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# Influence of Some Extraction Conditions Factor on Phenolic Content and Antioxidant Activity of *Solanum betaceum Cav.*

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## ABSTRACT

*Solanum betaceum Cav.* fruit is renowned for having antioxidant activity because it contains phenolic compounds. This study aimed to determine the effect of some condition factor namely solvent composition, maceration time, liquid-solid ratio, and the particle size of *S. betaceum Cav* fruit to the total phenolic content and antioxidant activity. The fruit was collected from Temanggung, Wonosobo, and Kopeng, Central Java, Indonesia. The research used single-factor experiments and simplex lattice design (SLD) as an optimization method. Total phenolic content was determined using Folin Ciocalteau reagent, while antioxidant activity was determined using 2,2-diphenyl-1-picrilhidrazil (DPPH) radical scavenger activity. The solvent combination which gave the highest responses was ethanol: water (60:40 $^{v}/_{v}$) with phenolic content of 7.48% $^{w}/_{w}$ EAG. Maceration for 8 hours will produce an extract with the highest total phenolic content (8.76% $^{w}/_{w}$ EAG). The optimal solvent ratio was at 10:1 $^{v}/_{w}$ with total phenolic content of 7.26 ± 0.20% $^{w}/_{w}$ EAG. The optimum particle size was 600-850 µm with a total phenolic content of 6.07 ± 0.18% $^{w}/_{w}$ EAG. Antioxidant activity with the DPPH free radical scavenger capture method from three regions did not show significant results.

**Keywords:** antioxidant activity; extraction conditions factor; *Solanum betaceum Cav*.; Temanggung-Wonosobo-Kopeng

## INTRODUCTION

Antioxidant compounds are considered essential for the body to help terminate the reaction of free radicals that are attributed to cell or tissue damage, autoimmune diseases, degenerative diseases, to cancer (Brewer, 2011). One of the fruits that are known to possess antioxidant activity is *Solanum betaceum Cav* (Orqueda *et al.,* 2017).

*Solanum betaceum Cav.* contains some chemical compounds including alkaloids, tannins, saponins, glycosides, flavonoids, and phenolics (Acosta *et al.,* 2015; Kadir, 2015). The phenolic compounds from plants are known to have antioxidant and anti-inflammatory activity. Phenolic compounds derived from plants are able to selectively interfere with the production or function of cytokines. Phenolic compounds have the capacity to modulate the immune response and have potential anti-inflammatory activity (Magrone *et al.,* 2016; Santangelo *et al.,* 2007).

In the development of extract as a raw material of food supplement product or traditional medicine, it is necessary to research about the most suitable extraction conditions to extract the active compound. Factors of extraction conditions influence the yield, i.e material size, extraction time, extraction temperature, type and amount of solvent (Spigno *et al*, 2007). The particle size of the powder and the optimum ratio of the optimum solvents can increase the number of active compounds present (Safaralie *et al.*, 2009; Sari, 2011).

The right solvent composition will generate an extract with maximum active compound content by means of solvent optimization. The solid-solvents ratio directly affects the effectiveness of the extraction. The particle size of raw material will affect border layer thickness, which will affect the effectiveness of the solvent to reach the active chemical content in cell material (Prior *et al.,* 2005). This research used a single factor experiment as an optimization method to define the optimum particle size and maceration time. This was a preliminary study to observe the optimal result of one variable at one time of extraction while the other factor was made constant (Chen *et al.,* 2007). Meanwhile, Simplex Lattice Design (SLD) was used as an optimization method for determining the optimum solvent composition.

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## METHODOLOGY

### Preparation of materials

Ripe *S. betaceum Cav* fruit was washed, cut and dried in an oven with a temperature of 50$^{O}$C to dry, then powdered.

### Determination of solvent composition

Fruit powder was weighed for 10.0 g and macerated with solvent: water; water: ethanol (50:50 $^{v}/_{v}$) and 96% ethanol respectively, with a ratio of 10:1 $^{v}/_{w}$ solvent. Total phenolic contents of all the three extracts ware used to determine the solvent composition which yielded the extract with the highest phenolic compound. The data were inputted into the Design Expert 10 software.

