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Antimicrobial Activity of Anthocyanins Extracted from Red Sorghum (*Sorghum bicolor.* L) Bran

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Abstract

Recently, natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of micro-organisms. This study described the antibacterial activity of anthocyanins extracted from red sorghum bran on selected bacteria. The anthocyanins extracted by using acidified ethanol have shown highest antibacterial activity compared to methanol extracts. Among the selected bacterial cultures, the highest antibacterial activity was recorded against Staphylococcus aureus. Moderate antifungal activity was observed against Aspergillus niger and Aspergillus fumigates.

Keywords: Red sorghum bran • Antibacterial activity • Anthocyanins

Introduction

Sorghum (*Sorghum bicolor* (L)) is the fifth leading cereal crop in the world and is used primarily in Asia and Africa as a food crop. Speciality of sorghum have high levels of phytochemicals, including proanthocyanins, 3-deoxyanthocyanins, phenolic acids, phytosterols and policosanols in their bran layers. In addition, sorghum bran is rich in dietary fiber. This sorghum bran are potentially useful ingredients in various functional food applications and were shown to produce desirable attributes (e.g., attractive natural color) without adversely affecting other sensory properties of foods such as bread, cookies and expanded snacks. Anthocyanins are belonging to water soluble plant pigments and representatives of flavonoids.

They are responsible for the blue, purple and red colour of many plant tissues. They play a definite role in the attraction of animals for pollination and seed dispersal, and hence they are of considerable value in the co-evolution of these plant- animal interactions [1]. Antibiotic resistant bacteria is still of worldwide concern. Since the use of antibiotics became wide spread over 50 years ago, bacteria have progressively developed resistance.

Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic therapy. The objective of the present study was to evaluate the antimicrobial activity of anthocyanins isolated from red sorghum bran.

Materials and Methods

Samples

Sorghum bicolor was collected from the village area (Coimbatore district, India) and raised in the college campus under normal climatic conditions [2]. The plant was identified and authenticated by Botanical Survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The bran was collected and was stored at -20°C.

Anthocyanin extraction

The bran of red Sorghum were extracted by incubating with two solvent systems like methanol and with 1% Hydrochloric acid in methanol, over night at room temperature, followed by a filtration through whatman filter paper no 4. Methanol was removed by a rotary evaporation under 35°C and the pigmented fraction extracts of anthocyanin extracted by methanol represented as methanol extract and anthocyanin extracted by using 1% Hydrochloric acid in methanol represented as acidified methanol extract were stored for a further study.

Isolation of anthocyanin

The concentrated filtrates were then loaded onto an amperlite XAD-7 resin column (1.5 × 40 cm)(sigma), and washed with distilled water, followed by an elution with 1% Trifluoroacetic acid in methanol. The fractions with the highest absorbance at 480nm were pooled and evaporated to remove methanol and dried under vaccum at 35°C.

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Microorganisms

Escherichia coli, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger* and *Aspergillus fumigatus* were the micro organisms used and they were stored at freeze temperature until use.

Inoculum preparation

The tested microorganisms were separately cultured on sterilized Muller-Hinton Agar (MHA) at 37°C for 24 hours by using streak plate method. Then, well-isolated overnight-cultured colonies of the same morphological type were selected from the cultured media. Each colony was touched with a flamed wire-loop and the growth was transferred into a sterilized test tube containing 5 ml steriled normal saline solution. The test tubes that contain the bacterial suspension were vortexed to be mixed well uniformly [3]. Then, the bacterial suspension was adjusted with 0.5 McFarland turbidity standards. The adjustment and comparison of turbidity of Inoculum tubes were performed by visually observing them with naked eye against a 0.5 McFarland turbidity equivalence standard with white background and contrasting blue lines in the presence of adequate light. The adjusted bacterial suspensions should be used as inocula within 15 minutes; otherwise, they are not used for testing purpose.

Determination of zone of inhibition

For the determination of zone of inhibition, Streptomycin and Nystin was taken as standard antibiotic for comparison of the results. The antibacterial activities of the methanol extract and acidified methanol extract of anthocyanin pigment extracted from red sorghum bran against G +ve and G -ve bacterial microorganisms. The zone of inhibition test was done by agar well diffusion method.

Determination of antibacterial activity

An agar well diffusion methods was employed for determination of antibacterial activity. The isolated anthocyanin extracts were tested for microbial resistance using such technique with an inoculum volume equivalent to 0.5% Mc Farland's Standard in Mueller-Hinton agar and examined after 24 hrs. The anthocyanin extracts containing 1 mg, 2.5 mg, 5 mg, 7.5 mg, 10 mg and 12.5 mg of isolated anthocyanin were dissolved in 1 ml DMSO. Negative controls were prepared using DMSO solution. Streptomycin was used as positive reference standards to determine the sensitivity of each bacterial species tested and Nystin was used as reference standard for fungal cultures. The inoculated plates were incubated at 37°C for 24 hrs. Antibacterial activity was evaluated by measuring the inhibition zones formed on the medium were evaluated in mm of the tested bacterial cultures and for fungal culture it was incubated at room temperature for 48 hours. All the tests were performed in triplicates [4].

