SYNTHESIS AND CHARACTERIZATION OF NEW POLYMERIC COATINGS FROM ANTHOCYANIN DYE AND STUDY BIOLOGICAL ACTIVITY

Mayson Thafir Hadi[1] Zainab A. Jabarah[2] Sana H. Awad[3]
Lecturer. Assist. Prof. Prof.
[1]Dept. Food Sci. Coll. of Agric. Eng. University of Baghdad
Dept. Com. Deser. Coll. of Agric. Eng. University of Baghdad
[3]Dept. Chem. Coll. of Sci. Women, University of Baghdad
[1]maysoon.d@coagri.uobaghdad.edu.iq [2]zainab.abd@coagri.uobaghdad.edu.iq
[3]sanaha@csw.uobaghdad.edu.iq

ABSTRACT

This study was aimed to prepared new polymeric coverings reinforced by the naturally occurring pigment anthocyanins and investigate their biological efficacy when combined with certain drugs against specific kinds of microbe's pathogens. FT-IR spectroscopy and mass spectrometry (MS) were used to identify the active groups of the anthocyanin pigment, which were situated the functional groups of anthocyanin in contrast to crystalline compounds, X-ray diffraction (XRD) examination also revealed the production of irregularly constituted compounds. Furthermore, all derivative compounds were had high inhibitory activity against the Gram-positive bacteria Staphylococcus aureus, as well as against the fungus Candida albicans in different concentrations.

Key words: red cabbage, succinic anhydride, salbutamol, phenylephrine, sulfamethoxazole.

تحضير وتشخيص اغلفة بوليميرية جديدة من صبغة الأنثوسيانين ودراسة فعاليتها البايولوجية

ميسون ظافر هادي[1] زينب عبد الرزاق جبارة[2] سناء هتور عواد[3]
مدرس أستاذ مساعد استاذ
[1]قسم علوم الأغذية، كلية علوم الهندسة الزراعية، جامعة بغداد.
[2]قسم مكافحة التصحر، كلية علوم الهندسية الزراعية، جامعة بغداد.
[3] قسم الكيمياء، كلية العلوم للبنات، جامعة بغداد.

المستخلص

هدفت الدراسة الحالية الى تحضير اغلفة بوليمرية جديدة من صبغة الأنثوسيانين الطبيعية ودراسة فعاليتها البايولوجية مع بعض الادوية ضد بعض أنواع الميكروبات المرضية. اذ تم تشخيص المجاميع الفعالة لصبغة الانثوسيانين، والتي تقع بين القمم الواضحة الى المجاميع الفعالة للانثوسيانين، باستخدام تحليل طيف الاشعة تحت الحمراء FT–IR وطيف الكتلة. كما واظهر تحليل حيود الاشعة السينية (XRD) الى ان المركبات المحضرة غير منتظمة التركيب مقارنة بالمركبات المتبلورة وأن جميع المركبات المحضرة لها بنية غير متبلورة بدلا من التركيب البلوري علاوة على ذلك، فان جميع المركبات المحضرة والمشتقة لها فعالية تثبيطيه عالية ضد البكتيريا الموجبة لصبغة كرام *Staphylococcus aureus* وكذلك ضد فطر *Candida albicans* وبتراكيز مختلفة.

الكلمات المفتاحية: اللهانة الحمراء، سكسينيك أنهيدريد، سال بيوتا مول، فينيل ابرين، سلفاميثاكسازول.