### Determination of maceration time

The maximum solvent composition resulted from the SLD method was used to determine the maceration time. 10.0 g dried fruit powder was macerated using 100 mL ethanol 50 %. This research used the dissolution method by taking 5 ml of macerating liquid and adding 5 ml of new solvent into an Erlenmeyer flask until maceration was completed. Sampling was conducted at 0, 1, 4, 8, 12, 24, 36, 48, 60 and 72 hours. The total phenolic content of each sample was plotted in rate and time relationship curve to determine the maceration time.

### Determination of liquid-solid ratio

The extraction as mentioned above was performed with a variation of the solvent ratio (4:1 $^{v}/_{b}$), (7:1 $^{v}/_{w}$), and (10:1 $^{v}/_{w}$). Maceration was done for 24 hours.

### Determination of optimum simplicia powder size

Fruit powder of *Solanum betaceum Cav* was sieved to obtain powder diameter size of ≥ 850 µm (≥20 mesh), 600-850 µm (20-30 mesh), 425-600 µm (30-40 mesh), 300-425 µm (40-50 mesh), and ≤ 300 µm (≤50 mesh). Each sample was accurately weighed for 10,0 g, and macerated for 24 hours with 10:1 liquid-solid ratio using ethanol 50 % as solvent.

### Test of DPPH free radical scavenger activity (Suh *et al.*, 2014)

Quercetin was used as a comparison since quercetin has already been known as a natural antioxidant substance. Each concentration of the test solution was plated for 50 µl, and was added with 1 ml of 0.4 mM DPPH solution and 3950 µl methanol. The mixture was mixed using vortex for 20 seconds and left for 30 minutes in darkness at room temperature. The absorbance of control solution was performed without adding the test solution and was measured using UV-Vis spectrophotometer at λ maximum of DPPH.

### Determination of Inhibition Concentration value ($IC_{50}$)

The absorbance values of DPPH solution before and after the addition of the test sample can be calculated as the percentage of Free Radical Scavenger (% FRS).

$$\% \text{ FRS} = \frac{\text{abs. DPPH control solvent} - \text{abs. sample}}{\text{abs. DPPH control solvent}} \times 100\%$$

A linear curve plot between %FRS versus sample concentration was made and then the linear regression equation $IC_{50}$ was determined. $IC_{50}$ was calculated by plotting 50% response using the regression curve equation.

### Determination of total phenolic content (Lutria *et al.,* 2007)

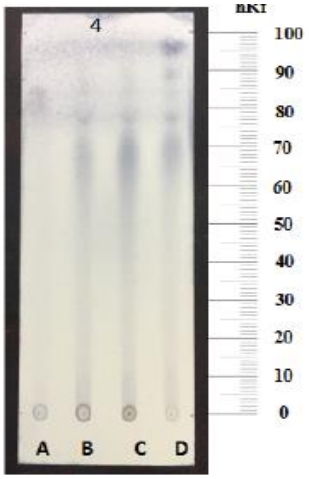
The total phenolic content was determined using the Folin-Ciocalteu assay with gallic acid (EAG) as a calibration standard. A calibration curve was created using different concentrations of standard gallic acid solutions, each time analysis was run. The total phenolic level in the extract was calculated from the standard calibration curve.

## RESULTS AND DISCUSSION

### Determination of solvent composition

The chromatogram profile of extract produce from different solvent compositions can be seen in Figure 1. Phenolic compounds in fruit extract of *S. betaceum* showed at hRf value of hRf 69, 78 and 88, indicating as dark blue color after being sprayed with Folin-Ciocalteu (Vemerris & Nicholson, 2006). According to Acosta-Quezada *et al,,* 2014, *S. betaceum* contain various compounds like alkaloid, tannin, saponin, glycoside, steroid/triterpenoid, flavonoid and phenolic. The phenolic content contained in *S. betaceum* fruit include caffeic acid, ferulic acid and rosmarinic acid (Espin *et al.,* 2016). The phenolic compounds structure contained in the fruit *S. betaceum* can be seen in Figure 2.

The total phenolic content was expressed with % w/w equivalent with gallic acid (% $^{w}/_{w}$ EAG). As can be seen in Table I, the highest phenolic content is present in water: ethanol (50:50 $^{v}/_{v}$) extracts (7.54% $^{w}/_{w}$ EAG). Statistical data using one way ANOVA showed a significance value of <0.05. Thus, it is conclusive that the three extracts have significantly different total phenolic contents.




