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Authors: Veriani Aprilia, Nurul Kusumawardani, Rizal Fauzi, Daru Estiningsih, Dwi Kusumawati

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Calcium oxalate levels, glucomannan levels, and antioxidative activities of different sized *Amorphophallus oncophyllus* particles and the maceration of *Strobilanthes crispus*

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Abstract. Porang (*Amorphophallus oncophyllus*) is a type of tuber widely planted in Indonesia. It has a high glucomannan content, but its use is limited due to its high calcium oxalate content. However, it has previously been shown that the maceration of porang with the ethanolic extract of *Strobilanthes crispus* (SC) can serve to reduce its calcium oxalate content. This study sought to determine the impact of sieving in combination with ethanolic extract of SC maceration on the calcium oxalate levels, glucomannan levels, and antioxidative activities of different sized particles of porang. The calcium oxalate levels were analyzed by means of the atomic absorption method, whereas the glucomannan levels were determined based on the yield of glucomannan derived from the ethanolic extraction process. The porang particles were then analyzed to assess their antioxidative activities using DPPH (1,1-diphenyl-2-picrylhydrazyl). The particles were grouped into six in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). The results revealed that porang with a particle size of <40 mesh that was macerated with SC exhibited lower calcium oxalate levels and higher glucomannan levels than porang with a particle size of >40 mesh. The antioxidative activity of the porang decreased after maceration with the ethanolic extract of SC.

1. Introduction

Diabetes mellitus (DM) is a cause of death worldwide, and its prevalence is known to be increasing. Thus, various efforts have been made by the Indonesian government to reduce the prevalence of DM, ranging from the procurement of low-cost drugs to the funding of various studies. Herbal therapy and the use of functional food diets or food medicines (nutraceuticals) have been suggested as affordable alternatives to established DM treatment, and such alternatives are expected to be associated with only minimal side effects [1].

Porang (*Amorphophallus oncophyllus*) contains glucomannan that is potential as a functional food [2]. Glucomannan is a water-soluble polysaccharide known to have many health benefits, including lowering the blood glucose level [3]. However, the use of pure glucomannan remains rare due to its high cost. In the case of Indonesia, there is limited factory capacity with regard to the production of glucomannan, which means that it must be imported from abroad. Rough porang has been suggested as an alternative means of sourcing consumable glucomannan with an equivalent function to the factory-produced variety. Yet, the use of porang is currently limited because it contains calcium oxalate, which is an irritant and might be a risk of kidney stone formation and kidney failure [4]. It has also been found to increase the creatinine and blood urea levels in rats [5,6].

Several attempts have been made to decrease the amount of calcium oxalate in porang, including the use of stamp mills, fractionation blowers, ball mills, and chemicals such as sodium chloride, ash, and ethanol [8] [9] [10] [11]. The use of a herbal method—that is, maceration with an ethanolic extract—represents another means of reducing the calcium oxalate content of porang. Indeed, prior studies have shown that it is more

effective than the use of ethanol alone [6]. The lowest level of calcium oxalate achieved in this way was 0.5% according to Indonesia Patent Registration Number S00202006668.

The maceration of porang with *Strobilanthes crispus* (SC) was investigated in a study involving hyperglycemia-induced rats, with the results proving that it was able to lower the rats' blood glucose levels to a greater extent than the provision of porang flour without maceration. In addition, maceration with SC has also been found to reduce blood glucose levels to an extent equivalent to the use of the commercial drug glibenclamide [7]. The mechanism behind the decrease in the blood glucose level was suggested to be associated with the SC fiber being able to absorb water up to 100 mL/g and form a viscous fluid [8], thereby prolonging satiety and potentially decreasing absorption of food or nutrient in the blood and small intestine. The decreased of glucose and secretion of insulin led to an improvement in insulin sensitivity and the protection of pancreatic structure in histopathological observation [1].

The effect of maceration with the ethanolic extract of SC on the glucomannan level and antioxidant activity of porang has not previously been investigated. Thus, the present study sought to determine its effect on the calcium oxalate levels, glucomannan levels, and antioxidative activities of different particle sizes of porang.

2. Methods

2.1. Production of porang macerated with SC

The porang utilized in this study was purchased in the form of dried chips from a farmer who is a member of the Porang Nusantara Activist Association branch of Boyolali, North Java, Indonesia. It was then ground, floured, and macerated with an ethanolic extract of SC in accordance with the procedure set out in Patent Registration Number S00202006668 [9]. The porang was grouped into six in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration).

2.2. Measurement of the calcium oxalate levels, glucomannan levels, and antioxidative activities

The calcium oxalate levels of the porang samples were measured by approximating the total calcium content of each sample using the atomic absorption spectrometer (AAS) (GBC Scientific Equipment Ltd-932 Plus AAS) method [10]. The glucomannan levels were analyzed by calculating the glucomannan yield (i.e., percentage of glucomannan produced from porang flour) that was extracted from each sample using ethanol according to a previously described procedure [2]. Moreover, the antioxidative activities of the samples were measured by means of a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay) [11].

3. Results and Discussion

3.1. Calcium oxalate levels

The use of maceration treatment in combination with sieving yielded different particle sizes, which had a significant effect on the achieved decrease in the calcium oxalate level ($p < 0.05$). More specifically, the calcium oxalate levels appeared to be lower in the macerated porang samples of <40 mesh (AX, AY, and AN) than in those of >40 mesh (BX, BY, and BN) ($p < 0.05$). This result was comparable with the results of other studies that showed a decrease in the calcium oxalate levels after sieving with cyclone separator [12]. In addition, maceration with the ethanolic extract of SC resulted in slightly lower calcium oxalate levels when compared with the absence of maceration. This finding differed from the results of previous studies, which showed that maceration with the ethanolic extract of SC declined the levels of calcium oxalate in porang in comparison with maceration solely in ethanol solvent [6] [9]. However, maceration with the ethanolic extract of SC has previously shown greater antidiabetic potency, which was found to be equivalent to that of commercial glibenclamide rather than that of porang without maceration in a study involving rats [7]. This study used two kinds of SC that could be used in industry. This combination of treatments also resulted in lower calcium oxalate levels than other treatments, such as cyclone separator which resulted in 3.97% - 5.71% of calcium oxalate [12].

