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Assessment of the Antioxidant Potential of Anthocyanin-Rich Extract of Eggplant (*Solanum melongena* L.) and Evaluation of its Antimicrobial Activity

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ABSTRACT

Eggplant peels (*Solanum melongena* L.), particularly the purple variety, contain high levels of anthocyanins, a type of flavonoid with promising medicinal properties. This study aims to investigate the antioxidant and antimicrobial potentials of anthocyanin-rich extract of eggplant peels. The anthocyanin-rich extract was obtained by maceration under acidic conditions. Preliminary qualitative phytochemical screening of the extract was done following standard procedures. The antimicrobial activity of the extract was evaluated against two bacterial (*Escherichia coli* and *Staphylococcus aureus*) and two fungal (*Aspergillus niger* and *Candida albicans*) strains using the agar well diffusion method. The antioxidant activity of the extract was assessed by the β-carotene bleaching assay. Preliminary phytochemical analysis confirmed the presence of anthocyanin, a type of flavonoid, in the purple-coloured peel extract. The extract demonstrated antibacterial activity against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacterial strains. The inhibition zone diameters were 22 mm and 18 mm at 100 mg/mL and 10 mg/mL, respectively against both bacterial strains. The minimum inhibitory concentration (MIC) was 0.1 mg/mL for *Staphylococcus aureus* and 0.01 mg/mL for *Escherichia coli*. The extract demonstrated a concentration-dependent antifungal activity against *Aspergillus niger* and *Candida albicans* with MIC of 0.1 mg/mL for both fungal strains. The extract showed potent antioxidant activity with percentage activity as high as 74.26%. The anthocyanin content in the extract contributes significantly to its antioxidant activity. These observations therefore suggest the potential for use of eggplant extract as natural antimicrobial and antioxidant agents.

Keywords: Eggplant, *Solanum melongena* L., Anthocyanin, Antioxidant, Antibacterial, Antifungal.

Introduction

Anthocyanins, natural pigments found in plants, impart red, blue, and purple hues to fruits, flowers, leaves, and other vegetative parts of plants. Beyond enhancing the visual appeal of plants, they serve crucial functions in pollination and seed dispersal. Additionally, Anthocyanins are utilized as natural colorants in various food and beverage products.[1,2]

Anthocyanins belong to the flavonoid category, one of the extensive groups of phenolic compounds. The fundamental structure of anthocyanins consists of a flavylium cation (C6-C3-C6), capable of linking various sugars, hydroxyl or methoxyl groups, leading to the identification of over 635 distinct anthocyanins to date.[3]

Anthocyanins have a diverse range of applications. They have a long history of use as dyes and food colorants. Anthocyanins are not only visually appealing but also possess significant health benefits. These bioactive compounds have been traditionally used in various medicinal practices as nutraceuticals to promote well-being and prevent diseases. Their effectiveness as nutraceuticals hinges on their bioavailability, the extent to which they are absorbed and utilized by the body. Optimizing bioavailability is crucial for maximizing the health benefits of anthocyanins.[4,5]

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Modern research has extensively investigated the therapeutic potential of plant anthocyanins. These compounds have been shown to exhibit antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity properties, and have shown promise in preventing cardiovascular diseases.[6] Thus, anthocyanins derived from edible plants hold significant potential as active pharmaceutical ingredient.

Anthocyanins are widely distributed throughout the plant kingdom, particularly in fruits, leaves, flowers, roots, and grains. A diverse array of anthocyanin and anthocyanidin compounds have been identified in fruits and vegetables. Among these, cyanidin-3-glucoside stands out as the most abundant anthocyanin. In plants, the formation of cyanidin-3-glucoside is influenced by low pH conditions.[7]

Specific structural features of anthocyanins, including the presence of chalcones and quinoidal bases with a double bond conjugated to the keto group, contribute to their potent antioxidant activity in neutralizing free radicals. Additionally, the glycosylated B-ring configuration of anthocyanins enhances their antioxidant potential. Ortho-hydroxylation and methoxylation further amplify the antioxidant capacity of anthocyanins.[8]

While anthocyanidin possesses a stronger antioxidant capacity, as measured by its ORAC value, anthocyanin exhibits a lower antioxidant potential. This difference in activity is attributed to the structural modifications of anthocyanin compared to anthocyanidin.[9] The addition of a sugar molecule at position C-3 in the heterocyclic C-ring of anthocyanin reduces its antioxidant effectiveness.[10] Conversely, acylation of anthocyanin with phenolic acid significantly enhances its antioxidant capability.[11] Diacylation of anthocyanin further amplifies its antioxidant activity, while 5-glycosylation results in a diminished antioxidant capacity.[9]