Description:
A=Gallic acid
B=Water Extract
C=Ethanol Water Extract
D=Ethanol Extract 96%

Figure 1. TLC profile using silica gel 60 $F_{254}$, chloroform: methanol (1:1 $^{v}/_{v}$) as solvent with Folin-Ciocalteu spray reagent (Nafingah, 2017)

The results of the total phenolic content from three different solvents were then analyzed using Simplex Lattice Design method with Design-Expert software version 7, which resulted in the following equation: Y = 6.84 [A] + 5.57 [B] + 4.21 [AB], where Y = Response of phenolic content, A=Fraction of ethanol component and B=Fraction of water component. The curve resulted from this equation is shown in Figure 3.

The Data of the total phenolic content included in SLD will provide solution response with high desirability value. The result of optimization of solvent composition from Design Expert shows that solvent composition that gave the maximum response was ethanol solvent: water (65:35 $^{v}/_{v}$) that solvent composition that gave the maximum response was ethanol solvent: water (65:35 $^{v}/_{v}$) with total phenolic content of 7.34% $^{w}/_{w}$ EAG and desirability value of 0.894. This result is in accordance with Design Experiment software result which was ethanol: water (60:40 $^{v}/_{v}$) as the optimal solvent composition. To ensure that the equation was valid, verification was carried out. The results can be seen in Figure 3.

Figure 3 shows slightly different result of the phenolic contents macerated using ethanol: water (60:40 $^{v}/_{v}$) and ethanol: water (65:35 $^{v}/_{v}$). This result was then statistically tested using paired t-test (SPSS 16.0), which shows a significance value of 0.79> 0.05 so it is concluded that there was no significant difference between the ethanol solvent composition: water (65:35 $^{v}/_{v}$) and (60:40 $^{v}/_{v}$). Based on this result, a further solvent composition of ethanol:water (60:40$^{v}/_{v}$) was further employed.

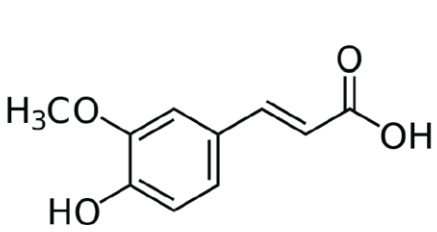
**Maceration Time Determination**

The optimum solvent composition obtained from the previous step was used to determine the optimum maceration time. The graph of total phenolic content versus sampling time is shown in Figure 4. It can be seen in Figure 4 that at the 8$^{th}$ hour the system has already gained the maximum total phenolic content (8.27% $^{w}/_{w}$ EAG), yet the phenolic content slightly decreased afterward.

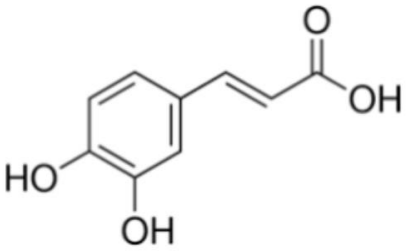
According to Wong (2013), the diffusion rate of phenolic compounds from the surface of solid to solvent is equal to the diffusion rate from solvent to solid surface so that the concentration of the phenolic compound in a solvent is at equilibrium. Consequently, further maceration time after 8 hours should be stable. Figure 6 shows that there was a decrease in the phenolic content in the extract. This is likely due to the addition of solvent volume at each sampling, which leads to the dilution of the total maximum phenolic content in the extract leading to a decrease in phenolic levels.

**Effect of liquid-solid ratio**

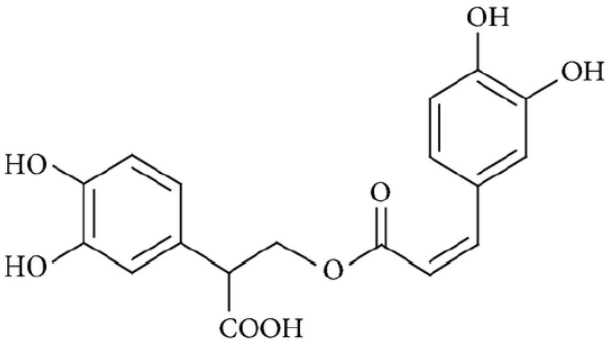
It is impossible to perfectly extract phenolic compounds with a small ratio of solvents, whereas excessive use of solvents will increase the cost of extraction (Wu, 2015). Determination of the optimum ratio of solvents was required for the extraction of *S. betaceum* based on the size of the total phenolic content. The average yield of three time total phenolic content sizes for each of the simplicia-solvent ratio can be seen in Figure 5. Figure 5 shows that the total phenolic content