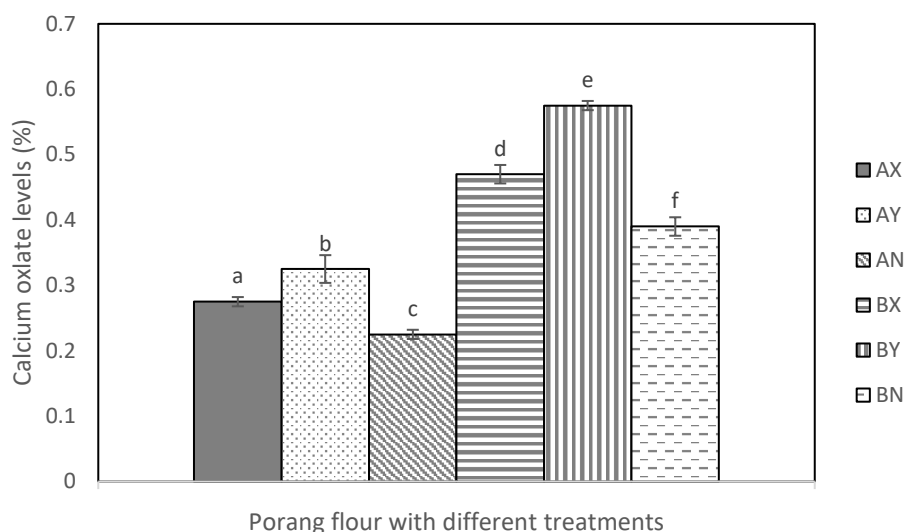


Figure 1. Calcium oxalate levels of differently treated porang samples: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p < 0.05$).

3.2. Glucomannan levels

Table 1 shows how the different particle sizes and maceration with the ethanolic extract of different brands of SC affected the glucomannan levels of the porang samples ($p < 0.05$). The highest glucomannan levels were seen in the AN group, although they were significantly different from those in the AY group. In prior studies, it was found that the glucomannan levels were influenced by the concentration of ethanol and the solvent/porang ratio [13]. However, the purification of porang by means of the physical grinding method could increase its glucomannan level [14]. In this study, the use of sieving reduced the impurities in the porang flour, as calcium oxalate is generally a light fraction or small-sized component [12].

Table 1. Glucomannan levels of differently treated porang sample

Treatments	Glucomannan levels (%)
AX	57.4±3.7b
AY	64.5±10.9bc
AN	70.9±2.26c
BX	51.2±0.9ab
BY	42.0±4.8a
BN	43.8±1.9a

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p < 0.05$).

3.3. Antioxidative activities

An antioxidative activity analysis was only performed on the macerated porang samples with a particle size of <40 mesh, as those samples had been shown to have lower calcium oxalate levels and higher glucomannan levels than the samples with a particle size of >40 mesh (see Table 1 and Figure 1). Table 2 describes the antioxidative activities of the macerated porang samples with various treatments. The results showed that the control group had the highest antioxidant activity (which was not subjected to sieving and soaking treatment) ($p < 0.05$). This finding could not prove the increase of antioxidant compounds from the ethanolic extract of SC to the porang, as the ethanolic solvent was rinsed twice after the maceration process. SC was found to have many antioxidant compounds, including polyphenol, catechin, alkaloid,

caffeine, tannin, and vitamin (C, B1, and B2) compounds [15]. The SC maceration treatment actually caused the natural antioxidant compounds in the porang flour to be wasted, possibly due to the washing of the ethanol, which is an organic solvent capable of extracting non-polar compounds, especially antioxidants [16]. Therefore, the maceration of porang with ethanol could decrease the antioxidative activity of porang and may not be suggested. It needs more study to select the right type of solvent to get high antioxidant activity. The high antioxidant activity in porang is potential its uses as functional food in maintaining of health.

Table 2. Antioxidative activities of differently treated porang samples

Treatments	Antioxidative activities (%)
AX	25.6±0.2a
AY	16.8±0.2b
AN	57.2±0.2c
CT	65.2±0.2d

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), CT (control, porang without treatment). Various letters demonstrate dissimilarity between groups ($p < 0.05$).

4. Conclusion

This study found that sieving treatment combined with maceration with the ethanolic extract of SC affected the calcium oxalate and glucomannan levels of porang flour. More specifically, the porang samples with a particle size of >40 mesh exhibited lower calcium oxalate levels and higher glucomannan levels. However, the combination of the two treatments significantly reduced the antioxidant activity of the porang flour. The further study is needed to find the right solvent of SC that may decrease calcium oxalate in porang and maintaining its antioxidative activity.

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4	What do higher antioxidative activities mean?	Done. Page 4, paragraph 1: The high antioxidant activity in porang is potential its uses as functional food in maintaining of health.
5	Add suggestion based on the result of this study	However, the combination of the two treatments significantly reduced the antioxidant activity of the porang flour. The further study is needed to find the right solvent of SC that may decrease calcium oxalate in porang and maintaining its antioxidative activity.

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2. Methods

2.1. Production of porang macerated with SC

The porang utilized in this study was purchased in the form of dried chips from a farmer who is a member of the Porang Nusantara Activist Association branch of Boyolali, North Java, Indonesia. It was then ground, floured, and macerated with an ethanolic extract of SC in accordance with the procedure set out in Patent Registration Number S00202006668 [9]. The porang was grouped into six groups in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration).

2.2. Measurement of the calcium oxalate levels, glucomannan levels, and antioxidative activities

The calcium oxalate levels of the porang samples were measured by approximating the total calcium content of each sample using the atomic absorption spectrometer (AAS) (GBC Scientific Equipment Ltd-932 Plus AAS) method [10]. The glucomannan levels were analyzed by calculating the glucomannan yield (i.e., percentage of glucomannan produced from porang flour) that was extracted from each sample using ethanol according to a previously described procedure [2]. Moreover, the antioxidative activities of the samples were measured by means of a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay) [11].