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INTRODUCTION

Anthocyanin is a water-soluble pigment found in plants that belongs to the flavonoids group (33). It encompasses many plant by-products metabolites such as pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and maldivin (19,33). Anthocyanin is a polyphenolic glycosylate that has been found in many fruits, vegetables, tea, nuts, olive oil, cacao, and cereals. It imparts distinct colors, such as red, purple, orange, and blue, to different sections and kinds of plants (22,34). Anthocyanins have a crucial function in producing attractive colors, as well as playing a vital part in pollination and dispersal of seeds (9). The primary activities of these pigments are to scavenge free radicals and exhibit antioxidant properties, which contribute to the enhancement of human health. Additionally, there are additional postulated functional pathways (2,35). Numerous studies have shown that anthocyanin pigments are synthesized in the cytoplasm and stored in the vacuoles of epidermal cells in different forms of accumulations (10,11,26). Lately, researchers have shown great interest in studying the characteristics of isolated anthocyanin pigments in connection to human health (7). Furthermore, many findings have shown that the action of anthocyanin may be improved when it is administered as a combination rather than individually (20). Anthocyanins consist of water-soluble glycosides that are connected to a sugar moiety (15,33). The diversity in the amount and placement of hydroxyl and methyl groups attached to anthocyanin molecules has resulted in the existence of about 25 natural anthocyanins and over 700 identified derivatives of anthocyanin to date (25). Furthermore, the diversity in the position and quantity of (OH) hydroxyl groups on the nucleus flavones was utilized to categorize anthocyanin (36, 38). Minimizing the impact of anthocyanins serves many purposes in limiting the negative consequences of reactive nitrogen and hydrogen species. Oxidation and reduction in biological liquids may lead to various negative health conditions in humans, including Parkinson's disease, Alzheimer's disease, heart and blood disorders, diabetes, and cancer (16). Suppressing these processes is enough to prevent the occurrence of such diseases. Salbutamol, phenylephrine, and sulfamethoxazole are often prescribed bacteriostatic antibiotics for the treatment of various illnesses. Currently, there are several therapeutic compounds being researched that rely on heterocyclic moieties (1). They are widely used in clinical medicine and function as pharmacological agents in different biological processes, such as antiviral activity, cancer therapy, herbicidal effects, antifungal properties, and the treatment of mycobacterial and tuberculosis infections (12). The aim of the current work was to create novel polymeric coverings reinforced by the naturally occurring pigment anthocyanins and investigate their biological efficacy when combined with certain drugs against specific kinds of microbe's pathogens.

MATERIAIS AND METHODS

Materials and Measurement: All chemicals are employed in their original form as obtained from suppliers. This includes red cabbage purchased from local markets in Baghdad, Iraq. The mixture consists of 98.8% succinic anhydride and either salbutamol, phenylephrine, or sulphamathaoxazole, all of which are 99.9% pure obtained from Merck. Fourier Transform Infrared (FT-IR) spectra (Shimadzu) spectrophotometer can be used of measuring wavelengths in the range of 4000 to 400 cm^{-1} using KBr discs. The melting point (m. p.) was determined using an electrothermal apparatus. SHIMADZU 6000XRD, and the analysis also includes mass spectra. The antibacterial activity was conducted at the Biology Department of the Phi Nano-science Centre, Nawah Atom Sci. Ltd. Co., in Baghdad, Iraq.

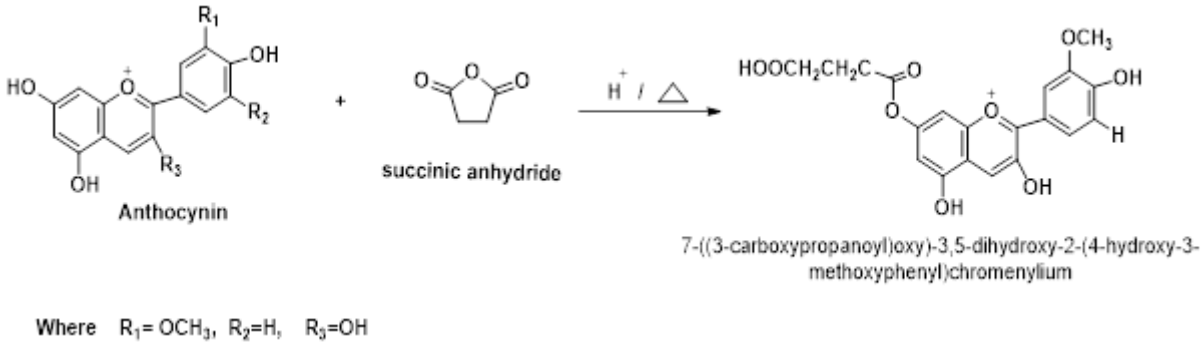
Extraction of anthocyanin from Iraqi red cabbage: (50 gm) of red cabbage was sliced and placed into a 1000 ml beaker which also contained 500 ml of 70% ethanol. The mixture was agitated for a duration of 3 hrs. After being stirred with a magnetic stirrer, the mixture was thereafter allowed to stand at room temperature for 24 hrs. The solution was passed through Whitman 0.1N filter paper to remove impurities. The resulting filtrate was then transferred to a dry and clean Petri dish. Subsequently, it was heated to 40°C in a vacuum oven for a duration of 2 days (3,21).

