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Research Article

Antioxidant and antimicrobial properties of *Garcinia mangostana*

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Aim of this study is to present microbiological quality, and antimicrobial and antioxidant activity of the mangosteen fruit in two forms: freeze-dried powder and fresh fruit. During the identification of the mangosteen microbiota using a MALDI-TOF MS Biotyper mass spectrophotometer, the presence of *B. cereus* and *Saccharomyces* spp. has been detected. The best antimicrobial activity was achieved against *Micrococcus* spp. Mangosteen fruit (*Garcina mangostana*) is characterized by a high content of polyphenols at the following levels: fresh fruit 3.22 ±0.68 mg GAE.g^{-1}; powder form 2.17 ±0.64 mg GAE.g^{-1}. Mangosteen shows a high antioxidant capacity of the fruit in the two forms presented in the work. It was 21.18% (fresh fruit) and 14.46% (freeze-dried fruit). Mangosteen also shows an antibacterial activity in relation to the strains of bacteria tested in our work.

Keywords: mangosteen fruit, microbiota, antioxidant and antimicrobial activity

1 Introduction

Mangosteen (*Garcinia mangostana*, *Garcinia mangosteen*) is a plant belonging to the Clusiaceae family. *Garcinia mangosteen* is referred to as the "Queen of fruits". It belongs to a special group of the so-called great fruits. The Garcinia type, in addition to *G. mangostana*, has up to 300 species, including: *G. dulcis*, *G. burgkiana*, *G. bancana*, *G. speciosa*, *G. antroviridis* (Dembitsky et al., 2011). It resembles tangerine in size and has a thick dark purple skin. When cut open, the skin is red inside. The inside of the fruit is white (Cieślik et al., 2017). The fruit is gaining popularity; it is desired all over the world. According to Kacaniova et al. (2018), Indonesia exports about 300 tons of fresh mangosteen per month, worth 350,000 USD Dollars, to Japan, Singapore, Saudi Arabia, China, and Europe. Gutierrez-Orozco and Fallia (2013) claim that in 2008, sales of mangosteen-based products exceeded $200 million, so mangosteen can be considered a fruit that brings huge income. Mangosteen can be eaten raw or used for processing: production of juices, preserves, and even wine. It is used also as an additional ingredient to various types of tea, ice cream, yoghurt, or desserts (Ketsa 2011; Suttirak & Manurakchinakorn, 2014). Mangosteen contains a large amount of minerals, antioxidants, and vitamins, and it is a low-calorie fruit. It has pro-health, anticarcinogenic, immunostimulating and hypolipemic properties. Asian medicine uses various parts of the mangosteen (leaves, peel, pericarp, bark) to prevent diseases such as skin disorders, urinary tract infections, diarrhoea, and acne (Pedraza-Chaverri et al., 2008). Fruit extract is widely used in the production of medicines, cosmetics, and pharmaceutical products. Mangosteen has many proven health-promoting properties. The fruit has the following effects: antimicrobial, anticarcinogenic, immunomodulating, antioxidant, hypolipemic. Due to the high content of polyphenols, xanthones, carotenoids, vitamins from the group vitamin B, and vitamin C, and low-calorie value, this fruit meets the requirements of a health-promoting food and a super fruit (Reilly, 1994). Relatively new on the Polish market, mangosteen is gaining more and more popularity due to its properties. Consumer awareness means that they reach for fruits and vegetables more and more often. Large retail chains introduce mangosteen fruit to their offer, which attracts new admirers. The aim of this study is to evaluate the microbiological quality, and the antimicrobial and antioxidant activity of the mangosteen fruit in two forms: freeze-dried powder and fresh fruit.

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2 Material and methods

2.1 Research material

The research material consisted of freeze-dried mangosteen fruit by kenayAG in a powdered form. The product was certified non-GMO. Food grade, organic, and fresh mangosteen fruit purchased from one of the retail chains from Thailand.

2.2 Microbiological quality

For microbiological diagnosis, mangosteen samples (5 g) were placed in 45 mL of saline (0.89%) and homogenized for a period of 30 minutes at 250 rpm, followed by serial dilutions (10^{-3}, 10^{-4}). Diluted samples were placed on Plate count agar and then incubated at 30 °C for 48 hours. Pure colonies were transferred to Tryptone soya agar and incubated again, and after 24 hours, bacterial biomass was collected and placed in Eppendorf's with 300 µL pure water and added 900 µL pure ethanol, and vortexed. The samples prepared in this way were preserved at -18 °C for further research. The Eppendorf's were centrifuged for 2 minutes at 13,000 rpm, the alcohol solution was removed. Volume of 10 µL of 70% formic acid and the same amount of acetonitrile were added to the biomass. The samples were centrifuged again, and then 1 µL of each sample was applied to the metal plate and allowed to dry at room temperature. After drying, 1 µL of the matrix was applied and allowed to dry again, and then the prepared plate was placed in the MALDI TOF MS spectrometry.

