Potential study of *Dillenia serrata* Thunb. fruit extract from Bali Botanical Garden’s collection

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Abstract

*Dillenia serrata* Thunb is a member of the *Dillenia* clan which is endemic to Sulawesi. *D. serrata* fruit is a seasonal fruit and can be used as a cooking ingredient as a sour taste, raw material for several food products such as candied fruit and syrup, and has the potential to be processed into chips and jam. Traditionally, the leaves and bark of *D. serrata* are also used by the local community as a sprue medicine, fever, wound medicine, treating swelling or inflammation and treating vomiting of blood. It is believed that there are still many untapped benefits from this plant. This study was conducted to determine the effectiveness of *D. serrata* fruit extract as an antimicrobial that causes disease in humans, and to determine its effectiveness as an antioxidant. The antioxidant test was carried out using the DPPH method while the antimicrobial test was carried out by the agar diffusion method (Kirby-Bauer). The ripe *Dillenia* fruit was extracted using methanol, the extract was then diluted in various concentrations for the DPPH test. The results showed that the *D. serrata* extract had the ability as an antioxidant. The antioxidant activity of fresh fruit extracts was higher than that of dried fruit extracts. *D. serrata* extract is effective in inhibiting the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans* bacteria.

Keywords: Antimicrobial, antioxidant, DPPH.

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Introduction

The *Dillenia* genus is a member of the *Dilleniaceae* family which is a tree, shrub, or shrub, which grows spread from Madagascar and the Seychelles Islands in the west, to the north to the Himalayas and southern China, across Southeast Asia and Australasia, to Fiji in the east (Rugayah et al., 1995). This plant is also commonly called simpur, sempur or sempu. The name *Dillenia* was given after the name of the German botanist, Johann Jacob Dillenius (1687-1747) (Quattrochi, 2012). These plants are usually in the form of shrubs, shrubs, and evergreen trees, reaching 40 m in height and up to 125 cm in diameter. The *Dillenia* species are monoeocious plants that produce attractive flowers and yellow fruit. They can grow from the coast to an altitude of about 2000 m above sea level. They mostly form large leaves and flowers with few inflorescences (Dickison, 1979). Around the world, this plant genus consists of about 60 species (Kader & Zeinab, 2015). Their bark is unique in its grayish, reddish brown color, and the wood of several *Dillenia* species can be used in furniture making (Hoogland, 1952). Several species of this genus also produce edible sweet and sour and astringent fruits (Hoogland, 1952; Jansen et al., 1992; Kerrigan et al., 2011; Lim, 2012; Saha & Sundriyal, 2012) and are cultivated as ornamental plants (Hoogland, 1952; Kerrigan et al., 2011).

Many of the benefits possessed by plants from this genus, certain species of *Dillenia* produce red dyes and traditional medicinal ingredients (Rugayah et al., 1995). *D. aurea* mixed with several other additives, can be used as a drug to treat thrush, intestinal thrush, and inflammation of the gums (Heyne, 1987). The fruit of several species of *Dillenia* can be either eaten, fresh or after processing, used as pickles or mixed in cooking. Certain fruits of *Dillenia* can also be used in shampooing, to get rid of head lice, or to wash clothes (Heyne, 1987; Rugayah et al., 1995). *Dillenia* plants are known to have good medical value so that people use them as medicine (Kader & Zeinab, 2015). Certain species such as *D. indica*, *D. obovata*, *D. ovata* and *D. suffruticosa* are commonly used as ornamental plants in gardens and roadsides (Rugayah et al., 1995).

*Dillenia serrata* Thunb. is a member of the *Dillenia* genus endemic to Sulawesi (Kainde, 2011; Pitopang, 2014). This species is well known by the local people of Sulawesi, especially the people of South Sulawesi and Central Sulawesi. The people of South Sulawesi know this plant by the name dengen, while the people of Central Sulawesi know it by the name jongi (Ilung, 2017). The shape, size and taste of *D. serrata* fruit are similar to oranges. When ripe, the fruit skin will open on its own like a flower petal. Although the fruit of *D. serrata* is ripe but the taste of the fruit is very sour when eaten directly, therefore this fruit is less attractive and has not been widely used so that it has not been widely cultivated. *D. serrata* fruit is a seasonal fruit with a relatively short shelf life.

