

## Antimicrobial Activity of the Anthocyanins Isolated from Purple Field Corn (*Zea mays* L.) Cob against *Candida* spp.

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**Abstract:** The phytochemical compounds from purple field corn (*Zea mays* L.)cob (PFCC), mainly grown in Phitsanulok, Thailand, were extracted with acidified 80% methanol and the anthocyanins were isolated from the extract through column chromatography with acidified absolute methanol. The PFCC extract (PFCCE) showed total anthocyanins glucoside as 6022 mg/100 g by colorimetric method. Three kinds of anthocyanins were detected by HPLC-DAD at 500 nm. The antimicrobial activity of the anthocyanins from PFCC was then evaluated via macrobroth dilution method against *Candida albicans* ATCC 90028, two clinical isolates of Fluconazole susceptible species (*C. albicans* and *C. tropicalis*) and three clinical isolates of Fluconazole resistant species (*C. glabrata*, *C. krusei*, and *C. parapsilosis*). As a result, the anthocyanins exhibited a potent inhibitory activity against all the tested species with the MIC in range of 0.625 – 2.5 mg/mL. Moreover, fungicidal activity of the anthocyanins was also observed against clinical isolates of *C. tropicalis* and *C. albicans* with the MFC in range of 0.625 – 1.25 and 2.5 mg/mL, respectively.

**Keywords:** Anthocyanins, Antimicrobial, *Candida* spp., Purple field Corn Cob

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### I. Introduction

The incidence of serious infections caused by yeast, particularly species of *Candida*, has increased dramatically during the past decade. Candidiasis, formerly dismissed as a simple infection occurring in denture wearers, is now one of the most common opportunistic infections in immune-compromised patients such as those undergoing chemotherapy, HIV-infected persons, diabetes mellitus, broad-spectrum antibiotic therapy, transplant recipients and topical use of corticosteroid. There are topical and systemic antifungal agents that may be indicated to control candidiasis, but, the development of resistance is an emerging trend that may threaten their clinical effectiveness [1]. *Candida* species are a common cause of bloodstream infections. Recent surveillance had shown an increase of infections caused by *C. glabrata*, which had reduced susceptibility to fluconazole [2]. For millions of people that traditional medicine serves as the only opportunity for health care, plants are vital sources [1].

Purple corn (*Zea mays* L.) cob was the byproduct during the corn processing. It is dark purple to almost black color due to its high content of anthocyanins. Anthocyanins are a group of natural occurred pigments acid-base indicator responsible for the red-blue color of many fruits and vegetables. Anthocyanins are of interest for natural colorants and also have important implications in the field of human health. Numerous studies indicated that the potential effect these flavonoids may play a role in reducing the incidence of cardiovascular disease, cancer, hyperlipidemias and other chronic diseases through the intake of anthocyanin-rich foods [3,4].

This kind of purple corn is mainly grown in Thailand. In 1997, the pod of purple field corn (*Zea mays* L.) was found in experimental field of Rajamangala University of Technology Lanna Phitsanulok (RMUTL). The genetics of this purple field corn is based on local corn which has a starch synthesis gene and easily adapted in Thailand without using purple corn hybrid from original or other country [5,6]. Purple field corn cobs are considered waste in the corn processing. However, it makes this a good source of anthocyanins [4]. Recent reports mainly analyzed the stability and structures of these anthocyanins. No information determining the antimicrobial activity of the extract of PFCC against clinical isolates *Candida* spp. via macro broth dilution methods.

### II. Materials And Methods

#### 2.1 Extraction and characterization of phytochemical compounds from purple field corn cob

Purple field corn cob powder of twenty grams extracted with method, containing 80 % methanol mixture with 1% of citric acid (1M) for 24 hours at 4-6 °C. The crude extract was obtained by filtering through Whatman No.1 filter by vacuum funnel. The solvent were evaporated methanol to dryness at 50 °C with a rotary evaporator, and the remain solvent were freeze-dried at -50 °C [7,4]. Anthocyanins would be of natural occurred pigments acid-base indicator responsible with hydrochloric acid and sodium hydroxide, the hue color would change from red to blue by changing the pH of the anthocyanins [3]. The major anthocyanins initially

characterized by TLC using butanol: acetic acid: water (4:1:4) as mobile solvent for suggested close of anthocyanins comparison with known standard  $R_f$  values [8].

