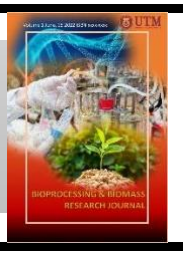
Bioprocessing and Biomass Technology

Journal homepage: https://bioprocessing.utm.my



Research Article

Effect of *Curcuma xanthorrhiza* Extracts on the Phytochemical Content, Antioxidant Activity and *Lactobacillus casei* Growth

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ARTICLE INFO

Article History:
Received 17 April 2024
Received in revised form 16 May 2024
Accepted 18 May 2024
Available online 30 June 2024

Keywords:
Curcuma xanthorrhiza,
antioxidant activity,
total phenolic content,
total flavonoid content

ABSTRACT

Curcuma xanthorrhiza (CX) has been reported as the most widely cultivated species in Southeast Asia. It is commonly found in the wild and has been traditionally used for medicinal purposes. The rhizome and root of CX contain constituents that offer various health benefits, including the treatment of skin inflammations and acne, as well as having antioxidant properties. Recently, numerous herbal products and traditional medicines made from CX have appeared in the market, available in forms such as capsules and bottled drinks. However, no research has yet validated the potential of growing *Lactobacillus casei (L. casei)* probiotic bacteria in CX extracts. The aim of this study is to determine the total phytochemical content of CX extracts, the antioxidant activity of CX extracts, and the prebiotic potential of CX. In this article, the chemical contents of CX, including total phenolics, total flavonoids, and antioxidant activity, have been studied using the Folin-Ciocalteu method, the aluminum chloride colorimetric method, and the DPPH free radical scavenging assay for antioxidant activity. The studies were conducted using three different methods of extracting CX: methanol-maceration, fresh juiced, and fresh boiled CX. The prebiotic potential of CX was studied using the *L. casei* probiotic strain. From the methanol-maceration extraction, the values of TPC and TFC are 111.55 ± 13.64 µg GAE/mg DW and 27.30 ± 7.75 µg QE/mg DW, respectively. Meanwhile, for the fresh juiced extraction, the TPC and TFC values are 30.513 ± 0.917 µg GAE/mg DW and 43.81 ± 5.13 µg QE/mg DW, respectively. Lastly, the boiled extraction method yields a TPC of 10.85 ± 0.21 µg GAE/mg DW and a TFC of 8.00 ± 0.49 µg QE/mg DW. At a concentration of 2000 µg/mL, the scavenging activity of the fresh juiced extraction reached a plateau of 89.33%. Meanwhile, at the same concentration, the scavenging effects of CX fresh methanol maceration and fresh boiled extraction are 61.39% and 49.81%, respectively. The *L. casei* strain was shown to be actively growing in CX extracts within the first 24 hours; however, after 24 hours, the growth remained almost constant. This may be due to the limited supply of CX extracts for the growth of the probiotic *L. casei* strain. Altogether, these results demonstrate that CX is a potential source of valuable bioactive compounds that could be used in medical, pharmaceutical, and food industries.

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INTRODUCTION

In Indonesia, *Curcuma xanthorrhiza* (CX), commonly known as Javanese turmeric, grows in Southeast Asia and can be found in both cultivated and wild areas. CX is traditionally used for medicinal purposes. The rhizome and root of CX contain constituents that offer benefits and have been used for the treatment of skin inflammations, acne, and possess antioxidant properties. Recent studies have shown that products derived from CX exhibit antioxidative, detoxifying, and anticarcinogenic characteristics. The chemical content in CX is promising, especially its high antioxidant activity, which provides benefits such as anti-aging and detoxifying effects. CX also has prebiotic potential for the growth of probiotic bacteria. According to Sjamsul et al. (2007), the rhizome of CX is one of the most common herbs in Indonesian jamus. This statement is supported by Sharin (2006), who states that CX is widely used as an ingredient in traditional health remedies such as "jamu" and "maajun," or as a specific treatment for certain health problems. Additionally, Sharin (2006) notes that the juice extract from CX rhizomes is used to treat rheumatism and indigestion, or to be applied to the body during childbirth.