Preparation of coating by condensation polymerization of anthocyanin with succinic anhydride: (3.6 g, 0.001 mol) of anthocyanin was dissolved in 60 ml of ethanol, along with (1.8 g, 0.001 mol) of succinic anhydride diluted in 30 ml of ethanol, in a round bottom flask. The mixture was then heated at 70ºC for about 6 h. afterwards, the final product underwent several washes with 10 ml of diethyl ether to remove any remaining unreacted components. Ultimately, the substance was dehydrated using a vacuum device at a temperature of 50°C (17). The reaction and its characteristics are shown in equation (1) and (table 1).

Substitution of amino drug with graft polymer-succinic anhydride: A flask containing 0.1 g, 0.001 mol) of 7-((3-carboxypropanoyl) oxy)-3,5dihydroxy-2-(4-hydroxy-3methoxyphenyl) chromenylium dissolved in 10 ml of DMF. 5drops of thionyl chloride, was added gradually to the solution. The mixture was then allowed to stand for 30 min, and subsequently refluxed with 0.1 g, 0.001 mol) of salbutamol, which was dissolved in 10 ml of DMF. The mixture underwent reflux for approximately 6 hrs. A precipitate was collected. The resulting residue was retrieved and washed with 10 ml of diethyl ether, then, was subjected to a vacuum oven at a temperature of 500 C, following the same technique employed in the preparation of the polymer medicine Phenylephrine or sulphamethaoxazol (6). The reaction and its characteristics were displayed in Table (1).

Table 1. Physical data of anthocyanin derivatives

Comp. No.	Formula weight	M. Wt.	M.P.°C	Wight of product	Yield%	Color
Anthocyanin peonidin	$C_{16}H_{13}O^{+}_{6}$	**301.27**	**29-33**	**0.4**	**80**	**purple**
[I]	$C_{20}H_{17}O^{+}_{9}$	**401.35**	**51-52**	**0. 6**	**65**	**Brown**
[II]	$C_{33}H_{36}NO^{+}_{11}$	**622.65**	**72-74**	**0. 5**	**69**	**rubicund**
[III]	$C_{29}H_{28}NO^{+}_{11}$	**566.54**	**81-86**	**1.8**	**83**	**pink**
[IV]	$C_{30}H_{26}N_3O^{+}_{12}S$	**652.61**	**74-75**	**1.2**	**75**	**ruddy**



Equation (1). Synthesis of 7-((3-carboxypropanoyl) oxy)-3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromenylium

RESULTS AND DISCUSSION

Red cabbage is extensively utilized in the medical field and the food industry due to its high content of anthocyanin pigment. This natural and potent pigment helps reduce the damaging effects of free radicals and serves as a natural substitute for antioxidants. However, concerns have been raised about the safety of these antibiotics from a health perspective (24). Through a triphasic synthesis process, we successfully synthesized three distinct anthocyanin derivatives.