2.3 Antimicrobial activity

Mueller-Hinton Agar was used for the substrate preparation for the tests. The zone of inhibition is directly proportional to the sensitivity of the bacteria to the biologically active ingredient. Depending on the size of this zone, we divide bacteria into susceptible, intermediate, and resistant. The Mueller-Hinton agar medium was used in the study, and the following bacterial strains were used: *Klebsiella* spp., *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus* spp., *Nocardia cordis*, and *Staphylococococcus citreus*. The inoculum of the 24 hour culture was diluted in physiological saline (0.89%) to obtain a density of 0.5 on the Mc Farland, which corresponds to 1.5×10^8 CFU.mL^{-1}, and the density of the suspension was read with an optical densitometer. Volume of 100 µL of inoculum was applied to the substrate and spread with a sterile spreader. After drying, discs impregnated with mangosteen solution were placed on the substrate. The dishes were incubated for 24 hours at 37 °C. After removal from the incubator, the zone of inhibition of the individual bacteria was measured in triplicate.

2.4 Determination of the antioxidant capacity by the DPPH radical reduction method

The most frequently used method for determining the antiradical activity is the use of the DPPH radical, 1,1-diphenyl-2-picrylhydrazyl. Decrease in absorbance recorded by the spectrophotometer occurs at the wavelength of 515 nm, and is recorded at periodic time intervals (Sanchez-Moreno et al., 1998). The test was performed in two repetitions. The result of the antioxidant capacity by the DPPH method was expressed as a percentage of radical inhibition, calculated from the formula (1):

$$\% \text{ inhibition} = \frac{(A0 - AA)}{A0} \times 100 \qquad (1)$$

where: $A0$ – radical absorbance. DPPH absorbance value of the mangosteen solution containing an antioxidant (Sanchez-Moreno et al., 1998)

Determination of total polyphenol content – method using the Folin-Ciocalteu reagent. The Folin-Ciocalteu method is used to determine the total polyphenol content. The reaction consists of the oxidation of salts of phosphomolybdic and phospho-wolframic acids contained in the sample of phenolic compounds in an alkaline environment. The reaction produces blue colour. The above-mentioned method takes advantage of the ability of the polyphenolic compounds to react with the Folin's reagent. The absorbance is then measured at the wavelength of 756 nm. Total polyphenol content was expressed from the calibration curve of gallic acid in mg GAE.g^{-1} fresh sample - $R2$ = 0.9978 (Singleton & Rossi, 1965).

3 Results and discussion

3.1 Identification of bacteria found in mangosteen using the MALDI-TOF MS Biotyper

Bacteria identification was carried out using the MALDI-TOF MS Biotyper mass spectrometer. During the tests, a high probability of the presence of bacteria *B. cereus* and yeast *Saccharomyces* spp. was detected. The surface of the fruit is covered with many microorganisms, originating from soil, water, air pollution, rodents, or birds. According to Sicard and Legras (2011) , the bacteria of the genus *Clostridium*, *Bacillus*, and *Micrococcus* are the most dominant group of the bacteria found in fruits. On the other hand, *Saccharomyces*, *Candida*, *Kloeckera*, *Pichia*, and *Cryptococcus* are the yeasts commonly found in various types of fruits. The collected information confirms the results that are summarized in Table 1.

Table 1 Microbiological quality of the mangosteen fruit

Mangosteen fruit (sample 1)			Mangosteen fruit (sample 2)		
Rank	**detected microorganisms**	**value**	**Rank**	**detected microorganisms**	**value**
++	*Bacillus cereus*	2.121	++	*Bacillus cereus*	2.043
++	*Saccharomyces* spp.	2.021	++	*Saccharomyces* spp.	2.014

3.2 Antimicrobial activity

The microbial zone of inhibition (Table 2) presents data on the zone of inhibition of the selected microorganisms found in mangosteen. The subject of the study was the powdered form of the fruit.

It was noted that the bacteria *B. cereus* and *Nocardia* spp. had the smallest zone of inhibition, reaching the average value of only 5.67 mm – the zone of inhibition was barely visible. The highest zone of inhibition was observed in the following bacteria: *Micrococcus* spp. (mean value: 9.33 mm) and *Klebsiella* spp., *S. aureus*, which had a diameter of 8.67 mm. Authors Yin et al. (2016) showed similar increase in the diameter of the zone of inhibition for *S. aureus* bacteria during the research on the zone of inhibition, with the difference when the peel or pulp of mangosteen fruit were the subjects of the study. According to the authors, the zone of inhibition for *B. cereus* was 7 mm, reaching a higher value. Yin et al. (2016) showed that the *S. aureus* bacterium had the zone of inhibition of 7 – 10 mm, depending on the examined part of the fruit, consistent with the test results presented in Table 2. In their studies on the zone of inhibition of mangosteen powder against *E. coli*, authors Soetikno et al. (2016) proved similar effects to those presented in this study. The zone of inhibition reported by the authors was 5.75 mm. They also observed the most favourable zone of inhibition for *E. coli*, which was at 10% concentration and reached 15 mm.