The fruit of *D. serrata* can be used as a cooking ingredient to give sour taste, raw materials for several food products such as candy, and syrup, and has the potential to be processed into sweets, chips and jam (Ilma, 2012; Hasniarti, 2012; Mustafa, 2017). The bark of *D. serrata* is traditionally used by the community as a medicine for fever and wound (Irnawati et al., 2017), treating vomiting of blood (Windardi et al., 2006), and

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treating swelling or inflammation (Jalil et al., 2015). The fruit of *D. serrata* contains citric acid, vitamin C and beta-carotene compounds (Hasniarti, 2012; Illing, 2017; Irnawati et al., 2017). In addition, this fruit contains secondary metabolic compounds such as alkaloid compounds, flavonoids, saponins, polyphenols and triterpenoids (Gandhi et al., 2013; Bandara et al., 2015; Illing, 2017). It is believed that there are still many untapped benefits from this plant. This study was conducted to determine the effectiveness of *D. serrata* fruit extracts grown in Bali Botanic Gardens as an antimicrobial that causes disease in humans, and to determine its effectiveness as an antioxidant.

**Methods**

**Plant samples and location**

*Dillenia serrata* plant samples were taken from the Bali LIPI Botanical Garden Plant Collection. This plant was originally collected from Bancea Village, Pamona Selatan District, Poso, and Central Sulawesi in 1984. In its area of origin, this plant was found to grow at an altitude of 650 m. above sea level. five plants were taken to be used as a living collection (Figure 1).

The research was conducted at the Plant Breeding and Potential Laboratory, Research Center for Plant Conservation and the LIPI Botanical Garden, Bali.

![Figure 1. Photograph of *D. serrata* fruit, flowers and trees](image)

**Tools and materials**

The tools used in this research are laminar air flow (LAF), rotary vacuum evaporator, autoclave, spectrophotometer, analytical balance, vortex mixer, mortar, a set of glassware and micropipettes. The materials used in this study were *D. serrata* fruit, absolute methanol, 95% technical methanol, DPPH (1,1-diphenyl-2-pikrihidrazil) and filter paper.

**Sample collection and extraction**

The fruit samples used in this study were ripe fruit with a bright yellow color. To make a fresh extract, 100 grams of *D. serrata* fruit are immediately chopped and crushed using a mortar. To make a dry extract as much as 100 grams of *D. serrata* fruit, sliced into thin slices, then oven at 45 °C for ± 3 days until completely dry. Each fresh and dry sample was mixed with 1000 ml of methanol. This bath was then left to stand for ± 3 days in a dark place and room temperature. After 3 days of immersion, it is filtered using filter paper and evaporated using a rotary vacuum evaporator to separate the solvent from the crude extract. The crude extract obtained is then used in further tests.

**Antioxidant activity test using the DPPH method**

The antioxidant activity test was carried out according to the method of Chow et al. (2003) with modifications. Each crude extract of *D. serrata* was made into stock solutions with a concentration of 1000 ppm. The stock solution was again diluted into several concentration series, namely: 50; 100; 150; 200; 250; 300; and 350 ppm. The test was carried out by mixing 1 ml of each extract concentration with 4 ml of 40 ppm DPPH solution. The mixture is shaken and left for 30 minutes at room temperature in a dark place. As a comparison, ascorbic acid was also calculated for its antioxidant activity, while the concentration variations were: 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm and 12 ppm. Furthermore, each mixture was measured its absorbance (A) using a UV-Vis spectrophotometer at a wavelength of 517 nm. Quantitative calculations are carried out by determining the inhibitory power of the sample free radicals which are calculated by the following formula:

\[
\% \text{ inhibition} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100\%
\]

**Antimicrobial test**

Antimicrobial activity testing was carried out using the agar diffusion method (Kirby-Bauer disc diffusion method) (Bauer et al., 1966) which was modified using NA media (nutrient agar). Some of the microbes tested were disease-causing microbes in humans, including *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus mutans*. Observations were made for 1-3 days to see whether there was an inhibition zone formed in the treatment. The inhibition zone diameter was measured to compare the effectiveness of each extract.

**Results**

The results of testing for antioxidant activity using DPPH showed that the DPPH solution which was originally purple turned yellowish, this indicates that the extract of *D. serrata* has the ability as an antioxidant. The antioxidant activity of fresh fruit extracts was higher than that of dried fruit extracts. Fresh fruit extract has a value of IC50: 516.70 and dry fruit extract has a value of IC50: 981.58. Meanwhile, ascorbic acid has an IC value of 50: 5.02. The antioxidant ability of *D. serrata* extract is classified as very weak, because its IC50 value is more than 200 ppm (Figure 2).

![Figure 2. The regression graph of antioxidant testing, (A) fresh fruit extract, (B) dried fruit extract](image)
The results of the antimicrobial test showed that the two extracts of D. serrata were effective in inhibiting the growth of Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus mutans but were not effective at inhibiting the growth of Candida albicans and Salmonella typhimurium. The extract is effective in inhibiting bacterial growth seen from the formation of an inhibition zone in the experiment, where this does not happen if the extract is not effective at inhibiting bacterial growth. The antioxidant activity of fresh fruit extracts was higher than that of dried fruit extracts. This can be seen from the ratio of the average diameter formed, where the fresh extract forms a wider average diameter than the dry extract (Table 1).