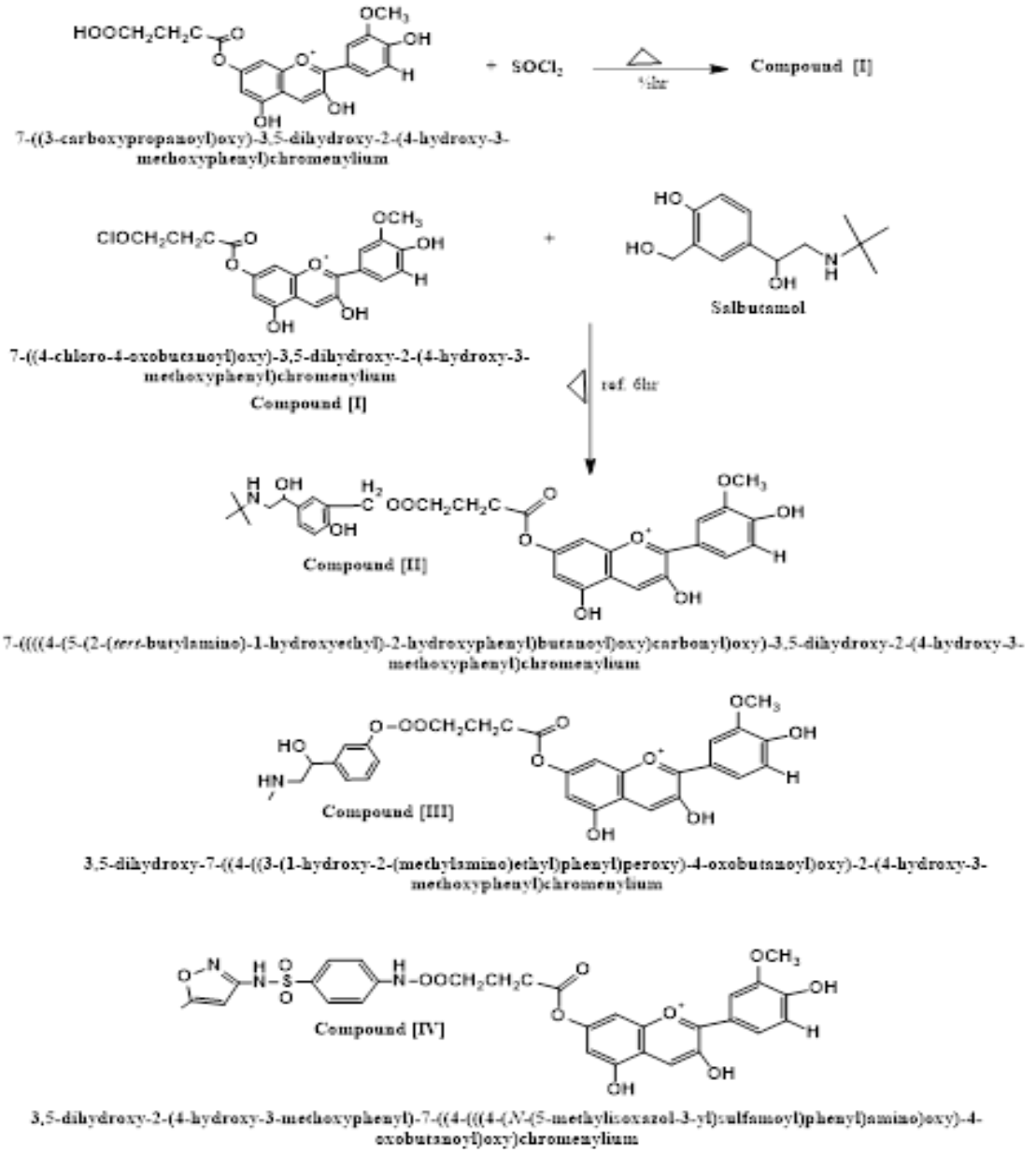
First stage: the extraction of anthocyanin, the red pigment from red cabbage, using the conventional method. The extracted anthocyanin is then characterized using FTIR (14,30,31,28).