Caffeic acid




Ferulic acid




Rosmarinic acid

Figure 2. Chemical structure of phenolic compound in *S. betaceum* Cav.

Table I. Level of phenolic compound of *Solanum betaceum* Cav Extract (Hanum, 2017)

| **Total phenolic content (% $^{w}/_{w}$ EAG)** | **Water** | **Water:ethanol (50:50 $^{v}/_{v}$)** | **Ethanol 96%** |
|---|---|---|---|
| I | 5.62 | 7.57 | 6.41 |
| II | 5.56 | 7.51 | 6.60 |
| II | 5.54 | 7.53 | 6.68 |
| Average (% $^{w}/_{w}$ EAG ± SD) | 5.57 ± 0.04 | 7.54 ± 0.03 | 6.56 ± 0.14 |

increased from a ratio 4:1 $^{v}/_{w}$ to a ratio of 10:1 $^{v}/_{w}$. The total phenolic content reached the highest point with a value of 7.26 ± 0.20% $^{w}/_{w}$ EAG at a ratio of 10:1 $^{v}/_{w}$, so the ratio of 10:1 $^{v}/_{w}$ was the optimum ratio of solvents to obtain the highest total phenolic content. Thus, this ratio was used for the determination of the size of the simplicia powder. The more solvents added, the greater the solvent's ability to dissolve the material which increases the amount of compound extracted by the solvent (Nakamura *et al.,* 2017). The compound will continue to increase until the solution becomes saturated. After passing the saturation point, the amount of the extracted compound will become constant.

Reduction of powder particle size aims to expand the contact surface between the powder and the solvent. In addition, it also minimizes the thickness of the boundary layer so that the diffusion rate increases. The average yield of three time total phenolic content size for each of the size of the simplicia powder can be seen in Figure 6.

Figure 6 shows that the total phenolic content increased from the powder size of ≥850 µm, to a maximum of 600-850 µm powder size

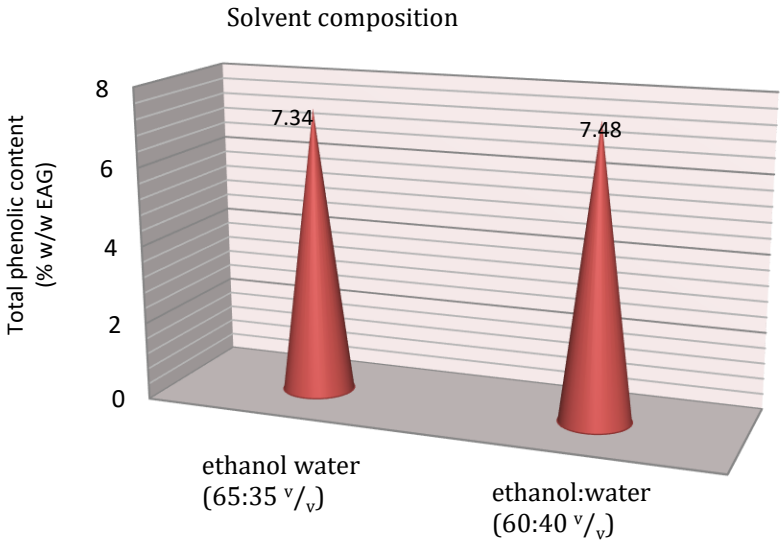
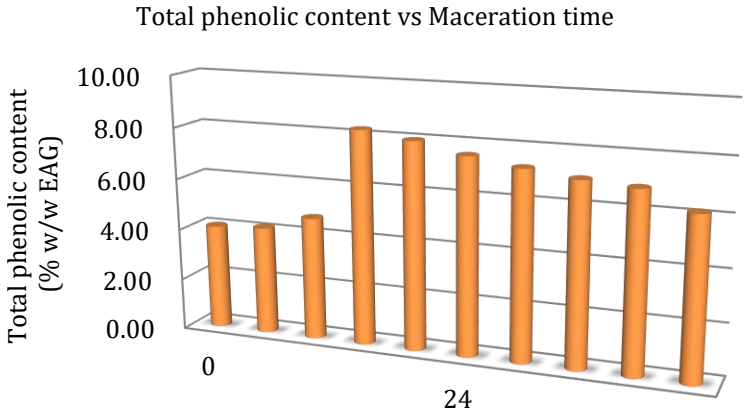



