Bekul Fruit, Potential Pharma food from Northern Region of Bali Island, Indonesia: Selected Phytochemical Analyses and Antioxidant Activity of Its Ethanol Extract

Agung Nova Mahendra¹, I Nyoman Tri Pramartha² and Agung Ari Chandra Wibawa³

¹Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia.  
²Medical Student, PSSKPD, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia.  
³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Mahasaraswati, Denpasar, Bali, Indonesia.  
*Corresponding Author E-mail: novamahendra@unud.ac.id

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Bekulfruit from Northern region (Buleleng regency), Bali, Indonesia, is commonly consumed fresh in the island of Bali or processed as local delicacy and used as part of religious offerings. Up to date, there is no data regarding the taxonomy, phytochemical composition, and antioxidant properties of this Balinese fruit. This study was aimed to investigate total phenolic content, tannin content and antioxidant activity of the ethanol extract of bekul fruit obtained from Banjar district, Buleleng regency, Bali. Total phenolic compound was quantified in terms of gallic acid equivalent (GAE) by using Folin-Ciocalteu method, meanwhile tannin content was determined in terms of tannic acid equivalent (TAE). IC₅₀ of the extract was determined using DPPH assay, and subsequently used in the calculation of antioxidant activity index (AAI) using the formula of Scherer and Godoy (2009). Bekul plant was revealed as Ziziphus jujuba Mill. Total phenolic and tannin content of the extract was 29.48 mg/100 g GAE and 91.06 mg/100 g TAE, respectively. The value of IC₅₀ was 77.40 mg/ml with antioxidant activity index (AAI) of 50.94. Ethanol extract of bekul (Ziziphus jujuba Mill.) fruit contains phenolic and tannin compounds. This extract is found to scavenge free radicals and possess very strong antioxidant activity in vitro. Taken together, these findings lead to the notion that bekul fruit from Northern region of Bali, Indonesia, is a promising pharma food.

**Keywords:** Ziziphus jujuba Mill. fruit, Ethanol extract, Total phenolics, Tannins, Antioxidant activity.

Endogenous antioxidant system is deployed by human body to mitigate the adverse effects of oxidative stress, thus maintaining the homeostasis. Unfortunately, in many pathological conditions, this system of mitigation is frequently overwhelmed and subsequently contribute to the genesis and progressivity of many diseases. Supplementation of exogenous antioxidants in these situations will enhance the capacity of human body to attenuate oxidative stress and restore the homeostasis, as reviewed elsewhere.¹ Natural products such as fresh fruits have long been known as good sources of exogenous antioxidants.² A local fruit known in Bali as bekul fruit has not been investigated for its biomedical potentials, especially as a source of antioxidants. North Balinese bekul fruit, cultivated in Banjar District, Buleleng Regency, Bali, Indonesia is...
variable in size and tastes sweet with a hint of sourness. The skin is green and glossy, similar to that of Malang apple from East Java region, Java, Indonesia. The fruit is usually eaten immediately after harvest under fresh condition, or mixed with a special sauce (shrimp paste-based sauce) and made into local delicacy known as rujakbekul (a kind of Indonesian traditional spicy salad with bekul fruit as its main ingredient). The fruit is also used as part of offerings in Balinese Hindu (Sanatana Dharma) religious ceremonies. Thus, bekul fruit is mainly used for local consumption and for religious purpose.

To the best of our knowledge, there is lack of information regarding the taxonomy, phytochemical composition, and antioxidant activity of North Balinese bekul fruit. Therefore, it is important to determine the species and investigate selected phytochemical properties of bekul fruit cultivated in North Bali. Specifically, a study on taxonomy, selected phytochemical constituents and antioxidant activity of North Balinese bekulfruit ethanol extract was conducted as an initial effort to explore its potential biomedical benefits.

MATERIALS AND METHODS

Species Determination

The whole live plant was obtained from a local bekul plantation in Banjar, Buleleng regency (North Bali region) and submitted to the office of Lembaga Ilmu Pengetahuan Indonesia/LIPI (Indonesian Institute of Sciences) at EkaKarya Bali Botanical Garden, Bedugul, Tabanan regency, Bali, for taxonomical analysis. The process of identification was done by three official botanists/taxonomists of LIPI. The plant was then kept in the botanical garden as trophy or voucher specimen.