Second step: involves applying a coating on anthocyanin using condensation polymerization with Succinic anhydride. The resulting product is then analyzed using FTIR and a graft polymer-succinic anhydride is then prepared (32).

Third step: Substitution of amino drug with graft polymer-succinic anhydride with salbutamol, Phenylephrine or sulphamathmazole in presence of ethanol to give compound [II, III and IV] respectively. FT-IR spectra of these compounds, indicated of band amide (N-H), also tow type of (amid-II and -I) of amide group (1628, 1627 of compounds [II and IIII], esteric (C=O) stretching bands in (1714, 1791 and 1716 cm^{-1}) of compounds [II, III and IV]. Stretching band of –COO carbonyl group and sulphonamido group S-NH in 1612 cm^{-1} and the hydroxyl group, all these data are showed in Table (2) (4,8,13,23).

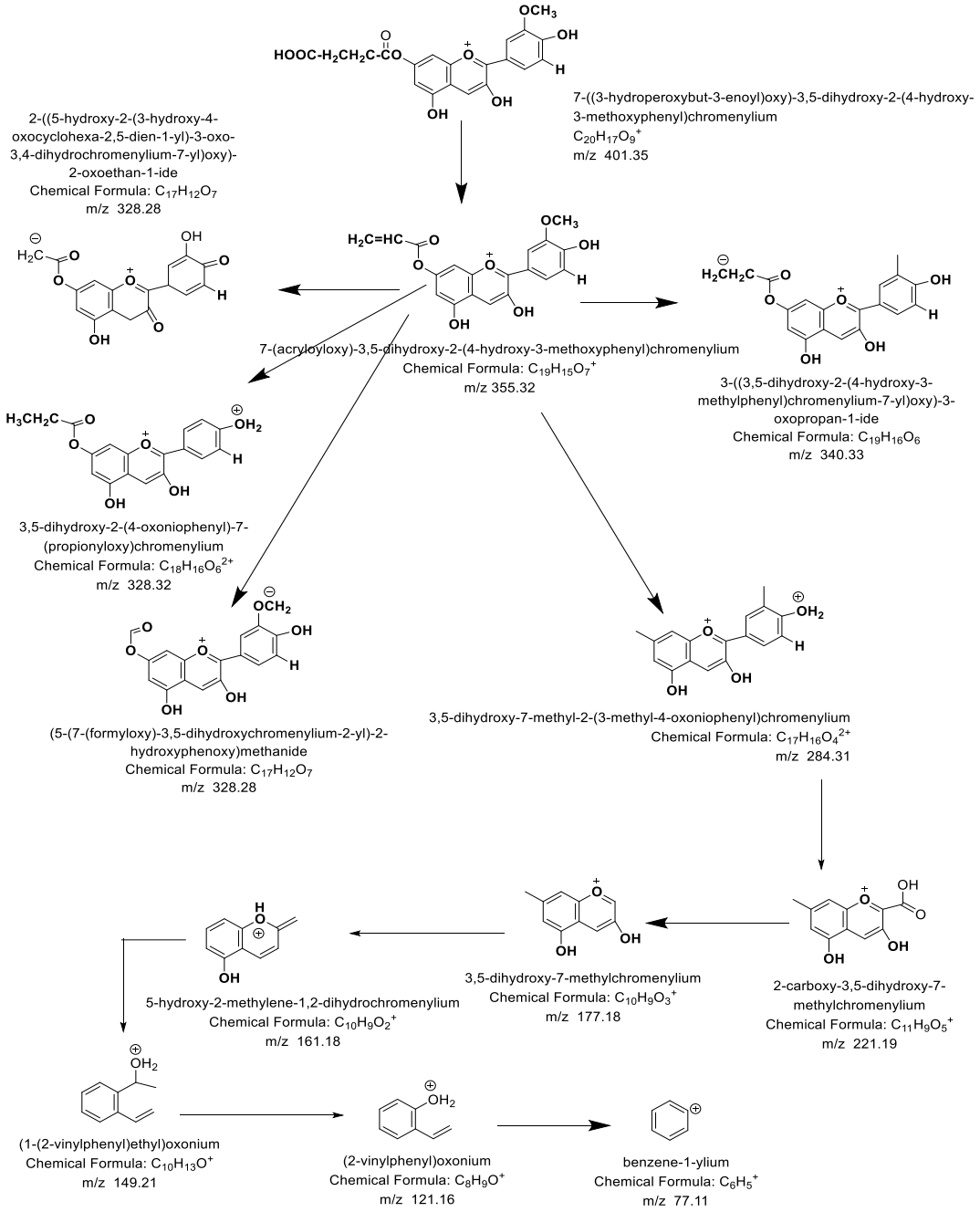
Table 2. FITR spectral data for anthocyanin derivatives [I], [II], [III] and [IV]