3. Results and Discussion

3.1. Calcium oxalate levels

The use of maceration treatment in combination with sieving yielded different particle sizes, which had a significant effect on the achieved decrease in the calcium oxalate level ($p < 0.05$). More specifically, the calcium oxalate levels appeared to be lower in the macerated porang samples of <40 mesh (AX, AY, and AN) than in those of >40 mesh (BX, BY, and BN) ($p < 0.05$). This result was comparable with the results of other studies that showed a decrease in the calcium oxalate levels after sieving with cyclone separator [12]. In addition, maceration with the ethanolic extract of SC resulted in slightly lower calcium oxalate levels when compared with the absence of maceration. This finding differed from the results of previous studies, which showed that maceration with the ethanolic extract of SC declined the levels of calcium oxalate in porang in comparison with maceration solely in ethanol solvent [6] [9]. However, maceration with the ethanolic extract of SC has previously shown greater antidiabetic potency, which was found to be equivalent to that of commercial glibenclamide rather than that of porang without maceration in a study involving rats [7]. This study used two kinds of SC that could be used in industry. This combination of treatments also resulted in lower calcium oxalate levels than other treatments, such as cyclone separator which resulted in 3.97% - 5.71% of calcium oxalate [12].

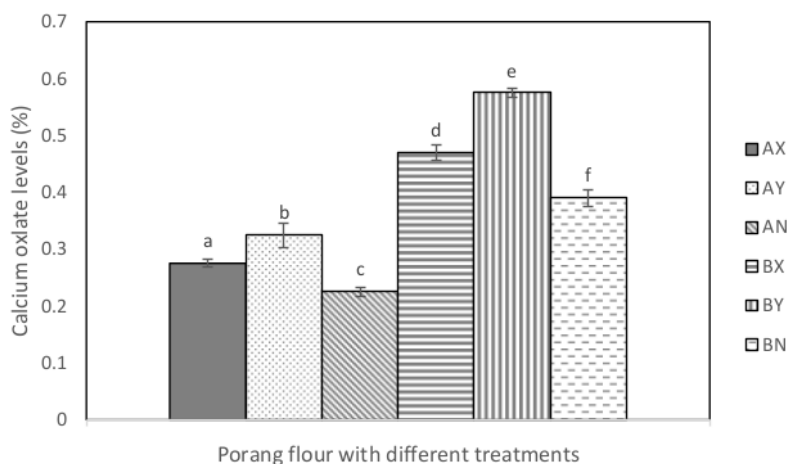


Figure 1. Calcium oxalate levels of differently treated porang samples: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p < 0.05$).

3.2. Glucomannan levels

Table 1 shows how the different particle sizes and maceration with the ethanolic extract of different brands of SC affected the glucomannan levels of the porang samples ($p < 0.05$). The highest glucomannan levels were seen in the AN group, although they were significantly different from those in the AY group. In prior studies, it was found that the glucomannan levels were influenced by the concentration of ethanol and the solvent/porang ratio [13]. However, the purification of porang by means of the physical grinding method could increase its glucomannan level [14]. In this study, the use of sieving reduced the impurities in the porang flour, as calcium oxalate is generally a light fraction or small-sized component [12].

Table 1. Glucomannan levels of differently treated porang sample

Treatments	Glucomannan levels (%)
AX	57.4±3.7b
AY	64.5±10.9bc
AN	70.9±2.26c
BX	51.2±0.9ab
BY	42.0±4.8a
BN	43.8±1.9a

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p<0.05$).

3.3. Antioxidative activities

An antioxidative activity analysis was only performed on the macerated porang samples with a particle size of <40 mesh, as those samples had been shown to have lower calcium oxalate levels and higher glucomannan levels than the samples with a particle size of >40 mesh (see Table 1 and Figure 18). Table 2 describes the antioxidative activities of the macerated porang samples with various treatments. The results showed that the control group had the highest antioxidant activity (which was not subjected to sieving and soaking treatment) ($p<0.05$). This finding could not prove the increase of antioxidant compounds from the ethanolic extract of SC to the porang, as the ethanolic solvent was rinsed twice after the maceration process. SC was found to have many antioxidant compounds, including polyphenol, catechin, alkaloid, caffeine, tannin, and vitamin (C, B1, and B2) compounds [15]. The SC maceration treatment actually caused the natural antioxidant compounds in the porang flour to be wasted, possibly due to the washing of the ethanol, which is an organic solvent capable of extracting non-polar compounds, especially antioxidants [16]. Therefore, the maceration of porang with ethanol could decrease the antioxidative activity of porang and may not be suggested. It needs more study to select the right type of solvent to get high antioxidant activity. The high antioxidant activity in porang is potential its uses as functional food in maintaining of health.

Table 2. Antioxidative activities of differently treated porang samples

Treatments	Antioxidative activities (%)
AX	25.6±0.2a
AY	16.8±0.2b
AN	57.2±0.2c
CT	65.2±0.2d

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), CT (control, porang without treatment). Various letters demonstrate dissimilarity between groups ($p<0.05$).

4. Conclusion

This study found that sieving treatment combined with maceration with the ethanolic extract of SC affected the calcium oxalate and glucomannan levels of porang flour. More specifically, the porang samples with a particle size of >40 mesh exhibited lower calcium oxalate levels and higher glucomannan levels. However, the combination of the two treatments significantly reduced the antioxidant activity of the porang flour. The

further study is needed to find the right solvent of SC that may decrease calcium oxalate in porang and maintaining its antioxidative activity.

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Calcium oxalate levels, glucomannan levels, and antioxidative activities of different sized *Amorphophallus oncophyllus* particles and the maceration of *Strobilanthes crispus*

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Abstract. Porang (*Amorphophallus oncophyllus*) is a type of tuber widely planted in Indonesia. It has a high glucomannan content, but its use is limited due to its high calcium oxalate content. However, it has previously been shown that the maceration of porang with the ethanolic extract of *Strobilanthes crispus* (SC) can serve to reduce its calcium oxalate content. This study sought to determine the impact of sieving in combination with ethanolic extract of SC maceration on the calcium oxalate levels, glucomannan levels, and antioxidant activities of different sized particles of porang. The calcium oxalate levels were analyzed by means of the atomic absorption method, whereas the glucomannan levels were determined based on the yield of glucomannan derived from the ethanolic extraction process. The porang particles were then analyzed to assess their antioxidant activities using DPPH (1,1-diphenyl-2-picrylhydrazyl). The particles were grouped into six groups in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). The results revealed that porang with a particle size of <40 mesh that was macerated with SC exhibited lower calcium oxalate levels and higher glucomannan levels than porang with a particle size of >40 mesh. The antioxidant activity of the porang decreased after maceration with the ethanolic extract of SC.

1. Introduction

Diabetes mellitus (DM) is a cause of death worldwide, and its prevalence is known to be increasing. Thus, various efforts have been made by the Indonesian government to reduce the prevalence of DM, ranging from the procurement of low-cost drugs to the funding of various studies. Herbal therapy and the use of functional food diets or food medicines (nutraceuticals) have been suggested as affordable alternatives to established DM treatment, and such alternatives are expected to be associated with only minimal side effects [1].