Figure 3. Graphic of total phenolic content of solvent composition verification (Hanum, 2017)




| | 0 | 1 | 4 | 8 | 12 | 24 | 36 | 48 | 60 | 72 |
|---|---|---|---|---|---|---|---|---|---|---|
| Series1 | 4.07 | 4.18 | 4.74 | 8.27 | 8.00 | 7.59 | 7.29 | 7.04 | 6.90 | 6.20 |

Figure 4. Curve of phenolic content of maceration time (Hanum, 2017)

and then it decreased to a powder size of ≤300 µm, It can be said that the powder size of 600-850 µm was the optimum powder size to obtain the highest total phenolic content. Powders with particle sizes of 600-850 µm contain the most seed parts, while the ≤300 µm particle size contains more flesh and fruit peels. This is based on microscopic observation of fragment identification of seeds, flesh, and fruit peel.

A microscopic observation with 100x magnification in the chloralhydrate medium can be seen in Fig. 7 and 8. Microscopic observations of the 600-850 µm powder size indicate the presence of identification fragments from the seeds of the fruit so that the powder size of 600-850 µm is likely to contain more seeds than flesh and fruit peels.

Based on Figure 8, the microscopic observation of the powder size of ≤300 µm indicates the presence of a fragment identification of fruit flesh and peel, so that the powder size of ≤300 µm is likely to contain more flesh and fruit peel than fruit seeds. Thavamonev *et al.,* (2018) argued that ethanol extract of fruit seed of *S.*

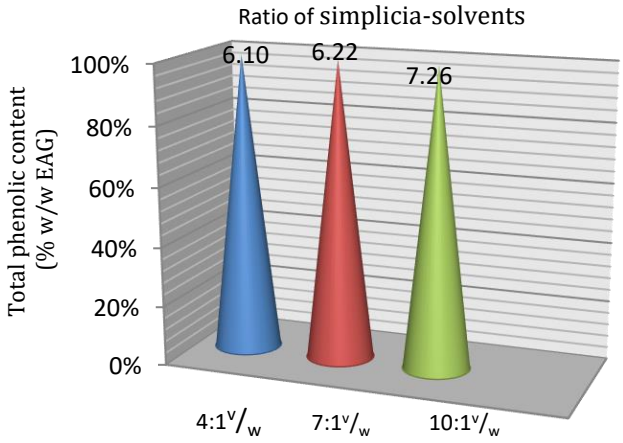



Figure 5. Relationship between the simplicia-solvents ratio and total phenolic content (Afini, 2017)

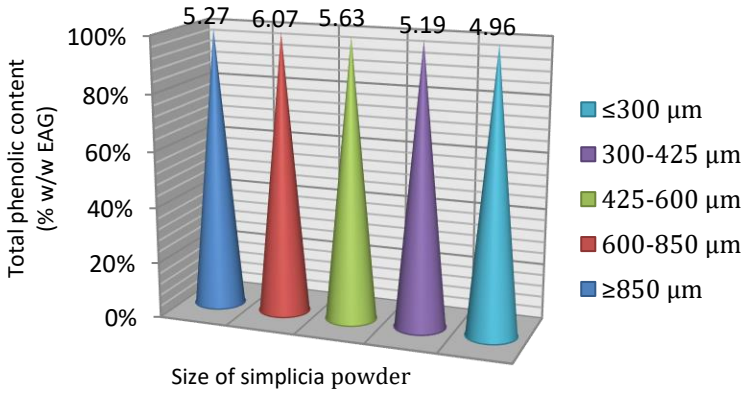



Figure 6. Relationship between the size of the simplicia powder and the total phenolic content (Afini, 2017)