Extracting anthocyanin pigments using conventional organic solvents like methanol and ethanol raises concerns about toxicity. While ethanol is generally regarded as a safe extraction medium, water-based

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extraction methods offer a more environmentally friendly approach. Subcritical water extraction, a technique employing acidified water (0.01% HCl, pH ~2.3) at high temperatures (110–160°C) under constant pressure (40 bars), has proven to be highly efficient in extracting anthocyanins from fruits. Alternatively, adding sulfur dioxide to water stabilizes the anthocyanin structure and enhances its diffusion coefficient, promoting anthocyanin solubility and extraction efficiency.[12, 13]

This paper proposes a method for upcycling a common food waste into a source of natural dyes with inherent antioxidant and antimicrobial properties, this paper introduces a sustainable solution for using anthocyanin extracted from eggplant peels, offers its immense potential in healthcare applications without harmful side effects.

Material and Methods

Plant material

Eggplant was purchased from local markets in Basra Governorate in May 2022. A voucher specimen number (BSRASCI00522) was provided for future referencing purposes. The plant was peeled, and the peels were collected, cut into small pieces and stored in an opaque glass container until ready for use.

Extraction of anthocyanin

Fresh pieces of eggplant peels (100 g) were macerated in 200 mL of 10% acetic acid for 24 hours with constant agitation. The extract was filtered and the residue was re-extracted by maceration in 200 mL of glacial acetic acid for another 24 h. The filtrates (10% acetic acid extract and glacial acetic acid extract) were combined to give a total extract with a violet hue. The total extract was concentrated to dryness using a rotary evaporator at 40°C. Then, the crude extract was treated with 200 mL of methanol, evaporated to dryness to give a solid methanol extract. The methanol extract was dissolved in 200 mL of distill water until complete dissolution, filtered and dried.[14]

Phytochemical screening

Preliminary qualitative phytochemical tests, including Molisch's test for carbohydrates, Benedict's test for glycosides, Dragendroff's test for alkaloids, frothing test for saponins, Liebermann-Burchard's test for steroids, Salkowsky's test for terpenoids, ferric chloride test for phenolic compounds, and Shinoda's test for flavonoids, were conducted on the eggplant peel extract following standard procedures.[15-17]

Determination of antibacterial activity

The antibacterial activity of the anthocyanin-rich extract of eggplant peels was evaluated against Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria, by agar well diffusion method. Various concentrations (100, 10, 1, 0.1 and 0.01 mg/mL) of the anthocyanin-rich extract of eggplant peels were prepared in distilled water. Wells were made in the inoculated Mueller-Hinton agar with the aid of a cork borer. The wells were filled with the extract, and the plates were incubated at 37°C for 24 h. Antibacterial activity was indicated by clear zone of growth inhibition.[18,19]

Determination of antifungal activity

The Antifungal activity of the anthocyanin-rich extract of eggplant peels was investigated using agar well diffusion method with modification.[20] The culture medium (Sabouraud's dextrose agar) was inoculated separately with two fungal strains (*Aspergillus niger* and *Candida albicans*). Various concentrations (100, 10, 1, 0.1 and 0.01 mg/mL) of anthocyanin-rich extract of eggplant peels were prepared in distilled water. Wells were made in the inoculated agar plates, and the wells were filled with extract, and incubated at 27°C for 72 h. The antifungal activity was estimated by measurements of the inhibition zone diameter by each extract concentration.

Determination of the antioxidant activity

The antioxidant activity of the anthocyanin-rich extract of the eggplant peels was assessed using the beta carotene bleaching assay as reported by Ahmeda *et al.* (2009).[21] Briefly, linoleic acid (0.02 mL), tween 20 (0.2 mL) and ß-carotene (1 mL of 0.1 mg/mL in chloroform) were mixed in a 100 mL round flask. The chloroform was evaporated at room temperature in the dark, and then 50 mL of distilled water was added to the mixture and shaken thoroughly. The mixture (3.8 mL) was then mixed with 0.2 mL of the anthocyanin-rich extract at concentration of 100 mg/mL or butylated hydroxy toluene (BHT) as standard. The control consists of 0.2 mL distilled water instead of extract or standard. The absorbance was read at 470 nm at time zero, and then samples were subjected to thermal autoxidation at 45°C for 2 h. Absorbance was measured at 15 min intervals for a duration of 105 min. Antioxidant activity (AA) was estimated using the equation below:

$$\% AA = 1 - [(Ai - At) / (Ai^* - At^*)] \times 100$$

Where; Ai: Absorbance value of sample at time zero.
At: Absorbance value after 105 min incubation of sample at 45°C.
Ai*: Absorbance value of control at time zero.
At*: Absorbance value after 105 min incubation of control at 45°C.
The graph of absorbance versus time was plotted and used to compare the antioxidant efficacy of the anthocyanin-rich extract of eggplant peels and BHT.[21]