## **2.2 Isolation and characterization of anthocyanins form crude purple field corn cob extracted**

Ten grams of crude extract were isolated by column chromatography (CC); the more polar substance was flushed with two column volumes acidified deionized distilled water. Washing the cartridge with two column volumes ethyl acetate and then the anthocyanins eluted with acidified methanol contained (1% (1 M) citric acid). The methanol was evaporated to dryness at 50 °C with a rotary evaporator and freeze-dried the solvent at -50 °C overnight kept in a bottle at -120 °C in the dark [9] and identity of the anthocyanins were detected by HPLC-DAD.

## **2.3 Preparation of anthocyanins and fluconazole for determination antifungal**

Stocked solution of anthocyanins was prepared between 0.3125 mg/mL - 2.5 mg/mL by pipetting 50 mg/mL were two fold dilution with 50% methanol and another pipetting 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL. Final anthocyanins concentration was 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL respectively, each concentration extract were prepared by micropipetting 100 µL volumes. The final concentration was 0.3125 mg/mL, 0.625 mg/mL, 1.25 mg/mL, and 2.5 mg/mL ready for placed into broth RPMI-1640 for MIC testing [10]. The drug control of fluconazole was prepared the final concentration were 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 µg/mL with 1% DMSO.

## **2.4 Determination of antifungal activity of anthocyanins isolated from purple field corn cob**

Standard strain of *C. albican* ATCC 90028 taken from Department of Microbiology Science, Faculty of Science, Burapha University, Chonburi, Thailand and five clinical isolates of five *Candida* spp. namely *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* taken from Somdej Pra Boromrajathevi na Sriracha Hospital, Thailand. The inoculums preparation to frozen stokes were directly inoculated onto Sabouraud dextrose agar (SDA) plates to produce confluent growth at 35 °C for 24 hours, to ensure purity and viability, and then subcultured again to select for isolated colonies. Five colonies of  $\geq 1$  mm in diameter were suspended in 5.0 ml of sterile 0.85% NaCl and mixed thoroughly on vortex mixer for 15 to 20 seconds[11,12,13].

After adjusting the suspensions to a 0.5 McFarland, the resulted suspension was  $1 \times 10^6$  to  $5 \times 10^6$  CFU/mL. Then added 1 mL of suspension to 19 mL of RPMI-1640 (1:20 dilution) the resulting suspension was  $1 \times 10^4$  to  $5 \times 10^4$  CFU/mL, then added inoculums-broth suspension 1 mL of  $1 \times 10^4$  to  $5 \times 10^4$  CFU/mL to 9 mL of RPMI-1640 (1:10 dilution). The final concentration was  $1 \times 10^3$  to  $5 \times 10^3$  CFU/mL. Using a 5 mL serological pipette, added 0.1 mL (100 µL) of the final inoculums to each four anthocyanins and nine fluconazole concentrations for 0.1 mL (100 µL) mixed with RPMI 0.8 mL tubes. The next process included three empty tubes designated as the growth, solvent and sterility controls; growth control had 0.1 mL added of final inoculums to 0.9 mL of broth, the sterility control had 1 mL of broth added to tube and the solvent control had 0.1 mL added of final inoculums to 0.1 mL of solvent and 0.8 ml of broth to the tube. After completing the vortex of the inoculums suspension was achieved to re-suspend the yeast. Using a 100 µL pipette, 0.01 mL (10 µL) of inoculums was placed on a SDA plate, covering the entire surface. All the tubes and invert plates were incubated at 35 °C for 24 to 48 hours, or until colonies are visible for accurate counting. After inoculating the tubes, they were read for MIC (Determining MIC from the lowest fluconazole and anthocyanins concentration with no turbidity and suspensions). The MICs for all anthocyanins and fluconazole were the lowest concentrations [11,14, 15,16].

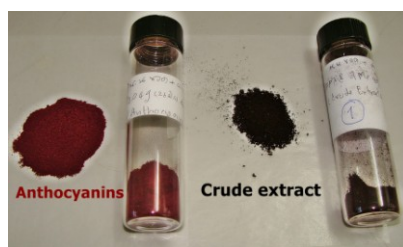
After MIC were read, using the entire contents concentrations above MIC to determine of MFC. A micropipette was used, and removed 20 µL from the MIC tube, each higher concentration tubes, and the growth control tube drop over SDA plate and incubated at 35 °C. Read plates when colonies on the growth control plate are visible, usually 24 h, and again at 48 h. The MFC was defined as the lowest anthocyanins and fluconazole concentration that resulted in a 99.9% reduction in the starting inoculums, any plate with one colony or fewer were negative. The lowest concentration for which sub-cultured negative was the MFC [15, 16]. All the experiments were repeated twice, including three controls.

