Food Research 8 (3) : 513 - 519 (June 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Antioxidant and antibacterial activity of nutgrass tuber (*Cyperus rotundus* L.) on extraction with different solvent polarity and particle sizes

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Article history:
Received: 19 May 2022
Received in revised form: 29 August 2022
Accepted: 15 June 2023
Available Online: 30 June 2024

Keywords:
Cyperus rotundus,
Nutgrass,
Tubers,
Antioxidant,
Antibacterial

DOI:
https://doi.org/10.26656/fr.2017.8(3).261

Abstract

Nutgrass tuber (*Cyperus rotundus* L.) is a wild weed often used as traditional medicine locally, having potential as an antioxidant and antibacterial. This study aimed to determine the effect of the type of solvent with different polarity and particle size on the antioxidant and antibacterial activity of nutgrass tuber (*C. rotundus* L.) extract and to determine the best type of solvent and particle size to produce nutgrass tuber extract with the highest antioxidant activity and antibacterial activity. The experiment design was a factorial randomized block design (RBD). A total of three different solvent treatments (ethanol, ethyl acetate and *n*-hexane) was the first factor while the particle size with three treatment levels of 40-, 60-, and 80-mesh sieves was the second factor. Data were then analyzed by analysis of variance (ANOVA) followed by Tukey's test. The results showed that the type of solvent, particle size, and their interactions were highly significant on the antioxidant activity and the antibacterial activity against *Staphylococcus aureus*, while the antibacterial activity against *Escherichia coli* had a significant effect by the interaction. For the antioxidant activity test, the best treatment was ethanol with particle size produced using the 80-mesh sieve resulting in an IC_{50} value of 192.18±0.43 ppm. The best antibacterial activity was the extract using ethyl acetate and the particle size produced using the 80-mesh sieve. Meanwhile, the antibacterial activity against *E. coli* and *S. aureus* was 3.53±0.19 mm^2/mL and 10.67±0.14 mm^2/mL (AU), respectively. This suggested nutgrass tuber extract with suitable solvent has the potency as an antioxidant and antibacterial.

1. Introduction

The inhibition of antibacterial compounds depends on the type of bacteria. Based on differences in the cell wall structure, Gram-positive and Gram-negative bacteria respond differently to antibacterial compounds (Breijyeh *et al*., 2020). *Escherichia coli* is a Gram-negative bacterium that often causes diarrhea and *S. aureus* is a Gram-positive bacterium that causes various skin diseases (Huda, 2013). These bacteria can develop resistance to antibiotics (Aminingsih *et al*., 2012). An alternative that can be done to overcome bacterial resistance to antibiotics is to use compounds in plants as a source of new antibiotics (Gupta and Birdi, 2017). Dwiyanti *et al*. (2020) previously reported that one of the readily available tropical plants that have antibacterial compounds is nutgrass tubers, so this study aimed to determine the antibacterial activity of nutgrass tubers.

Nutgrass or *Cyperus rotundus* is a weed capable of forming a complex root system in just 2–3 weeks and can produce 100 tubers in 100 days (Bangarwa, 2008). Nutgrass is very difficult to remove manually or using herbicides, having very detrimental effects on farming. Nutgrass tubers were traditionally used as a remedy for constipation, dysentery and other diseases. Dwiyanti *et al*. (2020) suggested the phenolic compounds, alkaloids, flavonoids, tannins, saponins, starch, glycosides, furochromones, and sesquiterpenoids content in nutgrass tubers as having the potency as antioxidants and antibacterial agent.

Antioxidant and antibacterial compounds from organic matters such as nutgrass tuber can be obtained by extraction, for example, maceration which is often used due to its simpler process, yields more extract and does not require high temperature which can reduce the risk of damage to the compounds contained (Istiqomah, 2013). Several factors could affect maceration: type of

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

solvent, particle size, ratio of the material to the solvent and maceration time (Winata and Yunianta, 2015). Research on nutgrass tubers extraction using maceration to obtain its antioxidant and antibacterial activity has never been conducted, so the optimum solvent type and particle size for this particular study on nutgrass tubers are not yet known. The effectiveness of the extraction using maceration is highly dependent on the solubility of the compound in the solvent, according to the principle of like dissolve like, where a compound will dissolve in a solvent having the same properties (Megha and Sabale, 2014). Meanwhile, particle size also has an effect on the extraction process, the smaller the particle size of the material, the more cells are damaged making it easier for the solvent to access and attract the active compound in the material (Ketaren, 1986; Jiang *et al*., 2020).