Porang (*Amorphophallus oncophyllus*) contains glucomannan that is potential as a functional food [2]. Glucomannan is a water-soluble polysaccharide known to have many health benefits, including lowering the blood glucose level [3]. However, the use of pure glucomannan remains rare due to its high cost. In the

case of Indonesia, there is limited factory capacity with regard to the production of glucomannan, which means that it must be imported from abroad. Rough porang has been suggested as an alternative means of sourcing consumable glucomannan with an equivalent function to the factory-produced variety. Yet, the use of porang is currently limited because it contains calcium oxalate, which is an irritant and might to be a risk of kidney stone formation and kidney failure [4]. It has also been found to increase the creatinine and blood urea levels in rats [5,6].

Several attempts have been made to decrease the amount of calcium oxalate in porang, including the use of stamp mills, fractionation blowers, ball mills, and chemicals such as sodium chloride, ash, and ethanol [8] [9] [10] [11]. The use of a herbal method—that is, maceration with an ethanolic extract—represents another means of reducing the calcium oxalate content of porang. Indeed, prior studies have shown that it is more effective than the use of ethanol alone [6]. The lowest level of calcium oxalate achieved in this way was 0.5% according to Indonesia Patent Registration Number S00202006668.

The maceration of porang with *Strobilanthes crispus* (SC) was investigated in a study involving hyperglycemia-induced rats, with the results proving that it was able to lower the rats' blood glucose levels to a greater extent than the provision of porang flour without maceration. In addition, maceration with SC has also been found to reduce blood glucose levels to an extent equivalent to the use of the commercial drug glibenclamide [7]. The mechanism behind the decrease in the blood glucose level was suggested to be associated with the SC fiber being able to absorb water up to 100 mL/g and form a viscous fluid [8], thereby prolonging satiety and potentially decreasing absorption of food or nutrient in the blood and small intestine. The decreased of glucose and secretion of insulin led to an improvement in insulin sensitivity and the protection of pancreatic structure in histopathological observation [1].

The effect of maceration with the ethanolic extract of SC on the glucomannan level and antioxidant activity of porang has not previously been investigated. Thus, the present study sought to determine its effect on the calcium oxalate levels, glucomannan levels, and antioxidative activities of different particle sizes of porang.

2. Methods

2.1. Production of porang macerated with SC

The porang utilized in this study was purchased in the form of dried chips from a farmer who is a member of the Porang Nusantara Activist Association branch of Boyolali, North Java, Indonesia. It was then ground, floured, and macerated with an ethanolic extract of SC in accordance with the procedure set out in Patent Registration Number S00202006668 [9]. The porang was grouped into six groups in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration).

2.2. Measurement of the calcium oxalate levels, glucomannan levels, and antioxidative activities

The calcium oxalate levels of the porang samples were measured by approximating the total calcium content of each sample using the atomic absorption spectrometer (AAS) (GBC Scientific Equipment Ltd-932 Plus AAS) method [10]. The glucomannan levels were analyzed by calculating the glucomannan yield (i.e., percentage of glucomannan produced from porang flour) that was extracted from each sample using ethanol according to a previously described procedure [2]. Moreover, the antioxidative activities of the samples were measured by means of a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay) [11].

3. Results and Discussion

3.1. Calcium oxalate levels

The use of maceration treatment in combination with sieving yielded different particle sizes, which had a significant effect on the achieved decrease in the calcium oxalate level ($p < 0.05$). More specifically, the calcium oxalate levels appeared to be lower in the macerated porang samples of < 40 mesh (AX, AY, and AN) than in those of > 40 mesh (BX, BY, and BN) ($p < 0.05$). This result was comparable with the results of other studies that showed a decrease in the calcium oxalate levels after sieving with cyclone separator [12]. In addition, maceration with the ethanolic extract of SC resulted in slightly lower calcium oxalate levels when compared with the absence of maceration. This finding differed from the results of previous studies, which showed that maceration with the ethanolic extract of SC declined the levels of calcium oxalate in porang in comparison with maceration solely in ethanol solvent [6] [9]. However, maceration with the ethanolic extract of SC has previously shown greater antidiabetic potency, which was found to be equivalent to that of commercial glibenclamide rather than that of porang without maceration in a study involving rats [7]. This study used two kinds of SC that could be used in industry. This combination of treatments also resulted in lower calcium oxalate levels than other treatments, such as cyclone separator which resulted in 3.97% - 5.71% of calcium oxalate [12].

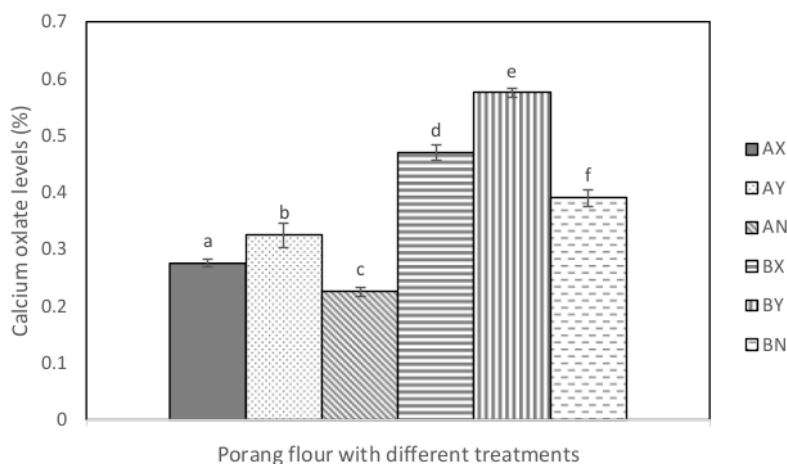


Figure 1. Calcium oxalate levels of differently treated porang samples: AX (particle size of < 40 mesh; maceration of X brand SC), AY (particle size of < 40 mesh; maceration of Y brand SC), AN (particle size of < 40 mesh; no maceration), BX (particle size of > 40 mesh; maceration of X brand SC), BY (particle size of > 40 mesh; maceration of Y brand SC), and BN (particle size of > 40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p < 0.05$).