Probiotics can be defined as living non-pathogenic microorganisms such as lactic acid bacteria or *Saccharomyces boulardii* yeast. Probiotics are also known as good bacteria that maintain the health of our gastrointestinal tract. They are especially beneficial for the gastrointestinal digestive system. Examples of lactic acid probiotic bacteria include *Bifidobacterium* species and *Lactobacillus* species, which can be consumed through foods and supplements. The benefits of probiotics include improving the balance of microbial composition in the gastrointestinal tract through mechanisms such as lowering intestinal pH and reducing the invasion and colonization of pathogenic microorganisms. In Malaysia, no research has been conducted to understand the probiotic growth potential in CX extracts. In this research, *Lactobacillus casei* (Strain Shirota) was used as the probiotic bacterium, and the growth potential of *Lactobacillus casei* in CX extracts was studied.

Antioxidants are "free-radical scavengers," substances that can slow or prevent damage to cells caused by free radicals. Free radicals are unstable molecules produced by the body in response to environmental factors. Antioxidants can be found naturally in CX or produced artificially. Research on natural antioxidants in plants has increased over the time. Antioxidants are also associated with reducing the risk of chronic diseases. However, the extent and chemical composition of different bioactive compounds vary with each species. In this research, the antioxidant activity in CX extracts was studied using the DPPH radical scavenging method.

The study of phytochemical content in CX extracts has also been conducted in this research. Total phenolic content (TPC) of CX extracts was measured to study the phenolic compounds contained in CX, as well as the total flavonoid content (TFC) in CX extracts. TPC and TFC were studied using the Folin-Ciocalteu method and the aluminum chloride colorimetric method, respectively. In conclusion, this research validates the study of phytochemical content in CX extracts, along with their antioxidant activity and the growth potential of *Lactobacillus casei*.

Studies of the chemical content in CX are usually conducted using methanol or ethanol extraction methods. However, in this research, the chemical content in CX was studied using three different extraction methods. This allows for a comparison between methanol-maceration extraction, fresh juiced CX, and fresh boiled CX. This research also validates the prebiotic potential of CX extract.

Thus, in the present study, CX was extracted with the aim of evaluating the antioxidant activities from different extraction methods. Methanol maceration, fresh juicing, and boiling extraction methods were used to examine their antioxidant activities by applying the DPPH free radical scavenging assay. Additionally, the extracts of CX were used to study the prebiotic potential using *Lactobacillus casei* probiotic bacteria.

METHODOLOGY

Preparation of *Curcuma xanthorrhiza* (CX)

Curcuma xanthorrhiza (CX) was used in this study. Fresh CX was hand-selected and purchased from a local supermarket at Pasar Larkin, Johor, while dried powdered CX was purchased from Sigma-Aldrich. The fresh CX was washed thoroughly to remove all dirt and impurities. It was then peeled and cut into small pieces. The fresh CX pieces were dried overnight in an oven at 60 °C until a constant weight was achieved.

Extraction procedure

Firstly, the methanol-maceration extraction involved dissolving dried powdered CX in methanol solution for 3 to 4 hours and filtering it using filter paper, following the method by Sharin (2006) with slight modifications. The clear CX extract was then subjected to rotary evaporation at 60 °C for 20 minutes. The CX extract was stored in a Schott bottle in the refrigerator for further use. The second method was fresh juiced extraction. 100 g of fresh dried CX was mixed with 200 ml of distilled water. This mixture was then blended for 5-6 minutes until it became a juice. The juice solution was filtered using filter paper and a vacuum filter. The clear CX juice was collected and stored in a Schott bottle in the refrigerator for further use. The third method was boiled extraction. 100 g of dried CX was mixed with 1000 ml of distilled water. This mixture was poured into a beaker and heated overnight at 60 °C with a magnetic stirrer. The solution was then filtered using a vacuum filter and stored in a Schott bottle in the refrigerator for further use.