## **III. Results And Discussion**

### **3.1 Percent yield of anthocyanins from PFCC**

The dark purple (Fig.1) powder ten grams of crude extracted purple field corn cob was added to the cartridge column chromatography. A solution of 1% citric acid (1M) in distill water was used to flushed sugars and acids was checked for fraction exhibiting done for dark color, after which ethyl acetate was used to remove the phenolic was checked for fraction exhibiting finish dark brown color. Finally, the anthocyanins were eluted using 1% citric acid (1M) in methanol, the product were magenta color powder 0.8 g (8% yield) after freeze

dried (Fig.1).



**Figure 1** Characteristic of crude extract (dark purple) and isolated PFCC anthocyanins (magenta color)

### 3.2 Characterization of phytochemical compounds from crude PFCCE

Anthocyanins were a major of crude extract has been showed acid-base indicator by color changed, pH 1-4 were red, pH 5-6 were purple pink, pH 7 were colorless, pH 8-9 were bluish green, pH 10-12 were yellow brown (Table 1).

**Table 1** Results of pH indicator characteristic from PFCCE

pH ranges	Color of anthocyanins
1-4	red
5-6	purple pink
7	colorless
8-9	bluish green
10-11	yellow brown

The acid-base indicator property of the PFCCE was also examined and being found as a predominant group of PFCC pigments [17]. This characteristic confounds isolation of specific anthocyanin. The initially screening again with using  $R_f$  value on TLC (Fig. 2) had pigment  $R_f$  values 0.81, 0.72, 0.69 and 0.67 close to anthocyanins  $R_f$  values comparison with  $R_f$  values of known standard [8].



**Figure 2** Thin Layer Chromatography of crude PFCCE

### 3.3 Characterization of anthocyanins form PFCCE

The PFCCE showed total of anthocyanins glucoside as 6022 mg/100g with colorimetric method by Central Laboratory (Thailand) CO., Ltd. Chiangmai Branch. Three kinds of anthocyanins were detected by HPLC-DAD at 500 nm. For this study we found the important three major type anthocyanins with reliable information which support in this education at retention times were 20.108, 23.892 and 26.263 minutes for suggest of cyaniding-3-glucoside, pelargonidin-3- glucoside and peonidin-3- glucoside respectively with compare of known standard (Fig 3).

