to the effect of roasting processing on the pharmacological effects and constituents of kedawung seeds. This study is part of the research to investigate the potential of roasted kedawung seeds. Therefore, the direction of this research is aimed at obtaining data related to the inhibitory effect of the enzyme alpha glucosidase and the potential toxicity of roasted kedawung seeds.

## MATERIALS AND MEHTODS

### Materials and Tools

The material used in this study were the Kedawung seed, ethanol 96% (Merck), ethyl acetate (Merck), $FeCl_3$ (Supelco), Mg (Pratama Sains Global), Pb acetate (Merck), Mayer reagent (Brataco), Chloroform (Merck), Phosphat Buffer Saline (Merck), HCl (Merck), α-Glucosidase from Saccharomyces cerevisiae (Merck), DMSO (Merck), p-nitrophenyl-α-D-glucopyranoside (Merck), $Na_2CO_3$ (Merck), Brine shrimp larvae and aquadest.

The equipment used in the study include oven (Memmert), rotary evaporator (Buchi), 96 Well plate (Iwaki), Elisa reader (Thermo Fisher) and Laminar Air Flow.

### Preparation of Roasted Kedawung Seed

Kedawung seeds were collected from the traditional market in Plered, Cirebon Regency as much as 1 kg and sorted to separate good and spoiled seeds. The seeds were then dried using an oven at 150°C for 15 minutes. The dried seeds were then

separated from the husk and powdered using a seed grinder.

**Maceration of Powdered Seed**

Extraction was carried out using a maceration method modified from previous studies. The maceration process is performed by soaking the simplisia powder in ethanol in a ratio of 1:4 (powder (kg) : solvent (litre)). The extraction process was carried out for 3 X 24 hours and remaceration (2 X 24 hours). The maceration process was accompanied by stirring every 12 hours. The macerate and filtrate were separated using a Buchner funnel and the solvent was evaporated using a rotary evaporator and waterbath (Hadi & Adiyas Putra, 2023)

**Phytochemical Screening**

Detection of saponin: 2 ml of liquid extract in ethanol was mixed with 3 ml distilled water. The mixture was vigorously stirred for 30 seconds. Positive results are characterised by the presence of 1 cm high foam that lasts for 5 minutes Bhattacharya et al., 2018)

Flavonoid test: 3 mL of liquid extract was added with 5 drop of Pb Acetate. The Positive results of flavonoids are marked by white precipitate (Singh & Mathur, 2016).

Alkaloid test: 3 ml liquid extract added with 2 drop of HCl 2N. Then the solution added with 3-4 drop of mayer reagent. The formation of white precipitate showed positif alkaloid (Bruck de Souza et al., 2020).

Terpenoid test: 3 ml of the extract was added to 7 ml of chloroform. After shaking, wait until 2 phases are formed. After that, 2 ml acetic anhydride was added to solution. Then, 3 drops of $H_2SO_4$(1N) were dropped through the test tube wall. Positive results are indicated by the appearance of a brownish green colour at the boundary between phases (Singh & Mathur, 2016).

**Brine Shrimp Lethality Test**

The test was conducted using 48-hour-old prawn larvae (*Artemia salina*). The larvae were placed in wells with liquid extracts in concentration series of 1000, 500, 100 and 10 ppm in 1% DMSO solution. Tests were performed with triplication. Larvae were observed for 24 hours by calculating the mortality of dead larvae. Data were analysed for heterogeneity using the Chi square method (pearson goodness of fit test) and then continued with probid regression analysis (Mastura et al., 2022)

**Inhibition of Alpha Glucosidase**

Stock solution of alpha glucosidase (0.5 U/ml) was prepared in phosphate buffer solution (pH 6.8). A total of 25 µl of enzyme solution was placed in 96 well plates in three repetitions (triplication). The assay was conducted by administering 25 µl of

Table 1. Phytochemical screening Kedawung seeds

| Phytochemical compound | Result |
|---|---|
| Saponin | + |
| Flavonoid | + |
| Terpenoid | + |

liquid extract at a concentration series of 1000, 500, 250, 125 ppm; positive control with acarbose; negative control with solvent alone. After that, p-nitrophenyl-α-D-glucopyranoside solution (PNPG in 0.1 M phosphate buffer, pH 6.8) was added and incubated for 30 minutes at 37 C. The reaction was stopped by the addition of 100 µl of $Na_2CO_3$ (0.2 M) (Sheikh et al., 2016; Suryanti et al., 2022). The absorbance was measured using an Elisa reader at a wavelength of 405 nm. The $IC_{50}$ value was calculated by the formula:

$$IC_{50} = \frac{Control\ abs - Sample\ abs}{Control\ abs} x\ 100$$

## RESULTS AND DISCUSSION

Kedawung seeds (Mimosacease family) are widely used as part of local wisdom (ethnomedicine) in several places in Indonesia. The utilisation of natural materials is inseparable from the processing and preservation methods used. In kedawung seeds, the preservation method that is often used is roasting. However, this process is believed to affect the phytochemical constituents of kedawung seeds. In this study, the identification of phytochemical compounds from roasted kedawung seed extracts was carried out using a simple method utilising precipitate formation or colour change due to the complexation reaction of metabolite compounds with the reagents used. The test results can be seen in Table 1.