Extraction of Bekul Fruit

Bekul fruits were air-dried and macerated in 80% ethyl alcohol for 48 hours. The macerate was then subjected to evaporation for removing water content, using a rotary vacuum evaporator. All procedures were done in Laboratory of Pharmacology and Therapy/Division of Drug Development and Laboratory Animal, Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University, Denpasar, Bali.

Total Phenolic and Tannin Content Analyses

Folin-Ciocalteu method was used in quantifying total phenolic content of bekul fruit ethanolic extract, as described in our previous study. A part (100 mg) of the extract was dissolved in 85% methyl alcohol, and then subjected to vortex and filtration. The resulting filtrate and standard solution were mixed respectively with Folin-Ciocalteu reagent and vortexed. The solutions were rested for 6 minutes and then mixed with 0.8 mL of 5% Na2CO3, vortexed and allowed to rest again for 30 minutes. These steps produced blue colour. The absorbances were measured at 760 nm. Linear regression curves were made to determine the formula y = ax + b, based on the absorbances and concentrations. The phenolic content of the sample was expressed as gallic acid equivalent (GAE) per gram of dry weight of the sample. UV-Vis spectrophotometry was used in the quantification of phenolics and tannins of bekul fruit ethanolic extract. Tannin content was expressed as tannic acid equivalent (TAE). Quantitative phytochemical analyses were done in Laboratory of Food Analysis, Universitas Udayana, Denpasar, Bali.

Determination of Antioxidant Activity

The assay we used in determining antioxidant activity of the extract is a slightly-modified version of the assay done by Aldarraji et al., as described previously in Dewi et al. Five concentrations of the sample were made using methyl alcohol as solvent. Each 100 mL of sample was mixed with 700 mL DPPH solution (0.1 mM), shaken well, and then incubated for 30 minutes in dark room (RT). Gallic acid was used as positive control. The absorbance values were read at wavelength of 517 nm. Percentage of DPPH free radical inhibition by the extract was calculated using the formula:

\[
\text{Inhibition (\%) = } \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]  

The results were made into a graph to obtain the linear regression equation and IC50 value. The antioxidant activity of the extract is expressed as antioxidant activity index (AAI), calculated using formula proposed by Scherer and Godoy.
AAI = \frac{\text{Final concentration of DPPH (µg/ml)}}{I_{50} \text{ (µg/ml)}} \tag{2}

Interpretation of AAI value was done based on the category proposed by Scherer and Godoy. If AAI < 0.5, a test substance was considered to have poor antioxidant activity; between 0.5 and 1.0, moderate antioxidant activity; between 1.0 and 2.0, strong antioxidant activity; and > 2.0, very strong activity.

**RESULTS**

**Plant Identification**

The result of species identification is presented in Table 1. The certificate of determination/identification was issued by Head of Exploration and Collection of Plants, EkaKarya Bali Botanical Garden – LIPI (certificate number: B-37/IPH.7/AP/I/2018).

**Table 1. The Taxonomy of Bekul**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Spermatophyte</td>
</tr>
<tr>
<td>Subdivision</td>
<td>Angiospermae</td>
</tr>
<tr>
<td>Class</td>
<td>Dicotyledinae</td>
</tr>
<tr>
<td>Order</td>
<td>Rosales</td>
</tr>
<tr>
<td>Family</td>
<td>Rhamnaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Ziziphus</td>
</tr>
<tr>
<td>Species</td>
<td>Ziziphus jujuba Mill.</td>
</tr>
</tbody>
</table>

**Total Phenolic Content**

The result of absorbance measurement for different concentrations of the sample is presented in Figure 1.

**Total Tannin Content**

The measurement of total tannin content was based on the reagent manufacturer’s standard (Sigma Aldrich). The procedure was initiated by making extract preparations having different concentrations. The result of tannin analysis is shown in Figure 2. The regression (r) value was found to be 0.9742 (with y = 0.114x + 0.0957).