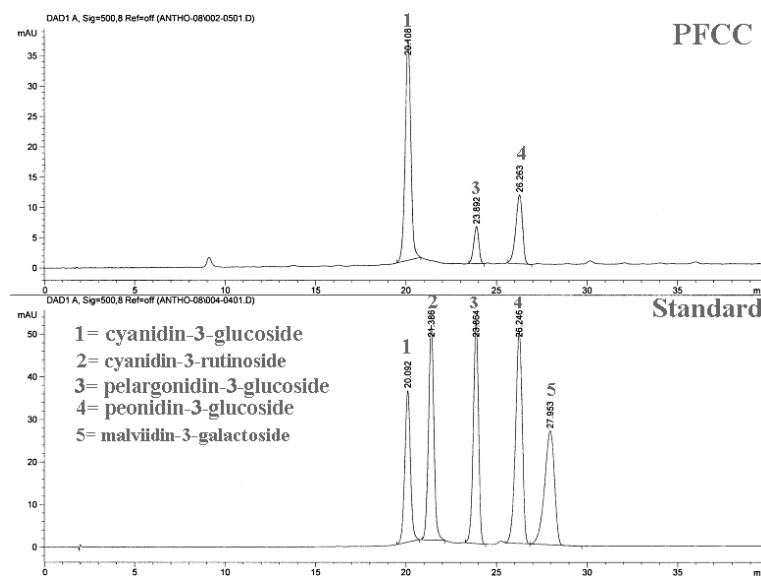


Figure 3 The HPLC-DAD profile of anthocyanin at 500 nm

### 3.4 Determination of antifungal activity of anthocyanins for macro broth dilution

The previously established CLSI of MIC interpretive break points for *Candida* spp. tested against fluconazole were based on an analysis of treatment in which MIC breakpoint of  $\leq 8$   $\mu\text{g/mL}$  define susceptibility (S) and of  $\geq 64$   $\mu\text{g/mL}$  define resistance (R), with MICs of between 16 and 32  $\mu\text{g/mL}$  reflecting dose-dependent susceptibility (SDD) [18,19]. The antimicrobial activity of the anthocyanins from PFCC was then evaluated via macrobroth dilution method against *Candida albicans* ATCC 90028, two clinical isolates of fluconazole susceptible species (*C. albicans* and *C. tropicalis*) and three clinical isolates of fluconazole resistant species (*C. glabrata*, *C. krusei*, and *C. parapsilosis*). As a result, the anthocyanins exhibited a potent inhibitory activity against all the tested species with the MIC in range of 0.625 – 2.5 mg/mL. Moreover, fungicidal activity of the anthocyanins was also observed against clinical isolates of *C. tropicalis* and *C. albicans* (Fig.4) with the MFC in range of 0.625 – 1.25 and 2.5 mg/mL, respectively (Table 2).

Table 2 MIC and MFC range of anthocyanins and fluconazole concentrations

Candida spp.	Anthocyanins (mg/mL)		Fluconazole ( $\mu\text{g/mL}$ )	
	MIC	MFC	MIC	MFC
<i>C. albicans</i> (ATCC 90028)	2.5	>2.5	1-2	>128
<i>C. albicans</i>	0.625-1.25	2.5	8-16	64
<i>C. glabrata</i>	0.625-1.25	>2.5	128	>128
<i>C. krusei</i>	2.5	>2.5	128	>128
<i>C. parapsilosis</i>	1.25-2.5	>2.5	128	>128
<i>C. tropicalis</i>	0.625-1.25	0.625-1.25	8-16	32

The activity of *Candida* spp. With MIC in range of 0.625-2.5 mg/mL inhibition activity of anthocyanins against *Candida* spp. was not depend on the fluconazole susceptibility pattern of microorganism.

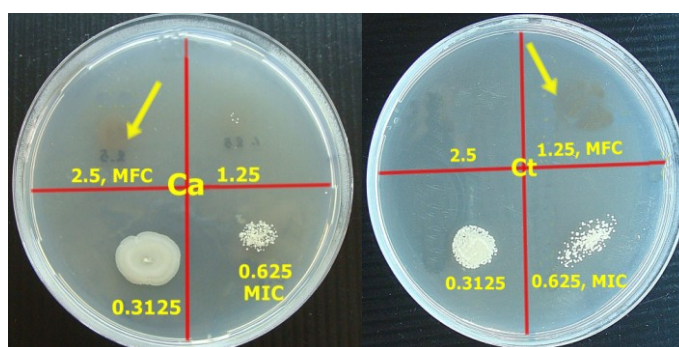


Figure4 Fungicidal activity of *C. albicans* (Ca) and *C. tropicalis* (Ct) (MFC = →)

#### IV. Conclusion

The bioactive of total anthocyanin-3-glucoside from PFCCE was activity again on *Candida* spp. namely *Candida albicans* ATCC 90028, and five urine specimen clinical isolated of five *Candida* spp.; two species are *C. albicans*, and *C. tropicalis*, the MFC rang was defined 2.5 mg/mL and 0.625-1.25 mg/mL respectively, and another four species inhibited the growth of *C. albicans* ATCC 90028, *C. glabrata*, *C. krusei*, and *C. parapsilosis*, the MIC was defined the lowest anthocyanins range as 2.5 mg/mL, 0.625-1.25 mg/mL, 2.5 mg/mL, and 1.25- 2.5 mg/mL respectively. It is now widely accepted that the resistance to fluconazole was relatively common in *Candida* spp. This experiment was founded three species which *C. glabrata*, *C. krusei*, and *C. parapsilosis* were resistance to fluconazole. In addition, the most interesting result was the susceptibility of *C. glabrata* to anthocyanins which is resistance to fluconazole. In the future, we are hopefully that anthocyanins from purple field corn cob extracts are vital sources, safety and suggested them as sources of new nontoxic pharmaceuticals for threat infection cause by *Candida* spp.

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