The phytochemical screening test of roasted kedawung seed extract showed positif results for all detection of metabolite. The phytochemicals have different properties which led to different pharmacology activity and characteristic of compounds. The saponin not only have non polar properties, it also show active surface properties due to bond between of hydrophilic (sugar) with (hydrophobic) sapogenin(Aryan et al., 2022; Reichert et al., 2018). The flavonoid and alkaloids both have unique structure that allows to form complexes with metal ions. The first have phenolic hydroxyl group that provided redox activity and cross linking with metal ions; while the later have nitrogen which have free lone pair electron that act as nucleophile. Following mechanism of both compound, it results in precipitates of metal with flavonoids (Pb complexes) and alkaloid (mercury complexes) (Kancherla et al., 2019; Spiegel et al., 2020; Walencik et al., 2024). The identification of terpenoids used Liebermann-Burchard method, which form colour change based on double bond system and presence of nonpolar bonds.

Table 2. Alpha glucosidase and toxicity of kedawung extract

| Test | $IC_{50}$ (ppm) | | $LC_{50}$ (ppm) |
|---|---|---|---|
| | Acarbose | Extract | |
| Alpha glucosidase | 0.25 | 265.24 | - |
| Toxicity BSLT | - | - | 78.18 |

The terpenoids were constructed by isoprene a functional groups which consist of two dienes and a methyl groups. This structure react with sulphuric acid and acetic anhydride without presence of water (Adu et al., 2019; Wutsqa et al., 2021).

Further analysis was conducted to investigate the effect of the roasting process on normality and alpha glucosidase enzyme inhibitory activity. The brin shrimp lethality test (BSLT) method is a rigid, simple, easy, fast and commonly used method for acute toxicity evaluation (Mastura et al., 2022). Menwhile, the alpha glucosidase enzyme inhibitory activity were conducted to investigate actual concentration of extract against alpha glucosidase; a enzyme that considered as a key of carbohydrate absorbtion in intestine (Akmal et al., 2025). The results obtained were represented by $LC_{50}$ values (lethal concentration that kills 50% of shrimp larvae) for toxicity, and $IC_{50}$ (Inhibotry concentration of 50% enzyme activity) (Athaillah et al., 2024; Bhatia et al., 2019; Sembiring & Putri, 2022). The $LC_{50}$ and $IC_{50}$ value could be seen in Table 2.

The BSLT is usually used as a preliminary test of acute toxicity before proceeding to sub acute or chronic toxicity. This method is done on the basis of counting the number of artemisia larvae that are still alive after being given a series of extracts and calculated by Probit analysis (Mastura et al., 2022). The results obtained showed the $LC_{50}$ value of the extract was 78.18 ppm. This value is recognised as being in the moderately toxic category which indicates the potential for harm to humans if consumed in large quantities (Meyer et al., 1982; Zakwan et al., 2023). This result is quite interesting, considering that roasting is one of the methods of processing kedawung seeds which is carried out for generations. This leads to speculation that there may be other steps in the roasting process that have been overlooked. In addition, other toxicity analyses that are recommended to be carried out are using in vitro acute toxicity tests with normal human cell lines or sub acute using other in vivo tests such as the zebra fish method.

The alpha glucosidase is an enzyme that used to absoprtion of glucose in gastrointestinal tract. The inhibition of this enzyme can reduce blood glucose levels, thus useful to prevent hyperglycemia in

diabetes mellitus patient. The drugs that categorized in this group were acarbose (McIver et al., 2025). The inhibitory ability of the extract on alpha glucosidase enzyme was tested using this drug as a positive control (Table 2).

The calculated $IC_{50}$ value of extract is 265.24 ppm showing a weak inhibitory effect. Interestingly, previous research shows that extracts from fresh kedawung seeds have a strong inhibitory effect on fractions with different solvent polarities. The best inhibitory effect is shown in the polar fraction and decreases as the polarity of the solvent decreases (Suryanti et al., 2022). This results test obtained shows a decrease in the ability to inhibit alpha glucosidase in kedawung seeds roast. This may be due to the destruction of thermolabile chemical compounds and the formation of new compounds during the hydration process as a result of high temperature heat application (Ajatta et al., 2019; Han et al., 2022). Therefore, it is necessary to study phytochemical content of roasting kedawung seed using more advanced instrument.

## CONCLUSION

The results of this study can be concluded that extract of kedawung seed that undergone roasting process has a moderate toxic properties. Meanwhile, it also has lower inhibiton effect of alpha glucosidase enzyme compared to fresh kedawung seeds extract.

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