3.2. Glucomannan levels

Table 1 shows how the different particle sizes and maceration with the ethanolic extract of different brands of SC affected the glucomannan levels of the porang samples ($p < 0.05$). The highest glucomannan levels were seen in the AN group, although they were significantly different from those in the AY group. In prior studies, it was found that the glucomannan levels were influenced by the concentration of ethanol and the solvent/porang ratio [13]. However, the purification of porang by means of the physical grinding method could increase its glucomannan level [14]. In this study, the use of sieving reduced the impurities in the porang flour, as calcium oxalate is generally a light fraction or small-sized component [12].

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4. Conclusion

This study found that sieving treatment combined with maceration with the ethanolic extract of SC affected the calcium oxalate and glucomannan levels of porang flour. More specifically, the porang samples with a particle size of >40 mesh exhibited lower calcium oxalate levels and higher glucomannan levels. However, the combination of the two treatments significantly reduced the antioxidant activity of the porang flour. The

further study is needed to find the right solvent of SC that may decrease calcium oxalate in porang and maintaining its antioxidative activity.

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Calcium oxalate levels, glucomannan levels, and antioxidative activities of different sized *Amorphophallus oncophyllus* particles and the maceration of *Strobilanthes crispus*

V Aprilia¹, N Kusumawardani², R Fauzi², D Estiningsih², D Kusumawati¹

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Abstract. Porang (*Amorphophallus oncophyllus*) is a type of tuber widely planted in Indonesia. It has a high glucomannan content, but its use is limited due to its high calcium oxalate content. However, it has previously been shown that the maceration of porang with the ethanolic extract of *Strobilanthes crispus* (SC) can serve to reduce its calcium oxalate content. This study sought to determine the impact of sieving in combination with ethanolic extract of SC maceration on the calcium oxalate levels, glucomannan levels, and antioxidant activities of different sized particles of porang. The calcium oxalate levels were analyzed by means of the atomic absorption method, whereas the glucomannan levels were determined based on the yield of glucomannan derived from the ethanolic extraction process. The porang particles were then analyzed to assess their antioxidant activities using DPPH (1,1-diphenyl-2-picrylhydrazyl). The particles were grouped into six in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). The results revealed that porang with a particle size of <40 mesh that was macerated with SC exhibited lower calcium oxalate levels and higher glucomannan levels than porang with a particle size of >40 mesh. The antioxidant activity of the porang decreased after maceration with the ethanolic extract of SC.

1. Introduction

Diabetes mellitus (DM) is a cause of death worldwide, and its prevalence is known to be increasing. Thus, various efforts have been made by the Indonesian government to reduce the prevalence of DM, ranging from the procurement of low-cost drugs to the funding of various studies. Herbal therapy and the use of functional food diets or food medicines (nutraceuticals) have been suggested as affordable alternatives to established DM treatment, and such alternatives are expected to be associated with only minimal side effects [1].

Porang (*Amorphophallus oncophyllus*) contains glucomannan that is potential as a functional food [2]. Glucomannan is a water-soluble polysaccharide known to have many health benefits, including lowering the blood glucose level [3]. However, the use of pure glucomannan remains rare due to its high cost. In the

case of Indonesia, there is limited factory capacity with regard to the production of glucomannan, which means that it must be imported from abroad. Rough porang has been suggested as an alternative means of sourcing consumable glucomannan with an equivalent function to the factory-produced variety. Yet, the use of porang is currently limited because it contains calcium oxalate, which is an irritant and might be a risk of kidney stone formation and kidney failure [4]. It has also been found to increase the creatinine and blood urea levels in rats [5,6].

Several attempts have been made to decrease the amount of calcium oxalate in porang, including the use of stamp mills, fractionation blowers, ball mills, and chemicals such as sodium chloride, ash, and ethanol [8] [9] [10] [11]. The use of a herbal method—that is, maceration with an ethanolic extract—represents another means of reducing the calcium oxalate content of porang. Indeed, prior studies have shown that it is more effective than the use of ethanol alone [6]. The lowest level of calcium oxalate achieved in this way was 0.5% according to Indonesia Patent Registration Number S00202006668.

The maceration of porang with *Strobilanthes crispus* (SC) was investigated in a study involving hyperglycemia-induced rats, with the results proving that it was able to lower the rats' blood glucose levels to a greater extent than the provision of porang flour without maceration. In addition, maceration with SC has also been found to reduce blood glucose levels to an extent equivalent to the use of the commercial drug glibenclamide [7]. The mechanism behind the decrease in the blood glucose level was suggested to be associated with the SC fiber being able to absorb water up to 100 mL/g and form a viscous fluid [8], thereby prolonging satiety and potentially decreasing absorption of food or nutrient in the blood and small intestine. The decreased of glucose and secretion of insulin led to an improvement in insulin sensitivity and the protection of pancreatic structure in histopathological observation [1].

The effect of maceration with the ethanolic extract of SC on the glucomannan level and antioxidant activity of porang has not previously been investigated. Thus, the present study sought to determine its effect on the calcium oxalate levels, glucomannan levels, and antioxidative activities of different particle sizes of porang.

2. Methods

2.1. Production of porang macerated with SC

The porang utilized in this study was purchased in the form of dried chips from a farmer who is a member of the Porang Nusantara Activist Association branch of Boyolali, North Java, Indonesia. It was then ground, floured, and macerated with an ethanolic extract of SC in accordance with the procedure set out in Patent Registration Number S00202006668 [9]. The porang was grouped into six in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration).

2.2. Measurement of the calcium oxalate levels, glucomannan levels, and antioxidative activities

The calcium oxalate levels of the porang samples were measured by approximating the total calcium content of each sample using the atomic absorption spectrometer (AAS) (GBC Scientific Equipment Ltd-932 Plus AAS) method [10]. The glucomannan levels were analyzed by calculating the glucomannan yield (i.e., percentage of glucomannan produced from porang flour) that was extracted from each sample using ethanol according to a previously described procedure [2]. Moreover, the antioxidative activities of the samples were measured by means of a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay) [11].