Determination of total phenolic content (TPC)

In the TPC test as described by Phuyal et al. (2020), the gallic acid standard solution was prepared by dissolving 50 mg of gallic acid in 50 ml of distilled water, followed by serial dilution into several different concentrations. Next, the Folin solution was prepared by mixing 1 ml of Folin reagent with 9 ml of distilled water. The 7.5% sodium carbonate solution was prepared by dissolving 3.7 g of sodium carbonate in 50 ml of distilled water. For the preparation of the CX sample,

40 mg of dried extracted CX was dissolved in 10 ml of distilled water. Then, 1.5 ml of the CX sample was pipetted and centrifuged at 5000 rpm for 5 minutes. The blank solutions were prepared by mixing 50 µl of CX sample solution with 50 µl of water and 50 µl of sodium carbonate solution. In a microplate, 50 µl of the sample was mixed with 50 µl of sodium carbonate solution and 50 µl of Folin solution. The mixture was then covered and allowed to stand for 30 minutes at room temperature in a dark place. The absorbance was read at 760 nm using a BIO-TEK ELX808 microplate reader. The gallic acid standard solution was run in triplicate.

Determination of total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Shraim et al. (2021), with quercetin as the standard solution. First, 50 mg of quercetin was dissolved in 50 ml of distilled water, followed by serial dilution into several different concentrations. Next, a 5% sodium nitrite solution was prepared by dissolving 2.5 g of sodium nitrite in 50 ml of distilled water. After that, a 10% aluminum chloride solution was prepared by dissolving 5 g of aluminum chloride in 50 ml of distilled water.

The CX sample solution was prepared by dissolving dried extracted CX in distilled water to a concentration of 4000 ppm. The blank solution was prepared by mixing 50 µl of the CX sample with 50 µl of water and 50 µl of sodium nitrite solution. In a microplate, 12 µl of sodium nitrite solution was mixed with 12 µl of aluminum chloride solution and 200 µl of sample solution. The mixture was then covered and allowed to stand for 30 minutes at room temperature in a dark place. The absorbance was read using a BIO-TEK ELX808 microplate reader at 510 nm. The quercetin standard solution was run in triplicate.

Evaluation of antioxidant activity via DPPH radical scavenging assay

The antioxidant activity was determined by the scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as reported by Baliyan et al. (2022) with slight modifications. First, the preparation of the DPPH solution involved dissolving 394 mg of DPPH in 100 ml of methanol to achieve a concentration of 10 mM, which was then diluted to 0.25 mM. Next, the ascorbic acid standard solution was prepared by dissolving 80 mg of ascorbic acid in 20 ml of distilled water, followed by serial dilutions ranging from 0 to 4000 µg/ml.

The CX sample solution was prepared by dissolving dried extracted CX in 20 ml of distilled water, also followed by serial dilutions ranging from 0 to 4000 µg/ml. The blank solution was prepared by mixing the sample with methanol solvent in a microplate. In a microplate reader, 100 µl of the sample solution was mixed with 100 µl of the DPPH solution and kept in the dark at room temperature for 40 minutes. The absorbance was then read at 517 nm using a Bio-Tek ELX808 microplate reader. The antioxidant activity of the samples was determined by the DPPH inhibition activity percentage calculated as:

$$\text{DPPH inhibition activity (\%)} = ((A_{contr}-A_{test})/A_{contr}) \times 100$$

Where, A_{contr} is a control sample absorbance in which DPPH solution without probiotic drinksm and A_{test} is the test sample absorbance in which DPPH solution plus probiotic drinks.

Probiotic strains and pre-culture preparation

In this study, *Lactobacillus casei* strain Shirota, obtained from Yakult drinks, was used. *Lactobacillus casei* pure culture was stored in MRS (de Man, Rogosa, and Sharpe) broth with 20% glycerol at -20 °C before use. Lactobacillus casei was grown in MRS broth and cultivated statically at 37 °C for 24 hours.