Norra *et al*. (2016) showed that ethanol solvent and particle size results from 60 mesh sieve were the best treatments to produce seaweed extract (*Sargassum* sp.) as a source of antioxidants. Hence in this study, three types of solvents with different levels of polarity: *n*-hexane (nonpolar); ethyl acetate (semipolar); and ethanol (polar) were tested and particle sizes results of 40, 60, and 80 mesh sieving which resulted in particle size of <420 µm, <250 µm and <177 µm, respectively (Merck KGaA, 2022). The antibacterial activity test was carried out using the disc diffusion method because of simplicity, and easiness, and does not require special tools (Katrin *et al*., 2015), meanwhile, the antioxidant activity test used the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method due to its simplicity and is sensitive to samples with small concentrations (Haryati *et al*., 2017). Thus, in this study, the objectives were to determine the effect of solvent type with different polarity and particle size on the antioxidant and antibacterial activity of nutgrass tubers and to determine the best type of solvent and particle size to produce nutgrass tuber extract with the highest antioxidant and antibacterial activity.

2. Materials and methods

2.1 Research materials

The nutgrass tubers used in this study were obtained from Candikuning Village, Tabanan-Bali, Indonesia. Only dark brown colored tubers, with white tuber flesh, having the hard texture of the nutgrass tubers were selectively used in this study, as can be seen in Figure 1. The bacteria tested were *E. coli* (strain ATCC 8739) and *S. aureus* (ATCC 19430) which were collections of the Bioindustry Laboratory of Udayana University. Chemicals used include *n*-hexane 96% (Smart lab), ethyl acetate 96% (Smart lab), ethanol 96% (Smart lab), gallic acid (Sigma-Aldrich), DPPH crystals (Himedia) and others.

Figure 1. Grass (left) and tuber (right) of nutgrass (*C. rotundus* L.).

2.2 Nutgrass tuber powder production

The nutgrass tubers used for this study were washed thoroughly, cut to 1 mm thickness, and then dried in the oven at 50°C for 2–3 hrs until the tubers could easily break with an estimated moisture content of ~12%. The dry ingredients were then finely mashed using a blender until became fine powder, then sieved using 40, 60, and 80 mesh sieves (Mostafa *et al*., 2018), resulting in approximately <420 µm, <250 µm, and <177 µm particle size, respectively (Sigma Aldrich, 2022).

2.3 Nutgrass tuber extract maceration

The nutgrass tuber powder results of 80, 60, and 40 mesh sieving each weighing 30 g were put into a maceration bottle, then 450 mL of 96% ethanol, 96% ethyl acetate, and 96% *n*-hexane resulting ratio of 1:15, macerated in a tightly closed container at room temperature (27°C) for 48 hrs continuously stirred with shaker. After the maceration, the solution was filtered using coarse filter paper to obtain filtrate 1 and dregs. The dregs obtained were later added with 50 mL of each solvent followed by stirring for 5 minutes, and were then filtered again using coarse filter paper to obtain filtrate 2. Filtrate 1 and filtrate 2 were then combined and filtered using Whatman filter paper no. 1. Each of the filtrate liquids obtained was then evaporated using a vacuum rotary evaporator at a temperature of 40°C, with a speed of 100 rpm, and pressure of 100 mbar. The extract obtained was weighed to calculate the yield of the extract and then stored in the bottle for further analysis (Suryani, 2012).

2.4 Medium and microbes preparation

For the antibacterial activity test, the nutrient agar (NA) powder medium was dissolved in distilled water in a ratio of 28 g: 1000 mL, and was then heated until the NA medium was completely dissolved, poured into the petri dishes and cooled down to create NA agar plate meanwhile the liquid nutrient broth (NB) was dissolved in distilled water in a ratio of 13 g: 1000 mL, then heated until the NB medium was completely dissolved and later

https://doi.org/10.26656/fr.2017.8(3).261

cooled down to room temperature. *Escherichia coli* and *S. aureus* culture stocks were rejuvenated by growing in each NA medium using the streak plate method and incubated at room temperature for 24 hrs at 37°C. *Escherichia coli* and *S. aureus* fresh cultures were prepared by taking a colony of the culture of the bacteria and inoculated into a test tube containing 10 mL of NB media and incubated at room temperature for 18–24 hrs at 37°C.