3. Results and Discussion

3.1. Calcium oxalate levels

The use of maceration treatment in combination with sieving yielded different particle sizes, which had a significant effect on the achieved decrease in the calcium oxalate level ($p < 0.05$). More specifically, the calcium oxalate levels appeared to be lower in the macerated porang samples of <40 mesh (AX, AY, and AN) than in those of >40 mesh (BX, BY, and BN) ($p < 0.05$). This result was comparable with the results of other studies that showed a decrease in the calcium oxalate levels after sieving with cyclone separator [12]. In addition, maceration with the ethanolic extract of SC resulted in slightly lower calcium oxalate levels when compared with the absence of maceration. This finding differed from the results of previous studies, which showed that maceration with the ethanolic extract of SC declined the levels of calcium oxalate in porang in comparison with maceration solely in ethanol solvent [6] [9]. However, maceration with the ethanolic extract of SC has previously shown greater antidiabetic potency, which was found to be equivalent to that of commercial glibenclamide rather than that of porang without maceration in a study involving rats [7]. This study used two kinds of SC that could be used in industry. This combination of treatments also resulted in lower calcium oxalate levels than other treatments, such as cyclone separator which resulted in 3.97% - 5.71% of calcium oxalate [12].

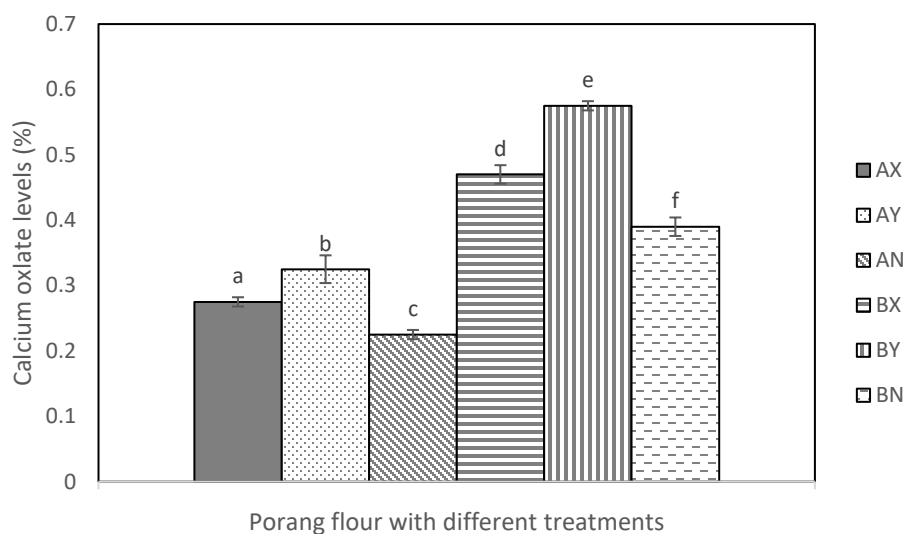


Figure 1. Calcium oxalate levels of differently treated porang samples: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p < 0.05$).

3.2. Glucomannan levels

Table 1 shows how the different particle sizes and maceration with the ethanolic extract of different brands of SC affected the glucomannan levels of the porang samples ($p < 0.05$). The highest glucomannan levels were seen in the AN group, although they were significantly different from those in the AY group. In prior studies, it was found that the glucomannan levels were influenced by the concentration of ethanol and the solvent/porang ratio [13]. However, the purification of porang by means of the physical grinding method

could increase its glucomannan level [14]. In this study, the use of sieving reduced the impurities in the porang flour, as calcium oxalate is generally a light fraction or small-sized component [12].

Table 1. Glucomannan levels of differently treated porang sample

Treatments	Glucomannan levels (%)
AX	57.4±3.7b
AY	64.5±10.9bc
AN	70.9±2.26c
BX	51.2±0.9ab
BY	42.0±4.8a
BN	43.8±1.9a

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p<0.05$).

3.3. Antioxidative activities

An antioxidative activity analysis was only performed on the macerated porang samples with a particle size of <40 mesh, as those samples had been shown to have lower calcium oxalate levels and higher glucomannan levels than the samples with a particle size of >40 mesh (see Table 1 and Figure 1). Table 2 describes the antioxidative activities of the macerated porang samples with various treatments. The results showed that the control group had the highest antioxidant activity (which was not subjected to sieving and soaking treatment) ($p<0.05$). This finding could not prove the increase of antioxidant compounds from the ethanolic extract of SC to the porang, as the ethanolic solvent was rinsed twice after the maceration process. SC was found to have many antioxidant compounds, including polyphenol, catechin, alkaloid, caffeine, tannin, and vitamin (C, B1, and B2) compounds [15]. The SC maceration treatment actually caused the natural antioxidant compounds in the porang flour to be wasted, possibly due to the washing of the ethanol, which is an organic solvent capable of extracting non-polar compounds, especially antioxidants [16]. Therefore, the maceration of porang with ethanol could decrease the antioxidative activity of porang and may not be suggested. It needs more study to select the right type of solvent to get high antioxidant activity. The high antioxidant activity in porang is potential its uses as functional food in maintaining of health.

Table 2. Antioxidative activities of differently treated porang samples

Treatments	Antioxidative activities (%)
AX	25.6±0.2a
AY	16.8±0.2b
AN	57.2±0.2c
CT	65.2±0.2d

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), CT (control, porang without treatment). Various letters demonstrate dissimilarity between groups ($p<0.05$).

4. Conclusion

This study found that sieving treatment combined with maceration with the ethanolic extract of SC affected the calcium oxalate and glucomannan levels of porang flour. More specifically, the porang samples with a particle size of >40 mesh exhibited lower calcium oxalate levels and higher glucomannan levels. However,

the combination of the two treatments significantly reduced the antioxidant activity of the porang flour. The further study is needed to find the right solvent of SC that may decrease calcium oxalate in porang and maintaining its antioxidative activity.

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1. Introduction

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2. Material and Methods

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2.2. Measurement of the calcium oxalate levels, glucomannan levels, and antioxidative activities

The calcium oxalate levels of the porang samples were measured by approximating the total calcium content of each sample using the atomic absorption spectrometer (AAS) (GBC Scientific Equipment Ltd-932 Plus AAS) method [10]. The glucomannan levels were analyzed by calculating the glucomannan yield (i.e., percentage of glucomannan produced from porang flour) that was extracted from each sample using ethanol according to a previously described procedure [2]. Moreover, the antioxidative activities of the samples were measured by means of a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay) [11].