Fermentation of the *Lactobacillus casei* strains in *Curcuma xanthorrhiza* extract

1% (volume/volume) of pre-cultured probiotic strain (0.2 ml) was inoculated into 50 ml conical flasks along with 0.2 ml of pasteurized CX extract and 17.8 ml of MRS broth. The fermentation samples were then incubated for 2 days at 37 °C. For microbiological and chemical analysis, samples were taken under aseptic conditions at 6, 12, 24, and 48 hours after the fermentation process started. The absorbance of each sample was measured at 600 nm using a microplate reader (Model UV-160, Shimadzu, Japan), with MRS broth used as a blank or control. The optimum conditions for fermentation were maintained at a temperature of 37 °C and a pH of 4. These conditions were selected because Lactobacillus can thrive at this pH and temperature (Panghal et al., 2017).

RESULTS AND DISCUSSION

Percentage of yield

Table 1 shows the percentage of extraction efficiency of CX extract.

Table 1 Extraction yield of CX by using different extraction methods

Extraction Methods	Extraction Yield (%)
Methanol-Maceration	16.33
Juiced	8.33
Boiled	4.33

The percentage yield for methanol-maceration extraction was the highest compared to fresh juiced and boiled extraction, with values of 16.33%, 8.33%, and 4.33% respectively. This variation in yield can be attributed to the differences in the extraction methods used.

Total phenolic content

The total phenolic content (µg GAE/mg dry weight) was obtained using the formula which was automatically derived from the gallic acid calibration plot.

Table 2 Total phenolic content

Method	TPC (µg GAE/mg dry weight)
Methanol-Maceration	111.55 ± 13.64
Juiced	30.513 ± 0.917
Boiled	10.85 ± 0.21

Referring to **Table 2**, different extraction methods have varying impacts on the total phenolic contents in CX. The highest extraction yield of total phenolic contents was 111.55 ± 13.64 µg GAE/mg DW obtained from methanol-

maceration extraction, followed by 30.513 ± 0.917 µg GAE/mg DW from fresh juiced extraction. Lastly, the boiled extraction method yielded total phenolic contents at 10.85 ± 0.21 µg GAE/mg DW.

Comparing the different extraction methods, it is evident that the method used plays a significant role in the extraction of phenolic compounds. From **Table 2**, it is clear that the methanol-maceration extraction method yielded the highest amount of phenolic compounds.

Phenolic compounds are secondary metabolites known for their ability to donate hydrogen, quench singlet oxygen, and function as metal chelators, thus acting as antioxidants. According to Zagoskina et al. (2023), phenolics in most land plants are classified as low molecular weight secondary metabolites.

The theory underlying this approach is the tendency of phenolic functional groups to undergo oxidation and reduction reactions. The reduction of phenolate ions by the reagent changes its color to blue, as reported by Yermeydan et al. (2024). When extracts contain higher amounts of phenolic compounds, the reduction in complexes increases, resulting in darker coloration and higher absorbance.

Antioxidant activity also increases with higher concentrations of plant samples. The results of the DPPH radical assay indicate a close correlation between antioxidant function and the level of phenolic compounds, suggesting that the antioxidant activities of CX are likely due to phenolic compounds.

Total flavonoid content (TFC)

The total flavonoid content (µg QE/mg dry weight) was obtained using the formula which was automatically derived from the quercetin calibration plot (**Table 3**).

Table 3 Total flavonoid content

Method	TFC (µg QE/mg dry weight)
Methanol-Maceration	27.32 ± 7.75
Juiced	43.81 ± 5.13
Boiled	8.00 ± 0.49

The total flavonoid content (TFC) is an essential parameter of a plant's antioxidant ability. As shown in **Table 3**, different extraction methods have varying impacts on the total flavonoid contents in CX. The highest extraction yield of total flavonoid contents, 43.81 ± 5.13 µg QE/mg DW, was obtained from fresh juiced extraction, followed by 27.30 ± 7.75 µg QE/mg DW obtained from methanol-maceration extraction. Lastly, the boiled extraction method yielded total flavonoid contents of 8.00 ± 0.49 µg QE/mg DW. Comparing different extraction methods, it is evident that the method used plays an important role in the extraction of flavonoid compounds. From **Table 3**, it is clear that the fresh juiced extraction method yielded the highest amount of flavonoid compounds.