3.3 Antioxidant activity of mangosteen fruit

The results of the antioxidant capacity of both fresh and powdered mangosteen are summarized below in Table 3. The data were put to the one-way ANOVA test (NIR test). It was discovered that the measurements differ from each other in a statistically significant way. It was shown that the antioxidant capacity of the fresh fruit is 5.5% higher than of the powdered form. Such a visible difference may occur due to the fact that the powdered form has undergone lyophilization. In their research, Kluszczyńska and Sowińska (2014), and Sanches-Moreno et al. (1998) discovered the dependence of lyophilization and other technological treatments on the antioxidant capacity. In their work, the authors showed that the freeze-dried fruit had the lowest antioxidant activity, defining the mentioned process as the least suitable method of fruit preservation. Kluszczyńska and Sowińska (2014) observed the highest losses of antioxidant activity in blueberry fruit, reaching 85.7%. The powdered form of mangosteen did not have such large losses, so it can be concluded that mangosteen is a fruit relatively resistant to freeze-drying.

Table 2 Antimicrobial activity of mangosteen

Bacteria	Zone of inhibition (mm)
***Klebsiella* spp.**	8.67 ±0.58
S. enterica	7.33 ±0.58
E. coli	6.67 ±0.58
S. aureus	8.33 ±0.58
B. cereus	5.67 ±0.58
***Micrococcus* spp.**	9.33 ±0.58
***Nocardia* spp.**	5.67 ±0.58
S. citreus	7.33 ±0.58

Table 3 Antioxidant activity determined by the DPPH method for fresh mangosteen fruit and powdered form

Fresh fruit (%)	Powder (%)
19.26 ±0.61[a]	13.76 ±0.32[b]

a, b – means marked with different letters in a row differ significantly at $p \leq 0.05$

Table 4 Total polyphenol content in fresh and powdered mangosteen

Fresh fruit (mg GAE.g^{-1})	Powder (mg GAE.g^{-1})
2.61 ±0.78^{a}	1.89 ±0.59^{b}

a, b – means marked with different letters in a row differ significantly at $p \leq 0.05$

In their work on the removal of free radicals and the pro-health effect of the mangosteen fruit, Gu et al. (2008) determined a similar result of antioxidant (Table 3). Depending on the method, the authors obtained a result of 14.20–19.25% of the antioxidant activity. In the method similar to that one presented in these studies, Gu et al. (2008) determined the antioxidant capacity at the level of 19.25%, achieving an almost identical result. Differences in the obtained results may be caused by the method of determination or the degree of freshness of the fruit.

3.4 Total polyphenol content in mangosteen

Total polyphenol content is presented in Table 4. All the results were tested for significance against the one-way variance ANOVA – NIR test. The data on the fresh fruit and the powdered form differed in a statistically significant way. According to Patthamakanokporn et al. (2008), the content of polyphenols in fresh mangosteen fruit should be 7.51 mg GAE.g^{-1} of the product. The value obtained by the author is definitely lower as it is presented in Table 4. The value was 2.61 mg GAE.g^{-1} of the product.

Authors Drużyńska et al. (2014) reported that the total polyphenol content in mangosteen was within 1.02 mg.g^{-1} of the fruit. However, this value is slightly lower. Such content of polyphenols may be caused by the long storage of the fruit (transport from tropical countries), and inadequate storage. The lower content of polyphenols in the powdered form – 1.89 mg GAE.g^{-1} of the product, as in the case of the antioxidant capacity, could be caused by the freeze-drying process. The differences between the fresh fruit and the powdered form (Table 4) were very low, oscillating around 0.72 mg.g^{-1} of the product. It can be said that the mangosteen fruit is basically resistant to freeze-drying processes.

Comparing to other tropical fruits, Drużyńska et al. (2014) found out that the total content of polyphenols in mango fruit was 1.20 mg.g^{-1} of the product, in lychee 1.47 mg.g^{-1}, and rambutan 0.63 mg.g^{-1}. The obtained results satisfactorily confirm the thesis that mangosteen is a "super fruit". Its total content of polyphenols is definitely higher than in other fruits.

4 Conclusions

Mangosteen fruit is characterized by a high content of polyphenols at the following levels: fresh fruit 2.61 ±0.78 mg GAE.g^{-1}; powder form 1.89 ±0.59 mg GAE.g^{-1}. Mangosteen shows a high antioxidant capacity of the fruit in both forms presented in the work. It was 19.26% (fresh fruit) and 13.76% (freeze-dried fruit). Mangosteen shows also an antibacterial activity in relation to the strains of bacteria tested in the work. During the identification of the mangosteen microflora using the MALDI-TOF MS Biotyper mass spectrophotometer, the presence of *B. cereus* bacteria and *Saccharomyces* spp. was detected.

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