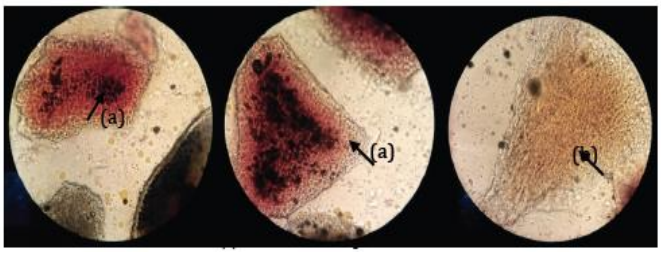



Figure 7. Powder sizes of 600-850 µm (Afini, 2017)
Description: (a) The endosperm parenchyma contains aleurone; (b) Perisperm

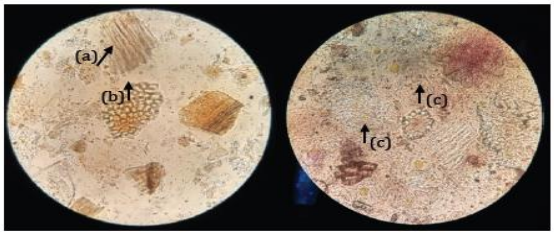



Figure 8. Powder sized fragments of ≤300 µm (Afini, 2017)
(a) Sclerenchymal fibers; (b) Endocarpium,;(c) Stone cell

Table II. $IC_{50}$ value of the three regions of *S. betaceum* Cav extracts from the three regions (Nafingah, 2017)

| Sample from the regions | Temanggung | Wonosobo | Kopeng |
|---|---|---|---|
| 1 | 74.31 | 68.81 | 69.10 |
| 2 | 64.62 | 83.07 | 64.58 |
| 3 | 64.22 | 70.78 | 67.88 |
| Average $IC_{50}$ ± SD (µg/mL) | 67.72 ± 5.71 | 74.22 ± 7.73 | 67.19 ± 2.34 |

*betaceum* contain more flavonoid compound than *S. betaceum* peel and flesh extract. Flavonoids belong to the polyphenol group, thereby increasing the total measured phenolic levels.

The total phenolic levels also rise because the compounds that should not diffuse become easy to diffuse due to the thicker boundary layer. According to Dai *et al.,* (2012), the compound may be dissolved ballast, which then it pollutes the essence. The amount and variation of the soluble compound increase but the capacity of the solvent still leads to the solubility of the compound. Therefore, the portion of the phenolic compound in the solvent reduces because it is occupied by another compound.

**The DPPH radical free scavenger test of ethanolic fruit extract *S. betaceum* Cav .**

The antioxidant test of the ethanolic extract of *S. betaceum* Cav fruit from the three regions was carried out by the DPPH radical scavenger test method based on the method carried out by Kedare & Singh (2011). The $IC_{50}$ values of the *S. betaceum* Cav ethanol extract obtained from Temanggung, Wonosobo and Kopeng can be seen in Table II.

The data of the $IC_{50}$ value of ethanolic extracts of *S. betaceum* showed that the extracts from the three regions were normally distributed and had a homogeneous variance (Table II). Based on the results of one way ANOVA statistical analysis, the significance value obtained was 0.315. The significance value was greater than 0.05 so that it can be concluded that the ethanolic extracts of *S. betaceum* from the three regions had an $IC_{50}$ value that was not significantly different. This possibly because the three regions have similar growing conditions for *S. betaceum* Cav. In this study, the environmental conditions of three regions were not specifically identified because the environmental data collection was not carried out.

## CONCLUSION

The solvent composition and maceration time that obtained the highest levels of phenolic compounds were ethanol: water (60:40 $^{v}/_{v}$) and 8 hour with a maximum phenolic content of 8.66% $^{w}/_{w}$ EAG. The liquid-solid ratio and particle size that obtained the highest phenolic compound of *Solanum betaceum Cav* fruit were 10:1 $^{v}/_{w}$ and 600-850 µm in diameter. Antioxidant activity with the DPPH free radical scavenger method on the extracts from the three regions, namely Temanggung, Wonosobo, and Kopeng did not show any significant results.

## ACKNOWLEDGMENTS

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## CONFLICTS OF INTEREST

There are no conflicts of interest.

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