Each bacterial suspension (100 µL) was dropped onto the NA agar plates and was spread using a bent glass rod and allowed to dry. Then 20 µL of nutgrass tuber extract was dripped onto sterile paper discs with a diameter of 6 mm and placed on media containing the test bacteria (*E. coli* and *S. aureus*). The diameter of the inhibition zone around the paper disk was observed after 24 hrs. The positive control was using 20 µL of 10 g/L of Cotrimoxazole as an antibiotic, and 20 µL of each solvent was added to the paper disc as a negative control. The inhibition zone area was calculated from the clear zone area from the paper disc area, where the diameter of the clear inhibition zone was averaged from 3 measurements.

2.5 Observed variables

The variables observed in this study were extraction yield (Hambali *et al*., 2014), total phenolic (Sakanaka *et al*., 2003), antioxidant activity (Dali *et al*., 2017), and antibacterial activity by diffusion test method (Sidabutar *et al*., 2015). The measurements of total phenolic content were conducted according to Sakanaka *et al*. (2003), antioxidant activity (Dali *et al.,* 2017), and antibacterial activity (Sidabutar *et al*., 2015) were repeated three times and the results were averaged, and each standard deviation (SD) were calculated. The area of the inhibition zone was calculated by subtracting the clear zone area from the paper disc area. The results of the inhibition zone measurements were then analyzed for antibacterial activity and calculated to determine the antibacterial activity (Arbitrary Unit/AU) and the antibacterial effectiveness of the test extract calculated using the following formula:

$$\text{Antibacterial Activity} = \frac{\text{LC} - \text{CP}}{\text{VL}} \; (\textit{Arbitrary Unit}/\text{AU})$$

Where LC = clear zone area (mm^2), LP = paper disc area (mm^2) and VL = bacterial sample volume (mL).

$$\text{Antibacterial Effectiveness (\%)} = \frac{\text{DE}}{\text{DA}} \times 100\%$$

Where DE = extract inhibition zone (mm) and DA = antibiotic inhibition zone (mm).

2.6 Data analysis

The data obtained were then analyzed using analysis of variance (ANOVA). When significant differences were detected by ANOVA, pairwise comparisons were performed using Tukey's test. The significance test was performed at probability level of $p < 0.05$.

3. Results and discussion

3.1 Yield of nutgrass tuber extract

The results of the analysis of variance (ANOVA) showed the type of solvent treatment, particle size, and interactions all had a very significant effect ($p \leq 0.01$) on the yield of nutgrass tuber extract obtained. The average value of the nutgrass tuber extract yield can be seen in Table 1.

Table 1. Average yield (%) of nutgrass tuber extract in the treatment of solvent type and particle size.

Solvent type	Particle size (mesh)		
	<420 µm (40)	<250 µm (60)	<177 µm (80)
Ethanol 96%	9.06±0.07^a	10.71±0.45^a	11.75±0.21^a
Ethyl acetate 96%	2.80±0.40^b	3.28±0.23^b	3.90±0.22^b
n-hexane 96%	2.30±0.12^b	2.54±0.16^b	3.10±0.08^b

Values are presented as mean±SD. Values with different superscripts within the same row or column are statistically significantly different 5% error level ($p \leq 0.05$).

The highest yield of nutgrass tuber extract was obtained from ethanol as the solvent with a particle size <177 µm from 80 mesh sieving which was 11.75±0.21 gram, while the lowest yield was obtained using *n*-hexane as the extraction solvent with a particle size <420 µm from 40 mesh sieve which was 2.30±0.12 gram. It seems that the more polar the solvent and the smaller the size of the material, the higher the yield of the nutgrass tuber extract was obtained.