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3. Results and Discussion

3.1. Calcium oxalate levels

The use of maceration treatment in combination with sieving yielded different particle sizes, which had a significant effect on the achieved decrease in the calcium oxalate level ($p < 0.05$). More specifically, the calcium oxalate levels appeared to be lower in the macerated porang samples of <40 mesh (AX, AY, and AN) than in those of >40 mesh (BX, BY, and BN) ($p < 0.05$). This result was comparable with the results of other studies that showed a decrease in the calcium oxalate levels after sieving with cyclone separator [12]. In addition, maceration with the ethanolic extract of SC resulted in slightly lower calcium oxalate levels when compared with the absence of maceration. This finding differed from the results of previous studies, which showed that maceration with the ethanolic extract of SC declined the levels of calcium oxalate in porang in comparison with maceration solely in ethanol solvent [6] [9]. However, maceration with the ethanolic extract of SC has previously shown greater antidiabetic potency, which was found to be equivalent to that of commercial glibenclamide rather than that of porang without maceration in a study involving rats [7]. This study used two kinds of SC that could be used in industry. This combination of treatments also resulted in lower calcium oxalate levels than other treatments, such as cyclone separator which resulted in 3.97% - 5.71% of calcium oxalate [12].

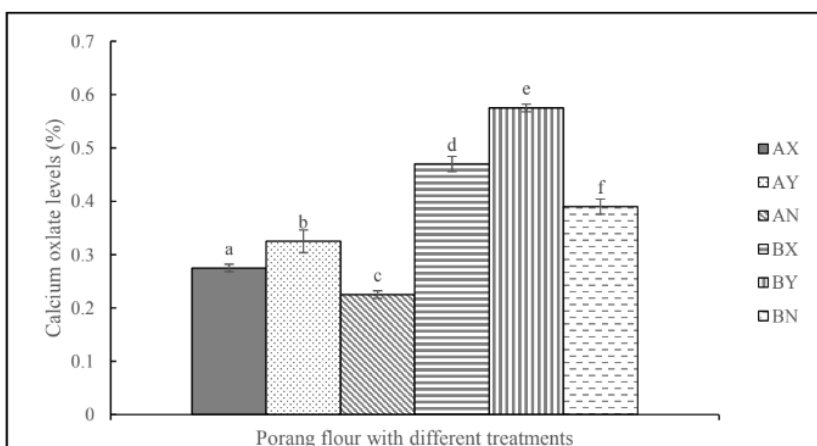


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3.2. Glucomannan levels

Table 1 shows how the different particle sizes and maceration with the ethanolic extract of different brands of SC affected the glucomannan levels of the porang samples ($p < 0.05$). The highest glucomannan levels were seen in the AN group, although they were significantly different from those in the AY group. In prior

studies, it was found that the glucomannan levels were influenced by the concentration of ethanol and the solvent/porang ratio [13]. However, the purification of porang by means of the physical grinding method could increase its glucomannan level [14]. In this study, the use of sieving reduced the impurities in the porang flour, as calcium oxalate is generally a light fraction or small-sized component [12].

Table 1. Glucomannan levels of differently treated porang sample

Treatments	Glucomannan levels (%)
AX	57.4±3.7b
AY	64.5±10.9bc
AN	70.9±2.26c
BX	51.2±0.9ab
BY	42.0±4.8a
BN	43.8±1.9a

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration). Various letters demonstrate dissimilarity between groups ($p<0.05$).

3.3. Antioxidative activities

An antioxidative activity analysis was only performed on the macerated porang samples with a particle size of <40 mesh, as those samples had been shown to have lower calcium oxalate levels and higher glucomannan levels than the samples with a particle size of >40 mesh (see Table 1 and Figure 1). Table 2 describes the antioxidative activities of the macerated porang samples with various treatments. The results showed that the control group had the highest antioxidant activity (which was not subjected to sieving and soaking treatment) ($p<0.05$). This finding could not prove the increase of antioxidant compounds from the ethanolic extract of SC to the porang, as the ethanolic solvent was rinsed twice after the maceration process. SC was found to have many antioxidant compounds, including polyphenol, catechin, alkaloid, caffeine, tannin, and vitamin (C, B1, and B2) compounds [15]. The SC maceration treatment actually caused the natural antioxidant compounds in the porang flour to be wasted, possibly due to the washing of the ethanol, which is an organic solvent capable of extracting non-polar compounds, especially antioxidants [16]. Therefore, the maceration of porang with ethanol could decrease the antioxidative activity of porang and may not be suggested. It needs more study to select the right type of solvent to get high antioxidant activity. The high antioxidant activity in porang is potential its uses as functional food in maintaining of health.

Table 2. Antioxidative activities of differently treated porang samples

Treatments	Antioxidative activities (%)
AX	25.6±0.2a
AY	16.8±0.2b
AN	57.2±0.2c
CT	65.2±0.2d

Notes: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), CT (control, porang without treatment). Various letters demonstrate dissimilarity between groups ($p<0.05$).

4. Conclusion

This study found that sieving treatment combined with maceration with the ethanolic extract of SC affected the calcium oxalate and glucomannan levels of porang flour. More specifically, the porang samples with a particle size of >40 mesh exhibited lower calcium oxalate levels and higher glucomannan levels. However, the combination of the two treatments significantly reduced the antioxidant activity of the porang flour. The further study is needed to find the right solvent of SC that may decrease calcium oxalate in porang and maintaining its antioxidative activity.

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Calcium oxalate levels, glucomannan levels, and antioxidative activities of different sized *Amorphophallus oncophyllus* particles and the maceration of *Strobilanthes crispus*

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Abstract. Porang (*Amorphophallus oncophyllus*) is a type of tuber widely planted in Indonesia. It has a high glucomannan content, but its use is limited due to high calcium oxalate content. However, it has previously been shown that the maceration of porang with the ethanolic extract of *Strobilanthes crispus* (SC) can serve to reduce its calcium oxalate content. This study sought to determine the impact of sieving in combination with ethanolic extract of SC maceration on the calcium oxalate levels, glucomannan levels, and antioxidant activities of different sized particles of porang. The calcium oxalate levels were analyzed by the atomic absorption method, whereas the glucomannan levels were determined based on the yield of glucomannan derived from the ethanolic extraction process. The porang particles were then analyzed to assess their antioxidant activities using DPPH (1,1-diphenyl-2-picrylhydrazyl). The particles were grouped into six in different particle size and maceration: AX, AY, AN, BY, and BN. The results revealed that porang with a particle size of <40 mesh that was macerated with SC exhibited lower calcium oxalate levels and higher glucomannan levels than porang with a particle size of >40 mesh. The antioxidant activity of the porang decreased after maceration with the ethanolic extract of SC.

