The antioxidant and antibacterial study of *Canarium indicum* L. latex extract

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Abstract. Recently, plant extracts are natural additives that are in great demand. Many biological activities of plant extracts in the fields of food and health, make research on plant extracts quite rapid. *Canarium indicum* L. is one of the most famous fruits in Indonesia. However, usually only the seed of *C. indicum* that was studied such as anti-inflammatory, antirheumatic, and hepatoprotective. In this paper, the antimicrobial and antioxidant activities of *C. indicum* latex were studied. The *C. indicum* extract were prepared by maceration method using ethanol for 48 hours. After the evaporation, the crude extracts were evaluated its biological activity. The antibacterial activity was performed using dilution method and the antioxidant activity was using DPPH assay. The scavenging activity of *C. indicum* latex extracts values in the range of 10.92 – 58.36% with the extract concentration of 100 to 800 ppm, respectively. The antibacterial activity of *C. indicum* latex extract was conducted against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*). The inhibition zone *C. indicum* was 6.39±0.06 mm against *S. aureus* and 2.38±0.62 mm against *E. coli* at extract concentration of 10000 ppm. The results obtained indicate that *C. indicum* latex extract is favourable to be applied in the fields of medicine and health.

1. Introduction

Antioxidants are substances or materials that could significantly inhibit oxidative process. Antioxidant can protect cells so that the harmful effect of free radicals does not occur. Several studies reported the bad effect of free radicals to living things include aging, stroke, diabetes, cancer and neurodegeneration [1-3]. Food and pharmaceutical industries usually used synthetic antioxidant, to prolong the product shelf life, especially to inhibit the lipid oxidation. Commonly used synthetic antioxidant is butylated hydroxytoluene, t-butyldihydroquinone and butylated hydroxyanisole [4]. Butylated hydroxytoluene was effective in fish products, animal fats, packaging material and low-fat food [5]. However, negative side effects of synthetic antioxidant such as its toxicological effect to various species was become a limitation of the using of synthetic antioxidant [6].

Secondary metabolites produced by plants, herbs, vegetable are usually containing chemical substance such as polyphenols, alkaloids, flavonoids, and terpenoids [7]. Secondary metabolites in plants are protecting their lives from UV radiation, dryness or microbial infection [8]. Recently, interest in secondary metabolites from plants focused on tannin, flavonoids and anthocyanin, that be proven to have an important role in prevention of several diseases [9]. These compounds were having high antioxidant properties [10]. Moreover, polyphenol also have good antibacterial activity, so that could be a candidate for antibiotics [11].
Bacteria as a single cell organism, have an important role in human life. Some of it have been used in various industries such as in pharmaceuticals and food. However, bacteria could be also dangerous to human body, since they can generate many diseases and infections [12]. Antibiotics or antimicrobial agents have been seriously needed and saved peoples from bacterial infections [13]. However, several microorganisms have an ability to resist the antibiotic [14], so that new antimicrobial agents with medicinal properties from nature have been studied extensively [15, 16]. Meanwhile, some infection and food spoilage by microbes were becoming problem in public health [17-19]. Several bacteria such as Bacillus subtilis, Escherichia coli and Staphylococcus aureus are identified as the cause of food spoilage [20]. Thus, search on antimicrobial compounds resistant bacteria is an urgent [21].

The genus of Canarium has approximately 100 species worldwide [22]. Canarium indicum L. or Kenari in Indonesian language, is one of the species of Canarium. It is an indigenous plant in Indonesia, especially in the eastern region such as Maluku islands [23]. The distribution of C. indicum plants spread in various countries, including Indonesia, the Philippines, Papua New Guinea, and Malaysia [24]. The seed of C. indicum is usually used as a snack food. The oil from C. indicum seed also comparable to other plants such as cashew, hazelnuts, and macadamia [25]. Moreover, the other parts of C. indicum such as leaves, stems and exocarp of C. indicum seed also had been studies for treating disease and natural food colouring [24]. However, the bioactivity studies about latex of C. indicum is still very rare. Because of that, the aim of this study is to determine the antioxidant and antibacterial activity of C. indicum latex extract. The results of this study hopefully can be a knowledge for the community, especially for those who have access to C. indicum trees.

2. Materials and methods

2.1. Sample collection
The sample of C. indicum latex was obtained from Papua Province, Indonesia. The sample was dried with indirect exposure of the sun. For the next extraction process, the dried latex was powdered.

2.2. Preparation of C. indicum latex extract
The powder of C. indicum latex was macerated using ethanol 95% for two day at room temperature (1:8 w/v). It was then filtered and evaporated using vacuum rotary evaporator to obtain ethanolic extract of C. indicum latex.