Dissolving power upon maceration is related to the polarity of the compound and the polarity of the solvent. The research of Banerjee *et al.* (2012) showed that polar solvents produced higher yields than non-polar solvents. The smaller particle size produced from sieving might cause more cell walls to be broken and damaged, easing the solvents to penetrate through cell walls and attract active compounds from the source materials (Jiang *et al*., 2020). This was also supported by the research of Marinda *et al*. (2020) which described particle size from 80 mesh sieving produced higher yield in comparison with particle size from using 40 and 60 mesh sieves, which was 10.78±0.61% in cocoa pod peel extract.

3.2 Total phenolic content measurement

For the total phenolic content measurement, using ethanol as the extraction solvent with a particle size of <177 µm using an 80 mesh sieve produced the highest total phenolic which was 184.15±0.99 mg, while the lowest total phenolic was produced using *n*-hexane as the extraction solvent with the particle size of <420 µm using 40 mesh which was 28.11±0.86. The result indicated the more polar the solvent used and the smaller the particle size of the nutgrass powder, the higher the total phenolic content of the nutgrass tubers was obtained. The results of the analysis of variance (ANOVA) showed that the type of solvent treatment of particle size and its interactions had a very significant effect ($p \leq 0.01$) on the total phenolic extract of nutgrass tuber. The average value of phenolic extracts of nutgrass tuber can be seen in Table 2.

Table 2. The average value of total phenolic (mg GAE) of nutgrass tuber extract on the treatment of solvent type and particle size.

Solvent type	Particle size (mesh)		
	<420 µm (40)	<250 µm (60)	<177 µm (80)
Ethanol 96%	109.08±0.34^{ab}	168.02±0.76^{a}	184.15±0.99^{a}
Ethyl acetate 96%	65.80±0.82^{c}	81.14±0.60^{bc}	103.20±0.51^{ab}
n-hexane 96%	28.11±0.86^{d}	30.49±0.88^{d}	50.80±1.12^{c}

Values are presented as mean±SD. Values with different superscripts within the same row or column are statistically significantly different 5% error level ($p \leq 0.05$).

The research from Roni *et al*. (2018) also defined that the ethanol extract of the wild plant Karamunting (*Melastoma malabathricum* L.) had the highest phenol content compared to ethyl acetate and *n*-hexane solvents. Nawaz *et al*. (2020) described that phenolic compounds tend to be soluble in polar solvents. The best treatment was obtained at a particle size of 80 mesh, this is due to the smaller the particle size of the material, the more cells are damaged thus the phenolic compounds in the material are more easily extracted (Nawaz *et al.,* 2016).

3.3 Antioxidant activity assay

The DPPH radical scavenging method was used for the antioxidant activity test in this study which was presented in IC_{50} values. The smaller the IC_{50} value indicates the greater the antioxidant activity due to only a small concentration of the extract was needed to reduce or neutralize free radicals by 50% (Zou *et al*., 2004). The results showed from Table 3 using ethanol solvent treatment with particle size of <177 µm from 80 mesh sieve produced the highest antioxidant activity despite a weak antioxidant category of 192.18±0.43 ppm (the smallest IC_{50} value), while the *n*-hexane solvent treatment with a particle size of 420 µm from 40 mesh sieve produces the lowest antioxidant activity with overall very weak antioxidant category, which is 367.89±0.95 ppm (largest IC_{50} value). The results suggested the more polar the solvent used for maceration and the smaller the particle size from sieving resulting the higher the antioxidant activity of the nutgrass tubers extract. This is in agreement with Swain *et al.* (2021) which showed that polar solvents produced higher antioxidant activity than semi-polar and non-polar solvents in *Cyperus articulates* extract.

Table 3. The average value of the antioxidant activity (IC_{50}) of nutgrass tuber extract in the treatment of solvent types and particle size.

Solvent type	Particle size (mesh)		
	<420 µm (40)	<250 µm (60)	<177 µm (80)
Ethanol 96%	197.33±0.35^{b}	194.84±0.08^{ab}	192.18±0.43^{a}
Ethyl acetate 96%	233.57±1.94^{e}	229.02±0.79^{d}	216.32±0.74^{c}
n-hexane 96%	367.89±0.95^{h}	352.82±0.01^{g}	309.07±0.11^{f}

Values are presented as mean±SD. Values with different superscripts within the same row or column are statistically significantly different 5% error level ($p \leq 0.05$).