**Free Radical Scavenging Activity and Antioxidant Activity Index**

Free radical scavenging capacity of bekulethanolic extract had been measured using 2,2 diphenyl-1-pycrilhidrazyl (DPPH) assay, and calculated using Formula 1. The IC$_{50}$ values were obtained from linear regression analysis of inhibition DPPH 0.1 mM. In this study, we obtained regression % inhibition y=0.6934x – 0.5106 with correlation coefficient value of 0.9485. The results of DPPH free radical scavenging activity and AAI

**Table 2. IC$_{50}$ and AAI of Bekul (Ziziphus jujuba Mill.) Fruit Ethanol Extract**

<table>
<thead>
<tr>
<th>Parameters of Antioxidant Properties</th>
<th>IC$_{50}$</th>
<th>AAI</th>
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<tr>
<td></td>
<td>77.40 µg/ml</td>
<td>50.94</td>
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**Fig. 1.** Concentration versus absorbance at 760 nm wavelength
quantification (based on calculation using formula 2) are shown in Table 2.

**DISCUSSION**

In current study, we aimed to reveal the taxonomy of North Balinese bekul, and to investigate total phenolics, tannin content and assessing *in vitro* antioxidant activity of bekul fruit ethanol extract. Taxonomically, *Ziziphus jujube* Mill. was revealed as the scientific name of bekul. This species is known as Asian native plant. Several relatives of bekul (*Z. jujube* Mill.) have been studied phytochemically, namely, *Z. lotus, Z. mauritiana, Z. mucronata, Z. spina-christi* and *Z. xylopylorus*. Bioactive phyto components and biomedicinal potentials of *Z. jujube* Mill. fruit had been discussed elsewhere. In a recent review, *Z. jujube* Mill. fruit is known to be ethnopharmaceutically used in China to treat blood deficiency (as “Qi” tonifier), and had been discussed both as a prophylactic and therapeutic natural product to combat anemia. Quantitative phytochemical analysis revealed that bekul ethanol extract contained phenolics as much as 29.48 mg/100 g gallic acid equivalent (GAE). Total phenols content in our study was much lower than that of 7 cultivars of Chinese *Z. jujube* fruit ethanol extract. This difference may be caused partly by the concentration of the ethanol used during the extraction. The ethanol concentration in our study was lower than that of Zhao *et al.*, i.e., 80% versus 95%, respectively. The ethanol extract of *Z. spina-christi* fruit from Oman was also shown to contain phenolic compounds and exhibit sound *in vitro* anti-inflammatory activity, compared to diclofenac sodium. Fruit from other species, i.e., *Z. mauritiana* obtained from Nigeria had been also shown to contain phenolic content, with higher amount than the specimen in our study. Total phenolic compounds from *Z. mauritiana* fruit aqueous extract was found to be 402.31±53.6mg GAE/100g. Methanol extract of *Z. xylopylorus* fruit from Karnataka, India, was observed to contain 6262 mg GAE/100g. The findings confirm that species and biogeographical factors are important determinants of secondary metabolite content, especially total phenolic compounds found in plants. The use of different solvents is also an important factor in determining the amount of extracted bioactive substances.

Tannin content in bekul fruit ethanol extract was revealed to be 91.06 mg/100 g TAE. Tannins in fruits of many plant species are a group protective compounds that act to protect plants against wide array of phytopathogens. Condensed tannins (and flavanols) are assumed to take predominant role in determining the antioxidant activity of ripe *Z. mauritiana* Lamk fruit. Since ripe bekul (*Ziziphus jujube* Mill.) fruits were used in current study, tannins may be assumed to contribute significantly to the antioxidant activity of its ethanol extract. There is currently lack of data regarding tannins content of *Z. jujube* Mill. fruit ethanol extract, using tannic acid as standard in the determination of tannin content. To tackle this issue we compare our result of tannin content determination to the findings of phytochemical (tannins) study on the relative of *Z. jujube* Mill., such as *Z. mauritiana* Lam. Similar to our finding, tannins is also present in ripe *Z. mauritiana* Lam. fruit obtained from Madya Pradesh, India. The ethanol extract of this Indian fruit was known...
to present in high amount (4+), but the exact amount awaits further investigation. Fruit juice of _Z. lotus_ was also revealed to contain both condensed and hydrolysable tannins. Moreover, procyanidin (condensed tannins) is present in the ethanol extract of another part of genus _Ziziphus_, such as shown in of _Z. mucronata_ Willd. subsp. _mucronata_ Willd bark.