Results and Discussion

Phytochemical constituents of anthocyanin-rich extract of eggplant peels

The result of qualitative analysis of anthocyanin-rich extract of eggplant (*Solanum melongena L.*) peels is presented in Table 1**.** The result indicated that the eggplant peel extract contained glycosides and phenolic compounds only, specifically flavonoids. No other phytochemical group of compounds were detected. The pigments that give plants their blue, red, or purple colour are anthocyanins, which exist as glycosides.[22] It can therefore be stated that the purple extract of eggplant peels obtained are mainly anthocyanin compounds. The study conducted by Condurache Lazăr *et al.*(2021) reveals that anthocyanins are responsible for the purple colour of eggplant peels.[23] The anthocyanins content in eggplant peels has been shown to be about 51.1 mg/100 g.[24]

Anti-bacterial activity

Table 2 shows the results of the antibacterial activity of anthocyanin-rich extract of eggplant peels. The anthocyanin-rich extract showed moderate effectiveness against *S. aureus* and *E. coli* at all concentrations used in the study, the inhibition zone diameter ranged from 10 mm to 22 mm. the minimum inhibition concentrations of the anthocyanin-rich extract were 1 mg/mL and 0.1 mg/mL against *S. aureus* and *E. coli*, respectively. The antimicrobial activity of extracts from uncooked and microwave-cooked eggplants was evaluated against *S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *E. coli*, and *C. albicans*, and it was found out that, at a concentration of 1 mg/mL, the light-cooked eggplant extract exhibited the highest antimicrobial activity, with inhibition zone diameters ranging from 15 mm to 20 mm against various pathogens. The medium-cooked sample showed slightly lower inhibition zones, while no antimicrobial activity was observed in the high-cooked sample. The uncooked sample also displayed antibacterial and antifungal activity.[25]

Table 1: Phytochemical constituents of anthocyanin-rich extract of fresh eggplant peels

Phytochemical content	Inference
Carbohydrates	-
Glycosides	+
Alkaloids	-
Steroids	-
Terpenoids	-
Saponins	-
Phenolic Compounds	+
Flavonoids	+

(+): indicate presence; (-): indicate absenc**e**

Antifungal activity

The results of the antifungal activity of the anthocyanin-rich extract of *eggplant peels are presented in* Table 3. The anthocyanin-rich extract showed low activity against *Candida albicans and Aspergillus niger* at all concentrations tested. The inhibition zone diameter ranged from *11 mm to 18 mm* against *Candida albicans, and* the MIC was *0.1 mg/mL. Against Aspergillus nige*r*, the inhibition zone diameter ranged from 9 mm to 16 mm, while the MIC was 0.1 mg/mL.* In addition, the results revealed that the inhibition zone diameter increased with increasing concentration of the extracts, this indicates that the antifungal effect of the extract is concentration-dependent. The antifungal efficacy of eggplant peel extract has been attributed to its phenolic content, and could offer a promising alternative to conventional fungicides against common pathogens.[26]

The overuse of antifungals arising from the increasing incidence of *C. albicans* infections is fueling the development of antifungal resistance. This trend is exemplified by the increasing resistance to azoles, a widely employed antifungal agent.[27] Growing concerns over side effects of synthetic medications have propelled a shift towards herbal alternatives, lauded for their minimal adverse effects and impressive therapeutic potentials. This has led to a surge in research exploring herbal remedies alongside conventional treatment. Notably, the Purple Eggplant (*Solanum melongena L.*) has emerged as a promising source of natural antifungal compounds, paving the way for adjuvant therapies.[28]

Purple Eggplant (*Solanum melongena L.*) harbors active ingredients with potent antibacterial and antifungal properties. This highly esteemed functional food boasts of a high concentration of flavonoids and phenolic acids, as documented in previous studies.[29-31] Purple Eggplant peels is rich in potent flavonoids, a class of secondary metabolites, capable of inhibiting *C. albicans* growth by directly attacking its cell membranes. These flavonoids exert their antifungal action through a multi-pronged approach: denaturing fungal proteins, disrupting the cell membrane's lipid layer, and causing irreversible cell wall damage. Their lipophilic nature allows them to bind directly to phospholipids in the fungal cell membrane, disrupting its permeability and interfering with vital cellular processes. Additionally, flavonoids bind to extracellular proteins in the fungal cell wall, compromising its integrity and inhibiting crucial enzymes. This dual action disrupts protein synthesis and alters the composition of the cell membrane, leading to increased permeability and leakage of vital intracellular materials. As a consequence, the fungus suffers from ATP depletion, impaired metabolism, stunted growth, and ultimately cell lysis.[32] Cellular injury in fungi triggers the detachment of their cell walls, significantly reducing the formation of both germ tubes and hyphae.[33]