The particle size of <177 µm 80 mesh sieving produced the extract of nutgrass tubers with the highest antioxidant activity, because the smaller the particle size of the material, the more cells were damaged thus the antioxidant compounds in the material were more easily extracted (Nawaz *et al*., 2016). Ethanol solvent (polar) and particle size <177 µm of 80 mesh sieve also produced the highest total phenolic which affected the antioxidant activity of the nutgrass tuber extract. Al-Dabbas *et al*. (2006) claimed that phenolic compounds contribute to antioxidant activity. Phenolic compounds have the ability as antioxidants because they can donate electrons on free radicals to become phenoxyl radicals or stable radicals (Ghasemzadeh and Ghasemzadeh, 2011).

3.4 Antibacterial activity assay

The results of different solvents upon maceration and particle size using ANOVA analysis showed a very significant effect ($p \leq 0.01$) on the inhibition diameter, antibacterial activity, and antibacterial effectiveness of nutgrass tuber extract against *S. aureus* bacteria. Meanwhile, for the test of *E. coli* inhibition, the interaction showed a significant effect ($p \leq 0.05$). The average value of inhibition, antibacterial activity, and antibacterial effectiveness of nutgrass tuber extract can be seen in Table 4.

Based on the data in Table 4, the highest average value of inhibition, antibacterial activity, and effectiveness was produced by ethyl-acetate as the

https://doi.org/10.26656/fr.2017.8(3).261

Table 4. The average value of the diameter of inhibition and antibacterial activity of nutgrass tuber extract against *E. coli* and *S. aureus* bacteria in the treatment of solvent type and particle size.

Combination treatment (Solvent type, particle size)	Inhibition Diameter (mm)		Antibacterial Activity AU (mm^2/mL)		Antibacterial Effectiveness (%)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Ethanol, 40 mesh	1.78±0.07^{bc}	6.92±0.49^{b}	70.00±2.78^{b}	271.48±19.43^{b}	14.46±0.57^{c}	32.16±1.94^{c}
Ethanol, 60 mesh	1.90±0.09^{b}	7.62±0.35^{ab}	74.58±3.70^{b}	298.95±13.88^{b}	15.41±0.76^{c}	35.42±1.25^{c}
Ethanol, 80 mesh	2.15±0.07^{b}	8.28±0.49^{a}	84.39±2.78^{ab}	325.12±19.43^{ab}	17.44±0.57^{bc}	38.52±1.87^{bc}
Ethyl acetate, 40 mesh	2.82±0.12^{ab}	9.40±0.47 ^{a}	110.55±4.63 ^{a}	368.95±18.50^{ab}	22.84±0.96^{ab}	43.74±2.68^{ab}
Ethyl acetate, 60 mesh	3.03±0.14^{ab}	9.77±0.33^{a}	119.06±5.55^{a}	383.34±12.95^{ab}	24.60±1.15^{ab}	45.44±2.04^{ab}
Ethyl acetate, 80 mesh	3.53±0.19^{a}	10.67±0.14^{a}	138.68±7.40^{a}	418.67±5.55^{a}	28.66±1.53^{a}	49.61±0.10^{a}
n-hexane, 40 mesh	1.12±0.16^{c}	3.05±0.40^{c}	43.83±6.48^{c}	119.71±15.73^{d}	9.06±1.34^{d}	14.20±2.02^{d}
n-hexane, 60 mesh	1.82±0.02^{b}	3.73±0.14^{c}	71.30±0.93^{b}	146.53±5.55^{c}	14.73±0.19^{c}	17.36±0.46^{d}

Values are presented as mean±SD. Values with different superscripts within the same row or column are statistically significantly different 5% error level ($p \leq 0.05$).

extraction solvent with the particle size of <177 µm from 80 mesh sieve, while the average value of inhibition diameter, antibacterial activity, and effectiveness was found to be lowest using *n*-hexane as the extraction solvent with the particle size of <420 µm from 40 mesh sieve. In comparison with other solvents, the ethanolic crude extract of nutgrass tubers showed moderate antibacterial activity. Previously, a similar study has been reported on the extract of *Aquilaria malaccensis* using ethanol as the extraction solvent which also showed moderate antibacterial activity against *E. coli* ATCC 1129 and *K. pneumoniae* ATCC 700603 (Jihadi *et al*., 2020).