2.3. FTIR spectroscopy analysis
The sample was mixing with KBR to make a KBr pellet. The FTIR spectrometer used was Shimadzu 8201 PC (Japan). The spectra were achieved in the range frequency of 4000 to 500 cm\(^{-1}\).

2.4. DPPH radical scavenging activity assay
The radical scavenging activity of C. indicum latex extract was performed using DPPH assay according to Indrianingsih et al (2020) with slight modification [26]. Several concentration of C. indicum latex extract were prepared using methanol as solvent. It was then reacted with DPPH solution (1.01 mM) for 30 minutes at room temperature. The room was in the dark condition to avoid the effect of light on DPPH since it was sensitive to light. The absorbance of the final samples was measured using Elisa Reader at 517 nm. The DPPH radical scavenging activity was calculated by the equation as follows:

\[
\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]

where \(A_0\) is absorbance of the control and \(A_1\) is absorbance of the sample. The assays were carried out in triplicates. Ascorbic acid was used as the standard control for this assay.

2.5. Antibacterial activity assay
The ability of C. indicum latex extract to inhibit the bacteria was performed using agar well diffusion method according to literature with some modification [27]. The extract was dissolved in DMSO at
several concentration (500; 1000; 10,000 ppm). One hundred microliter of bacteria suspension was inoculated in nutrient agar plates. The extract solution was then added into the wells in the solid media. After incubation at 37°C for 24 hours, the diameter of inhibition zone was measured. In this study, ampicillin was used as positive control while DMSO was used as negative control.

3. Results and Discussion
In this study, C. indicum latex extract was obtained by maceration process using ethanol. The latex of C. indicum was rarely studied, however, the nut of C. indicum were extensively studied. Several literatures reported the composition of C. indicum nut and kernel. Carbohydrate, vitamin E, and fatty acids (palmitic acid, stearic acid, oleic acid and linoleic acid) were obtained from kernel of C. indicum [28]. The yield of C. indicum latex extract showed in Table 1 as of 44.01 % (w/w).

| Sample  | Dry weight (g) | Extract weight (g) | Yield (%w/w) |
|---------|----------------|--------------------|--------------|
| C. indicum | 61.69         | 27.16              | 44.01        |

The capability of C. indicum latex extract to scavenge free radical was evaluated using DPPH radical scavenging assay. This method is the commonly used to perform the antioxidant activity capability of plant extracts. The antioxidant of C. indicum latex extract can be seen in Figure 1.

![Figure 1](image)

**Figure 1.** The antioxidant activity of C. indicum latex extract using DPPH assay.

Based on the DPPH assay, the higher the concentration of C. indicum latex extract, the higher the radical scavenging activity. The C. indicum latex extract has high activity as of 10.92% at 100 ppm, 12.74 % at 200 ppm, 27.12% at 400 ppm and 58.36 % at 800 ppm. The IC 50 of C. indicum latex extract is 697.45 ppm, meanwhile the antioxidant activity of ascorbic acid as standard as of 76.7 at 25 ppm. A study by Djarkasi et al. (2017) reported that C. indicum nut contains phenolic and flavonoid compounds so that it possessed a high antioxidant activity. The quantification of phenolic substances in C. indicum L. methanolic extract as of 7.4 to 8.8 mg GAE/g. Meanwhile, the vitamin E content of C. indicum nut around 439.36 ppm [29]. It had positive correlation between the antioxidant activity with phenolic and vitamin E contents. It also presented that tannins extracted from the leaves of Canarium album L showed potent antioxidant activity with IC50 of 56.8 µg/ml using DPPH assay [30]. A research also isolated the
pure compounds from *Canarium album* L. that were hyperin, ellagic acid and brevifolin that also had good antioxidant activity [31].

In this research, antibacterial activity of *C. indicum* latex extract was evaluated using agar well diffusion method. The capability of *C. indicum* latex extract to inhibit the bacteria was evaluated by its inhibition zone diameter against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*. Ampicillin was used as the positive control. The results of antibacterial activity of *C. indicum* latex extract are presented in Table 2. At the concentration of 500 and 1000 ppm, *C. indicum* latex extract did not show inhibition activity of bacterial growth for both *E. coli* and *S. aureus*. At concentration 10,000 ppm, *C. indicum* latex extract inhibited *E. coli* growth with inhibition zone diameter as 2.38±0.62 mm and inhibited *S. aureus* growth with inhibition zone as of 6.39±0.06 mm. Meanwhile DMSO that was used as negative control showed no inhibition zone diameter against the bacteria. *C. indicum* latex extract showed moderate antibacterial activity against *E. coli* and good antibacterial activity against *S. aureus*.