Table 1. Antibacterial test of D. serrata fruit extract

<table>
<thead>
<tr>
<th>No.</th>
<th>Extract type</th>
<th>Candida albicans</th>
<th>Pseudomonas aeruginosa (gram-negative)</th>
<th>Salmonella typhimurium (gram-negative)</th>
<th>Staphylococcus aureus (gram-positive)</th>
<th>Streptococcus mutans (gram-positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dilenia (fresh fruit extract)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Dilenia (dried fruit extract)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Information:
1) +: able to inhibit growth
2) -: unable to inhibit growth

**Discussion**

The antioxidant activity of the fruit extracts of *D. serrata* may be caused by compounds that act as antioxidants such as: proanthocyanidin, vitamin C (ascorbic acid), and 3,5,7-Trihydroxy-2-(4-hydroxy-benzyl)-chroman-4-one. Proanthocyanidin is a polyphenolic compound isolated from the fresh fruit of *D. indica* which shows significant antioxidant activity (Fu et al., 2015). The 3,5,7-Trihydroxy-2-(4-hydroxy-benzyl)-chroman-4-one compound which is a flavonoid group is a compound that is an antioxidant in the extract of *D. indica* (Kaur et al., 2016). Irnawati et al. (2017) stated that *D. serrata* juice taken from Lamomea Village, Konda District, South Konawe Regency, Southeast Sulawesi, contains 1.09% vitamin C with weak antioxidant activity against DPPH free radicals with a value The IC50 is 161.63 mg/L. Meanwhile, Syahruni&Nur (2015) stated that *D. serrata* fruit extracts taken from the Malili area, East Luwu Regency, South Sulawesi, showed that ethanol extract, water fraction, ethyl acetate fraction, and hexane fraction had antioxidant activity with an IC50 value respectively 200.9 ppm, 103 ppm, 108.4 ppm and 135.2 ppm.

The antioxidant activity of *D. serrata* extracts grown in the Bali Botanic Garden was classified as very weak. The weak ability of the extract is thought to be due to the low content of antioxidant compounds in the extract. You et al. (2011) stated that the concentration of chemical compounds in plants can be very different depending on various factors such as genetics of cultivar species, season, and location of growth. Cantin et al. (2012) stated that there are large differences in phytochemical profiles between genotypes / cultivars of the same species. The stage of fruit maturity was also reported to affect the total phenolic and anthocyanin contained in the fruit (Wang & Lin, 2000). Several other studies have also shown that environmental conditions, field maintenance systems, and growing seasons have an impact on antioxidant levels (Howard et al., 2003; Crespo et al., 2010; Kruger et al., 2011).

The antioxidant activity of dried fruit extracts was lower than that of fresh fruit extracts. This may be due to the drying of the fruit using the oven. When temperatures set too high in the extraction process it can cause damage to several groups of plant compounds (Margaretta et al., 2011; Yuliantari et al., 2017; Chairunnisa et al., 2019).

When compared with the IC50 value of ascorbic acid, *D. serrata* extract has a much lower activity; this is presumably because *D. serrata* extract is still a crude extract (not a pure compound) which still contains many other compounds that are not antioxidants. With ascorbic acid which is a pure compound that does have antioxidant properties. The antioxidant effect on *D. serrata* may not only result from a single antioxidant vitamin, but can be generated from a synergistic combination of various antioxidants (Cao et al., 1998).

*D. serrata* extract can inhibit bacterial growth possibly because the extract contains compounds that are anti-bacterial. Nick et al., (1995) stated that the antibacterial activity of *D. papuana*, which is in the same genus as *D. serrata*, is derived from triterpenoid acid compounds. Triterpenoid acid compounds such as Betulinic acid, Dillenic acid A, Dillenic acid B, Dillenic acid C, Dillenic acid D, Dillenic acid E, 3-Oxooolean-1,12-dien-30-oic acid, and 3-Oxooolean-12-en -30-oic acid derived from the plants *D. papuana* and *D. philippinensis* is a compound reported to have antioxidant properties (Nick et al., 1994;Nick et al., 1995b; Ragasa et al., 2009).

Plants that are closely related tend to produce similar metabolites, so it is possible that *D. serrata* also produces triterpenoid acid compounds which are anti-bacterial. Each secondary metabolite compound has a different way of working (Ningsih, 2017). The mechanisms of action of secondary metabolites include inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Ernawati, 2015).