Results and Discussion

Extraction and purification of anthocyanin from red sorghum bran

Anthocyanins were extracted from red sorghum bran with 1% Hydrochloric acid in methanol and purified by Amberlite XAD-7 chromatography. The anthocyanin obtained after methanol evaporation was a solid.This extraction method was used previously by Zhang 2004 to recover high levels of litchi anthocyanins. The Amberlite XAD-7 resin column was also used previously and shown to have high affinity for the anthocyanins.

Anthocyanin content

The total anthocyanins extracted by acidified methanol extracts were 4.7 ± 0.20 mg/g (mg cyanidin 3-glucoside equiv/g) were on average 59% higher than aqueous acetone extracts and 28% higher than methanol extracts. Several authors reported that aqueous acetone was better than various alcoholic solvents for extraction of fruit procyanidins, anthocyanins, and other phenols. However, since acidified methanol preservers better the extracted anthocyanins in their original form, it can be the solvent of choice for quantification and purification of anthocyanins.

Antibacterial activities of anthocyanin extracted by methanol solvent

One out of five bacteria used Staphylococcus aureus is G +ve and four (Escherichia coli, Klebsiella pneumoniae, Klebisella oxytoca, Pseudomonas aerginosa) are G -ve. There was significant variation in the antibacterial activities of anthocyanin extracts. In all four G -ve bacteria's the methanol extract of anthocyanin showed no inhibitory zone even at 12.5 mg concentrations. For Staphylococcus aureus, the zone of inhibition values of anthocyanin extracts were between 5-9 mm. The results of methanolic extracts on bacteria are presented in Table 1. The moderate antifungal activity was observed in Aspergillus fumigates and Aspergillus niger. (Table 1).The results observed in anthocyanin extracted from red Sorghum bran methanol extracts indicating that G +ve strain was more sensitive than G -ve . This observation can be attributed in the difference in the structure of bacterial cell wall. The less complex structure of the cell wall in the G +ve bacteria makes it more permeable to the antibacterial compounds.

Antibacterial activites of anthocaynin extracted by acidified methanol solvent

There was a significant variation in the antibacterial activities of anthocyanin extracted from acidified methanol. The acidified methanol extracts of anthocyanin samples showed inhibitory effect in G +ve and G -ve bacteria. The highest antibacterial activity was observed in K. oxytoca with zone of inhibition of 3- 6 mm with the concentration ranging from 1 to 12.5 mg. The moderate antifungal activity was observed in Aspergillus fumigates and Aspergillus niger. (Table 1). Phenolic compounds and anthocyanin content was higher in the extracts of skin and berry extracts of biliberry and blue berry showed highest inhibitory zone against G -ve bacteria than other extracts, which coincides with our results [5].

Micro organism	Concentration (mg/ml)	Zone of inhibition (mm)	Zone of inhibition by streptomycin (1 mg/ml)	Zone of inhibition by nystin (1 mg/ml)
Staphylococcus aureus	1	4 ± 0.01	1.70 ± 0.02	-
	2.5	5 ± 0.01		
	5	6 ± 0.01		

	7.5	8 ± 0.01		
	10	9 ± 0.02		
	12.5	10 ± 0.02		
Klebsiella oxytoca	1	3 ± 0.01	1.2 ± 0.01	-
	2.5	3 ± 0.01		
	5	4 ± 0.01		
	7.5	5 ± 0.01		
	10	5 ± 0.01		
	12.5	6 ± 0.01		
Escherichia coli	1	0	1.6 ± 0.02	-
	2.5	0		
	5	0		
	7.5	0		
	10	0		
	12.5	0		
Aspergillus niger	1	0	-	1.6 ± 0.02
	2.5	0		
	5	0		
	7.5	0.05 ± 0.01		
	10	0.06 ± 0.01		
	12.5	0.07 ± 0.01		
Aspergillus fumigates	1	0	-	1.87 ± 0.02
	2.5	0		
	5	0		
	7.5	0.06 ± 0.01		
	10	0.07 ± 0.01		
	12.5	0.07 ± 0.01		

Table 1. Antimicrobial influence of anthocyanin extracted from red sorghum bran using acidified methanol solvent.

Conclusion

The present study confirms the potential antimicrobial activity of the extract of red sorghum bran. The presence of anthocyanin as major active constituents may be responsible for these activities. Further studies are necessary to isolate characterize the active constituents of the plant to evaluate their modes of action and render this species interesting for future.

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Competing Interests

Authors have declared that no competing interests exist.

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