Antioxidant activity

Free radicals, known for their damaging effects on cells, are thought to be major players in both aging and disease progression. Antioxidants serve as primary defense against the harmful effects of free radicals, thus safeguarding health and maintain well-being.[34] Phenolic compounds act as reducing agents, free radical scavengers, metal ions chelators, hydrogen atom donors, and even singlet oxygen quenchers. The reactive oxygen molecules, present in the body, can oxidize lipids, proteins, and even nucleic acids, potentially triggering the development of degenerative diseases such as heart disease.[35]

The antioxidant activity of anthocyanin-rich extract of eggplant peels is likely attributed to the hydroxyl groups present in its phenolic compounds, which can effectively neutralize free radicals. The antioxidant activity of the anthocyanin-rich extract was determined by β-carotene bleaching assay, the relationship between absorbance and time as presented in Table 4 and Figure 1 was monitored for the extract and this was compared with that of BHT the standard used in this assay. The results showed that the eggplant peel extract exhibited an antioxidant activity of 74.26%, while BHT displayed a higher activity of 84.62% (Table 4).

Eggplants are rich in antioxidants and various phytochemicals, particularly in their peels. The most significant class of phenolic compounds in eggplants are flavonoids, with anthocyanins being the most prominent. These anthocyanins impart vibrant colours ranging from orange-red to deep purple to fruits and vegetables.[36]

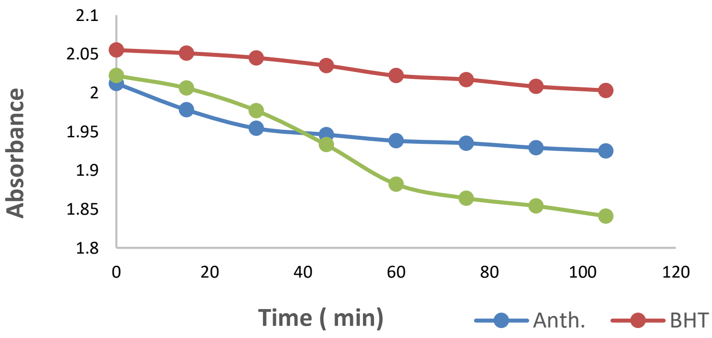

Figure 1: Antioxidant activity of anthocyanin-rich extract of eggplant peels

Table 2: Antibacterial activity of anthocyanin-rich extract of eggplant peels

Sample	Conc. of anthocyanin extract (mg/mL)	Inhibition Zone Diameter (mm)	
		E. coli	***S. aureus***
1	100	22	22
2	10	18	18
3	1	10	16
4	0.1	0	11
5	0.01	0	0

Table 3: Antifungal activity of anthocyanin-rich extract of eggplant peels

Sample	Conc. of anthocyanin-rich extract (mg/mL)	Inhibition Zone Diameter (mm)	
		niger	***C. albicans***
1	100	16	*18*
2	10	16	*16*
3	1	13	*13*
4	0.1	9	*11*
5	0.01	0	*0*

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Table 4: Antioxidant activity of anthocyanin-rich extract of eggplant peels compared with BHT

Sample	AI	AT	AI*	AT*	% AA
Anth	2.012	1.925	2.022	1.684	74.26
BHT	2.055	2.003	2.022	1.684	84.62

Anth = Anthocyanin-rich extract, BHT = Butylated hydroxytoluene, AI =: Absorbance of sample at time zero, AT = Absorbance of sample after 105 min, AI* = Absorbance of control at time zero, AT* = Absorbance of control after 105 min, AA = Antioxidant activity.

Recently, eggplants have gained significant attention as a powerful functional food. Their high content of phenolic compounds, which are known for their antioxidant properties, has earned them a place among the top ten vegetables with the highest antioxidant efficacy.[37] Eggplants has an abundance of anthocyanins in their peels, while their flesh is particularly rich in phenolic acids. Notably, delphinidin derivatives are the dominant phenolic compounds in the peel, while chlorogenic acid isomers are abundant in the flesh.[29]

Eggplant skin is a richer source of phenolic compounds than eggplant pulp. It contains both a greater variety and a higher concentration of most individual phenolic compounds found in either part of the fruit.[38]

Conclusion

From the results of the present study, the anthocyanin-rich extract of the peels of eggplant (*Solanum melongena L.*) possesses antimicrobial activity. The extract also showed high antioxidant activity which could be attributed to its anthocyanin contents which are known for their potent antioxidant properties. Therefore, eggplant is a promising source of natural antimicrobial and antioxidant agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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