**Antibacterial and anti-fungal activities of Dillenia spp.**

Several other *Dillenia* species are also reported to have antibacterial and antifungal properties such as *D. indica* (Apu et al., 2010; Alam et al., 2011; Jaisswal et al., 2014). *D. papuana* (Nick et al., 1995a), *D. pentagyna* (Haque et al., 2008), *D. sufruticoso* (Waite et al., 2004) and *D. sumatrana* (Grosvenor et al., 1995b). Extracts of
D. indica, D. papuana, D. pentagyna, D. suffruticosa, and D. sumatrana were reported to have growth inhibition against Gram positive and negative bacteria (Sabandar et al., 2016). However, they showed weak inhibition against the fungi Aspergillus fumigatus, A. niger, Candida albicans, C. arriza, C. crusei, Penicillium sp., Rhizopusoryzae, Saccharomyces cerevisiae and Trichoderma viride (Nick et al., 1995a; Wiart et al. al., 2004; Haque et al., 2008; Apu et al., 2010; Smitha et al., 2012). Seven types of triterpenoids from the Dillenia plant have been shown to have antimicrobial action (Nick et al., 1995b; Ragasa et al., 2009). Strong growth inhibition against Escherichia coli, Bacillus subtilis and Micrococcus luteus has been demonstrated by Dillenia papuana (Nick et al., 1995b). The same growth inhibition of Dillenia philippinensis was also reported against E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, and B. subtilis (Ragasa et al., 2009). These findings suggest that the Dillenia plant has potential as an antimicrobial agent supporting its traditional use for the therapeutic treatment of microbial infections related to diseases such as diarrhea, dysentery, septic chemical infections and skin-related diseases (Sabandar et al., 2016).

Utilization of Dillenia spp.

Dillenia species have been widely used as medicinal plants by indigenous peoples in South and Southeast Asian countries (Lim, 2012), including India, Nepal, Sri Lanka, Bangladesh, Laos, Thailand, Vietnam, Malaysia, Indonesia, the Philippines, and Papua New Guinea. Almost all parts of the Dillenia plant have traditionally been used for medicinal purposes. Fresh and dry ingredients from various parts of the Dillenia plant are processed into stew, poultices, fruit juices and mucus for the treatment of diarrhea, wounds, cancer, diabetes, fever, cough, rheumatism, urinary disorders, skin diseases, and aches, as well as hair tonics. D. excelsa, D. ovata, and D. parviflora have traditionally been used to cure diarrhea (Burkill, 1966; Srinith et al., 2009; Quattrocchi, 2012).

Skin-related diseases such as leucoderma, skin itching, skin rashes, and eczema can be treated using the leaves, fruits, and bark of D. andamanica, D. indica, D. ovata, and D. pentagyna (Prasad et al., 2008; Boer et al., 2012; Quattrocchi, 2012; Bhat et al., 2014). In addition, the bark of D. aurea and D. parviflora, as well as the leaves of D. suffruticosa, in the form of a paste or poultice are applied to the skin to heal wounds (Mat-Salleh&Latiff, 2002; Quattrocchi, 2012; Junsongduang et al., 2014). Juice and stew of fruit and stem bark, as well as the leaves of D. indica, D. pentagyna, and D. suffruticosa, are used to reduce cancer growth, particularly breast and stomach cancer (Ahmad &Holdsworth, 1995; Sharma et al., 2001; Das et al., 2008; Prasad et al., 2008; Rosangkima et al., 2008; Dubey et al., 2009). In addition, fruit juices of D. indica and D. philippinensis were given orally to cure fever and cough symptoms (Angami et al., 2006; Macahig et al., 2011; Quattrocchi, 2012). Mixed fruit and petal juice of D. indica and powdered stem bark of D. pentagyna is given daily for the treatment of diabetes (Dubey et al., 2009; Pavani et al., 2012; Ripunjoy et al., 2013). During pregnancy, the root of D. indica is often used to abort the uterus (Quattrocchi, 2012), while the bark of D. papuana and D. pentagyna is used to assist labor during labor and to avoid infection after delivery. (Nick et al., 1994; Dubey et al., 2009). Processed D. pentagyna and D. suffruticosa in the form of a paste applied to the joint area to relieve rheumatism (Quattrocchi, 2012; Hanum & Hamzah, 1999). In addition, the juice and fruit mucus of D. indica, D. pentagyna and D. philippinensis are used to clean hair, treat hair loss and eliminate dandruff (Saikia et al., 2006; Macagih et al., 2011; Rahman et al., 2011a). Dillenia fruit with sweet and sour taste, consumed directly or juiced with sugar as a fresh and healthy drink. In addition, the bark and roots of some Dillenia plants were reported to be neutralizers of food poisoning (Grosvener et al., 1995b; Islam et al., 2014).

In conclusion, the fruit extract of D. serrata grown in the Bali Botanic Garden has the ability as an antioxidant with very weak activity. The antioxidant activity of fresh fruit extracts was higher than that of dried fruit extracts. Extract of D. serrata is effective in inhibiting the growth of Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus mutans but it is not effective in inhibiting the growth of Candida albicans and Salmonella typhimurium.

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