Antioxidant activity by DPPH assay

The scavenging activities of CX extracts on the DPPH radical were shown in **Figure 1**.

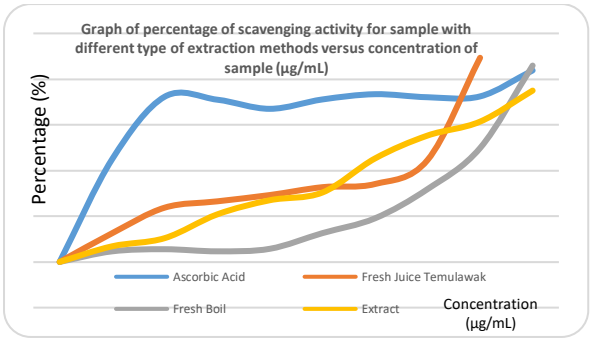

Figure 1 Percentage of scavenging activity of CX extracts.

At various concentrations tested, all of the CX extracts from different types of extraction reacted directly with quenched DPPH radicals to varying degrees, with increased activity observed at higher concentrations. Overall, based on **Figure 1**, CX extracted by fresh juiced extraction showed higher scavenging activity compared to CX extracted by methanol-maceration and fresh boil extraction methods. At a concentration of 2000 µg/mL, the scavenging activity of fresh juiced extraction reached a plateau of 89.33%. Meanwhile, at the same concentration, the scavenging effects of CX from fresh methanol-maceration and fresh boil extraction were 61.39% and 49.81%, respectively.

As stated by Baliyan et al. (2022), the antioxidant activities of CX, as measured by the DPPH radical, range between 30% and 90% (percent inhibition compared with control). This variation might have been caused by differences in potency or in the concentrations of reducing substances, mainly phenolics. Phenolics are the main antioxidant components, and their total contents are proportional to the antioxidant activity. However, the scavenging behavior was found to exhibit a different pattern compared to total phenolic content (TPC) and total flavonoid content (TFC) in this analysis. The variations in these results can be attributed to several factors, including differences in the plant matrix and the higher antioxidant activity of extracts containing phenolic compounds with a greater number of hydroxyl groups (Ziyatdinova & Kalmykova, 2023).

Prebiotic Potential in *Curcuma Xanthorrhiza* Extracts

CX were said to have prebiotic potential of growing probiotic strain. The potential of probiotic growth was determined by fermentation process of CX extracts and *Lactobacillus casei* strain. The samples were taken at 6, 12, 24 and 48 hours after cultivated at 37 °C (**Figure 2**).

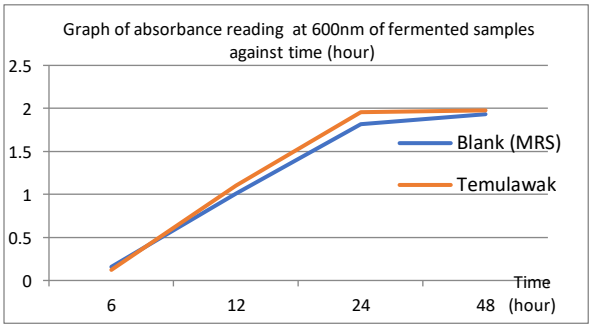

Figure 2 Graph of absorbance reading at 600 nm of fermented samples against time (hour).

Lactobacillus casei strain has been shown to actively grow in CX extracts within 24 hours, but after this period, the growth

rate remained nearly constant. This could be due to the availability of CX extracts supporting the growth of the probiotic Lactobacillus casei strain. Research by Rahman et al. (2018) indicates that the viability of *Lactobacillus casei* declines after 48 hours and remains constant for up to 5 days of fermentation time. The highly acidic conditions in the growth media may contribute to the decreased viability rate of *Lactobacillus casei*, thereby slowing the growth of the probiotic bacteria (Ng et al., 2023). This study demonstrates that CX extracts have prebiotic potential for the growth of probiotic Lactobacillus casei bacteria.