Table 2. Antibacterial activity of *C. indicum* latex extract against *E. coli* and *S. aureus*.

| Bacterial strain | Sample concentration (ppm) | Well 1 | Well 2 | Mean       |
|------------------|-----------------------------|--------|--------|------------|
| *E. coli*        | 500                         | -      | -      | -          |
|                  | 1000                        | -      | -      | -          |
|                  | 10,000                      | 2.815  | 1.935  | 2.38±0.62  |
| *S. aureus*      | 500                         | -      | -      | -          |
|                  | 1000                        | -      | -      | -          |
|                  | 10,000                      | 6.435  | 6.345  | 6.39±0.06  |
| Ampicillin       | 500                         | 3.07   | 3.11   | 3.09±0.03  |
| DMSO             | -                           | -      | -      | -          |

Literature studies showed that the research on antibacterial or antimicrobial properties from *C indicum* extract are still very rare. However, some literature has studied the bioactivity of the genus of Canarium. It was about only 12% of total Canarium species that had been studied for its biological activities included pharmacological activity [30]. Antibacterial activities against *Vibrio cholera* with MIC as of 0.62 mg/ml was reported from the dichloromethane extract of *Canarium schweinfurthii* while its ethyl acetate extract was active against *P. vulgaris* and *S. aureus* with MIC value as of 5 mg/ml and 10 mg/ml, respectively. The ethanol extract of *C. schweinfurthii* was also active against *V. cholera* and *P. vulgaris* with MIC values of 0.62mg/ml and 10mg/ml respectively [32]. The essential oil of *C. schweinfurthii* also active against *S. aureus*, *S. pyogens* and *S. enterica* with an inhibition zone of 18 mm, 25 mm and 27 mm, respectively [33]. Another species of Canarium, that was *Canarium patentinervium* also inhibited significantly against *S. aureus*, *B. cereus* and *P. aeruginosa* from its ethanol and hexane extract of barks and leaves [34]. Some mechanism of how the antibacterial peptides destroy the bacteria was reported by a literature, that is the antimicrobial agents could bind to bacterial DNA after reach the inner cells through membrane. The membrane itself can disrupt or not was possible.

The functional group of *C. indicum* latex extract were analysed using FTIR spectroscopy. The FTIR spectra between 4000 to 500 cm⁻¹ represent the chemical component on its functional groups of *C. indicum* latex ethanolic extract (Figure 2; Table 3.).
Figure 2. The FTIR spectra of *C. indicum*.

The functional groups detected in *C. indicum* latex extract were consist of OH (could be come from phenol and alcohols), C-H of alkane, C = C of aromatic, and C = O from aldehyde, ketone, ester, or carboxylic acid. The OH (hydroxyl) group was observed by absorption peak at 3425.58 cm\(^{-1}\) (broad), CH alkane was observed by absorption peak at around 2862.36-2924.58 cm\(^{-1}\) with high intensity, C = C group was observed by absorption peak at 1635.64 cm\(^{-1}\) (sharp) and C = O was indicated by the absorption peak at 1705.1 cm\(^{-1}\) (sharp).

**Table 3. Interpretation of FTIR spectra of *C. indicum* latex.**

| Functional group vibration                  | Wavenumber (1/cm) | Intensity  |
|--------------------------------------------|-------------------|------------|
| O-H, phenol, alcohols                      | 3425.6            | broad      |
| C-H, alkane                                | 2862.4-2924.6     | sharp      |
| C=C, aromatics                             | 1635.6            | sharp      |
| C=O, aldehyde, keton, ester, carboxylic acid| 1705.1            | sharp      |

The FTIR spectra of *C. indicum* showed that it possibly contained phenolic compounds. Another study revealed that several secondary bioactive metabolites from the genus Canarium L. contained terpenes include monoterpenes, carotenoids, sterols, triterpenes, coumarins, furans, carboxylic acids and phenol [30]. Single compounds also had been isolated from *Canarium album* L and the study of its biological activity showed that they had good antioxidant activity [31]. Djarkasi *et al.* (2017) also reported that *C. indicum* nut contain several amino acids such as arginine, leucine, aspartate and glutamate [29]. The various amino acids in *C. indicum* nut indicated that it can be had maintain human health since amino acids had the function to regulate human metabolism [35]. Another study showed that besides antioxidant and antibacterial capability, the extract of Canarium species also had an ability as an anti-inflammatory agent [28], an anti-atherosclerosis agent [36] and to maintain blood glucose levels [37].

4. Conclusion

In conclusion, *C. indicum* latex collected from Papua Province, Indonesia exhibit a potent antioxidant and antibacterial activity. *C. indicum* latex extract showed moderate antibacterial activity against *E. coli* and good antibacterial activity against *S. aureus*. The antioxidant activity of *C. indicum* latex extract was moderate compare to ascorbic acid as positive control. It is likely that the antioxidant and antibacterial activity was attributed to the phenolic compounds of *C. indicum* latex extract. More functional use and bioactivity of the *C. indicum* latex extract still need to further be performed.
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