Bekul fruit ethanol extract was showed to exhibit IC50 value of 77.40 µg/ml and very strong antioxidant activity (AAI > 2.0). Our specimen exhibited lower DPPH free radical scavenging activity than methanol extract of _Z. xylopylorus_ (Retz.) Willd. from Karnataka, India showed IC50 value 36.79 µg/ml, but higher activity than the extract of Nigerian _Ziziphus mauritiana_ fruit (IC50 value = 338.45 µg/ml). Bekul fruit ethanol extract was revealed to possess very strong antioxidant activity, based on the AAi value. Phenolics and tannins are secondary metabolites that ubiquitously exist in plants and their fruits. In current study, phenolic compounds and tannins may contribute synergistically to the significant in vitro antioxidant activity of bekul ( _Ziziphus jujube_ Mill.) ethanol extract.

It is possible that other parts of bekul plant may also contain significant amount of phenolic compounds and other bioactive substances. Choi _et al._ found that South Korean _Z. jujube_ seeds fractionated using different solvents exhibited substantial amount of total phenolics (ranging from 7.90 ± 0.47 mg GAE/g to 102.05 ± 2.42 mg GAE/g fraction). Both of our findings and that of Choi _et al._ promote the importance of conducting comparative phytochemical studies on various parts of North Balinese bekul plant, applying various solvents in the extraction process. Other factor that can be attributed to the variation of phytochemical composition, is the ripening stage of fruit. A study revealed that phenolic and tannin profiles of _Z. mauritiana_ Lamk fruits can be influenced by ripening or maturation stage. Therefore, it is also essential to consider this factor in studying the phytochemical composition of various parts of North Balinese bekul plant.

In order to be incorporated safely in clinical practice, a natural product must not be proved as genotoxic agent. Genotoxicity is a disruptive feature of an agent that may cause damage to DNA and/or cytocomponents that regulate the behaviour and functions of chromosomes. Recently, Indian _Z. jujuba_ fruit ethanol extract had been known to exhibit non-genotoxicity, both _in vitro_ and _in vivo_. Moreover, the extract had been also revealed to attenuate DNA alkylation _in vivo_, thus can be viewed as a phyto-antigenotoxin. The findings can be considered as preliminary signs of promising application in clinical settings, especially in the context of therapeutic safety. It is mandatory to extend this type of study in clinical settings to elucidate the safety profile of _Z. jujube_ fruit extract before assessing its health-promoting efficacy in humans.

Attenuated amount of endogenous antioxidants may predispose human body to oxidative stress and its associated damages. Oxidative stress is known to play significant roles in pathological entities such as cardiovascular disease (CVD), acute and chronic kidney disease (CKD), neurogenerative diseases (NDs), diabetes and cancer. Oxidative stress is also known to be associated with inflammaging in a diverse array of pathological states, as reviewed elsewhere. Since oxidative stress is linked to many crippling health problems, it is important to adhere to healthy lifestyle to promote health and halt the progression of diseases. One of the most convenient health-promoting ways is to consume natural products, such as fruits rich in antioxidants or high in antioxidant activity. Bekul ( _Z. jujube_ Mill.) fruit from northern region of Bali, Indonesia, is a promising example of such natural products.

**CONCLUSION**

North Balinese bekul was found to be _Ziziphus jujube_ Mill. The ethanol extract of its fruit had been revealed to possess phenolic compounds and tannins. The extract also exhibited free radical scavenging activity and very strong antioxidant property, based on DPPH assay and AAI value, respectively. Taken together, these findings indicate that bekulfruit from Northern region of Bali, Indonesia, is a pharma food with promising antioxidant activity.
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Conflict of interest

The authors declare that they do not have any conflict of interest.

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