These results are probably due to the antibacterial compounds in nutgrass tubers tending to dissolve in semi -polar solvents and were optimized in the smaller particle size of the material where more cells are broken and damaged therefore, the antibacterial compounds in the material are more easily extracted (Nawaz *et al.,* 2016). This is in accordance with the research of Al-Dabbas *et al*. (2006) that antibacterial activity against *S. aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *E. coli, Bacillus cereus* and *Salmonella enterica* serovar Enteritidis which was extracted using ethyl acetate as solvent produced the antibacterial inhibition zone in all treatments against *E. coli* was classified as weak antibacterial ≤ 5 mm, while *S. aureus* belonged to medium antibacterial 6–10 mm. The diameter of the inhibition zone of the nutgrass tuber can be seen in Figure 2.

The diameter of the inhibition zone of *S. aureus* was higher than its activity against *E. coli*. The difference is due to the *S. aureus* Gram-positive bacteria that have simpler cell structures which makes it easier for antibacterial compounds to enter the cell. While, *E. coli* and *Salmonella enterica* serovar Typhi are Gram-negative bacteria that have a more complex cell wall structure, with the outer cell wall layer in the form of lipoproteins and lipopolysaccharides which have a selection system against foreign substances and the inner layer is peptidoglycan (Huda, 2013). Research by Kishore and Alluraiah (2013) also claimed that the inhibitory power of *Cyperus iria* (L.) extract against *S. aureus* was greater than the inhibitory power of *E. coli* and *S. enterica* ser. Typhi.

The antibacterial potency of the nutgrass tuber extract compared with the positive control of co-trimoxazole was indicated by the value of its antibacterial effectiveness against *E. coli* and *S. aureus*. The highest antibacterial effectiveness was found in the treatment with ethyl acetate solvent and 80 mesh particle size, which were 28.66±1.53 and 49.61±0.10, respectively. The lowest antibacterial effectiveness was found in the *n*-hexane solvent treatment and the particle size of 40 mesh was 9.06±1.34 and 14.20±2.02, respectively. The phenolic compound is one of the

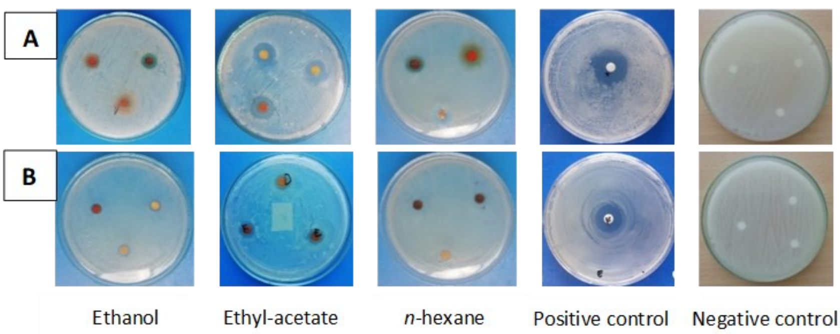

Figure 2. Inhibition zone of nutgrass tuber extract against (A) *S. aureus* and (B) *E. coli.*

compounds that can act as antibacterial, but in this study, the increase in phenolic compounds did not significantly affect the antibacterial activity of nutgrass tubers. This is caused not only by phenolic compounds that can act as antibacterial but non-phenolic compounds such as alkaloids, steroids, and terpenoids can also act as antibacterial.

4. Conclusion

The best antioxidant activity was produced by using ethanol as a solvent with a particle size of <177 µm by using the 80-mesh sieve with IC_{50} of 192.18±0.43 ppm. The best antibacterial activity was produced by using ethyl acetate as a solvent and a similar particle size of <420 µm by utilizing the 80-mesh sieve. The antibacterial activity against *E. coli* and *S. aureus* was 3.53±0.19 mm^2/mL and 10.67±0.14 mm^2/mL (AU), respectively. In conclusion, it can be claimed that the nutgrass tuber extract which is readily available locally has the potency as an antioxidant and antibacterial against tested pathogenic microbes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to express their gratitude to Udayana University for providing laboratory facilities and technical assistance.

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