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1. Introduction

Diabetes mellitus (DM) is a cause of death worldwide, and its prevalence is known to be increasing. Thus, various efforts have been made by the Indonesian government to reduce the prevalence of DM, ranging from the procurement of low-cost drugs to the funding of various studies. Herbal therapy and the use of functional food diets or food medicines (nutraceuticals) have been suggested as affordable alternatives to established DM treatment, and such alternatives are expected to be associated with only minimal side effects [1].

Porang (*Amorphophallus oncophyllus*) contains glucomannan that is potential as a functional food [2]. Glucomannan is a water-soluble polysaccharide known to have many health benefits, including lowering the blood glucose level [3]. However, the use of pure glucomannan remains rare due to its high cost. In the case of Indonesia, there is limited factory capacity with regard to the production of glucomannan, which

means that it must be imported from abroad. Rough porang has been suggested as an alternative means of sourcing consumable glucomannan with an equivalent function to the factory-produced variety. Yet, the use of porang is currently limited because it contains calcium oxalate, which is an irritant and might to be a risk of kidney stone formation and kidney failure [4]. It has also been found to increase the creatinine and blood urea levels in rats [5,6].

Several attempts have been made to decrease the amount of calcium oxalate in porang, including the use of stamp mills, fractionation blowers, ball mills, and chemicals such as sodium chloride, ash, and ethanol [2,7–9]. The use of a herbal method—that is, maceration with an ethanolic extract—represents another means of reducing the calcium oxalate content of porang. Indeed, prior studies have shown that it is more effective than the use of ethanol alone [6]. The lowest level of calcium oxalate achieved in this way was 0.5% according to Indonesia Patent Registration Number S00202006668.

The maceration of porang with *Strobilanthes crispus* (SC) was investigated in a study involving hyperglycemia-induced rats, with the results proving that it was able to lower the rats' blood glucose levels to a greater extent than the provision of porang flour without maceration. In addition, maceration with SC has also been found to reduce blood glucose levels to an extent equivalent to the use of the commercial drug glibenclamide [10]. The mechanism behind the decrease in the blood glucose level was suggested to be associated with the SC fiber being able to absorb water up to 100 mL/g and form a viscous fluid [2], thereby prolonging satiety and potentially decreasing absorption of food or nutrient in the blood and small intestine. The decreased of glucose and secretion of insulin led to an improvement in insulin sensitivity and the protection of pancreatic structure in histopathological observation [1].

The effect of maceration with the ethanolic extract of SC on the glucomannan level and antioxidant activity of porang has not previously been investigated. Thus, the present study sought to determine its effect on the calcium oxalate levels, glucomannan levels, and antioxidative activities of different particle sizes of porang.

2. Material and Methods

2.1. Production of porang macerated with SC

The porang utilized in this study was purchased in the form of dried chips from a farmer who is a member of the Porang Nusantara Activist Association branch of Boyolali, North Java, Indonesia. It was then ground, floured, and macerated with an ethanolic extract of SC in accordance with the procedure set out in Patent Registration Number S00202006668 [7]. The porang was grouped into six in different particle size and maceration: AX (particle size of <40 mesh; maceration of X brand SC), AY (particle size of <40 mesh; maceration of Y brand SC), AN (particle size of <40 mesh; no maceration), BX (particle size of >40 mesh; maceration of X brand SC), BY (particle size of >40 mesh; maceration of Y brand SC), and BN (particle size of >40 mesh; no maceration).

2.2. Measurement of the calcium oxalate levels, glucomannan levels, and antioxidative activities

The calcium oxalate levels of the porang samples were measured by approximating the total calcium content of each sample using the atomic absorption spectrometer (AAS) (GBC Scientific Equipment Ltd-932 Plus AAS) method [8]. The glucomannan levels were analyzed by calculating the glucomannan yield (i.e., percentage of glucomannan produced from porang flour) that was extracted from each sample using ethanol according to a previously described procedure [2]. Moreover, the antioxidative activities of the samples were measured by means of a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay) [9].

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3. Results and Discussion

3.1. Calcium oxalate levels

The use of maceration treatment in combination with sieving yielded different particle sizes, which had a significant effect on the achieved decrease in the calcium oxalate level ($p < 0.05$). More specifically, the calcium oxalate levels appeared to be lower in the macerated porang samples of <40 mesh (AX, AY, and AN) than in those of >40 mesh (BX, BY, and BN) ($p < 0.05$). This result was comparable with the results of other studies that showed a decrease in the calcium oxalate levels after sieving with cyclone separator [11]. In addition, maceration with the ethanolic extract of SC resulted in slightly lower calcium oxalate levels when compared with the absence of maceration. This finding differed from the results of previous studies, which showed that maceration with the ethanolic extract of SC declined the levels of calcium oxalate in porang in comparison with maceration solely in ethanol solvent [6] [7]. However, maceration with the ethanolic extract of SC has previously shown greater antidiabetic potency, which was found to be equivalent to that of commercial glibenclamide rather than that of porang without maceration in a study involving rats [10]. This study used two kinds of SC that could be used in industry. This combination of treatments also resulted in lower calcium oxalate levels than other treatments, such as cyclone separator which resulted in 3.97% - 5.71% of calcium oxalate [11].

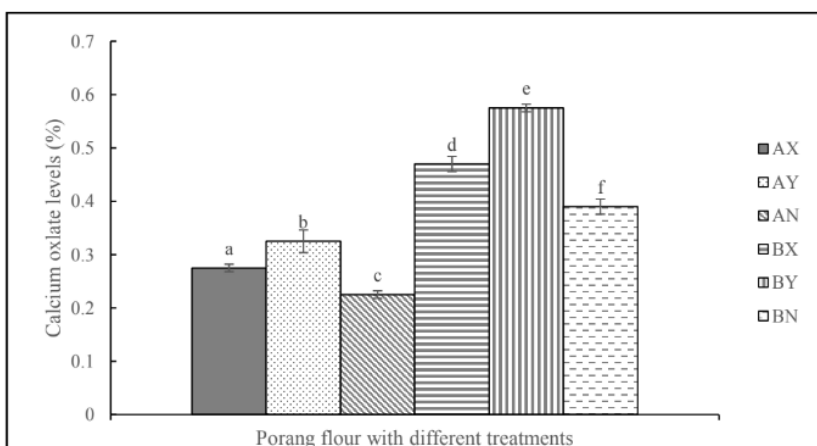


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