CONCLUSION

The objective of this study was achieved by observing the results of total phenolic and flavonoid content in CX extracts, as well as the DPPH radical scavenging activity. CX was extracted using methanol-maceration, fresh juiced, and fresh boiled extraction methods. Based on the results, CX extracted by methanol-maceration showed higher phenolic content (TPC) compared to other extracts. Meanwhile, for total flavonoid content (TFC), CX extracted by the fresh juiced method showed the highest content compared to methanol-maceration and fresh boiled extraction methods. The study also demonstrated the prebiotic potential of CX for the growth of Lactobacillus casei probiotic bacteria. Lastly, the DPPH results showed that CX extracts from the fresh juiced method exhibited higher scavenging activity compared to extracts from methanol-maceration and fresh boiled methods. These results suggest that CX is a potential source of beneficial bioactive compounds, possessing radical scavenging and antioxidant activities. Therefore, the use of CX in nutraceutical and functional food products as an alternative natural antioxidant to synthetic antioxidants would be highly beneficial. These findings indicate that CX can be classified as a healthy food, non-toxic with radical scavenging and antioxidant activities, and with prebiotic potential for the growth of *Lactobacillus casei* probiotic bacteria strain.

Acknowledgement

This research was financially supported by the Ministry of Education through UTM Fundamental Research Grant Vot. No: Q.J130000.3846.22H83.

References

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, *27*(4), 1326.

Ng, K. S., Bambace, M. F., & Schwab, C. (2023). Microbially-Produced Short Chain Carboxylic Acids are Ancient Food Biopreservatives with Complex Mode of Action. *Current Opinion in Food Science*, 101066.

Panghal, A., Virkar, K., Kumar, V., Dhull, S. B., Gat, Y., & Chhikara, N. (2017). Development of probiotic beetroot drink. *Current Research in Nutrition and Food Science Journal*, 5(3) 3776–3782.

Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *The Scientific World Journal*.

Rahman, I. A., Lazim, M. I. M., Mohamad, S., Peng, K. S., Othaman, M. A., Manan, M. A., & Asri, M. A. M. (2018). The Influence of Lactobacilli in GABA and Amino Acid Profile of Fermented Mature Coconut Water. *The Open Food Science Journal*, *10*(1).

Sharin, R. (2006). LC-MS/MS profiling and characterirization of active components from medicinal gingers *Curcuma xanthorrhiza* and *Zingiber zerumbet*. Thesis Master. University Putra Malaysia.

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *Lwt*, *150*, 111932.

Sjamsul, S.A., Hakim, E.H., Makmur, L., Syah, Y.M., Juliawaty, L.D. & Mujahidin, M. (2007) Chemistry and Uses of Indonesian Plant Drugs, Vol. I, ITB Publisher, pp. 143-152.

Yermeydan Peker, M., Koç, O. K., Üzer, A., & Apak, R. (2024). Folin–Ciocalteu Reagent-Loaded Acrylamide-Based Hydrogel Sensor for Antioxidant Capacity Measurement with the Molybdenum Green Method. *ACS Applied Polymer Materials*, *6*(3), 1864-1877.

Zagoskina, N. V., Zubova, M. Y., Nechaeva, T. L., Kazantseva, V. V., Goncharuk, E. A., Katanskaya, V. M., & Aksenova, M. A. (2023). Polyphenols in Plants: Structure, Biosynthesis, Abiotic Stress Regulation, and Practical Applications. *International Journal of Molecular Sciences*, *24*(18), 13874.

Ziyatdinova, G., & Kalmykova, A. (2023). Electrochemical Characterization of the Antioxidant Properties of Medicinal Plants and Products: A Review. *Molecules*, *28*(5), 2308.