Comp. No.	ν –OH	ν C=O	ν C=O	ν SO_2–	ν N–H Amide	ν CH– Alpha.	ν –CH Aroma.
Peonidin	**3425**	**1725**	**1632**	**-**	**-**	**2989**	**3100**
[I]	**3426-3384**	**-**	**1693**	**-**	**-**	**2983**	**3197**
[II]	**3428-3404**	**1714**	**1628**	**-**	**3239**	**2952**	**3183**
[III]	**3417**	**1791**		**-**	**3310**	**2857**	**3103**
[IV]	**3434-2500**	**1716**	**1627**	**1330-1157**	**3278**	**2928**	**3138**



Scheme (2). Synthesis of amino drug with graft polymer-succinic anhydride

Mass spectra MS/MS and suggested chemical structures of compound [II] the peonidin-based anthocyanin with succinic anhydride is shown in (fig.1, scheme 3). The apparent data indicate that the (M)+ m/z 401, which is due to the synthesis of 7-((3-hydroperoxybut-3-enoyl)oxy) -3,5- dihydroxy -2- (4-hydroxy-3methoxyphenyl) chromenylium, these compound was fragmentation ascertained as molecular ion [M]+ m/z 340 at the base peak indicates the compound 3-((3,5-dihydroxy-2-(4-hydroxy-3-methylphenyl)chromenylium-7-yl)oxy)-3-oxopropan-1-ide, which was very close to the theoretical value m/z 340.33 with less error 0.33 ppm, which confirms the validity of suggest molecular structure.



Scheme 3. Suggesting fragmentation of compound [II]

In addition, $(M)^+$ ion at m/z 355 is observed in the MS spectrum at peak 3 was suggest to 7-(acryloyloxy)-3,5- dihydroxy -2- (4-hydroxy-3methoxyphenyl) chromenylium was very close to the theoretical value m/z 355.31 with less error 0.31ppm, while the typical fragment are showed tow suggest compounds in $(M-328.28)^+$ and $(M-328.32)^+$ were obtained by fragmentation, which were attributed to the loss of 2- (5-hydroxy-2-(3-hydroxy-4-oxocyclohexa-2,5-dien-1-yl) - 3 - oxo- 3, 4-dihydrochromenylium-7-yl)oxy)-2-oxoethan-1-ide, or (5-(7-(formyloxy)-3,5-dihydroxychromenylium - 2-yl)-2-

hydroxyphenoxy) methanide, and 3,5-dihydroxy -2- (4- oxoniophenyl)- 7 (propionyloxy) chromenylium respectively. While the following composition was proposed for the compound with $(M-284)^+$ to 3,5- dihydroxy- 7- methyl -2- (3- methy l- 4- oxoniophenyl) chromenylium, these compounds complete the chain of fragments, so we propose that the compound split into a series of the following compounds (M= 221, 177, 161, 147, 121 and 77)$^+$, these peaks of which are shown in the fig. (10), until it reaches benzene-1-ylium (17,18 ,27,29).

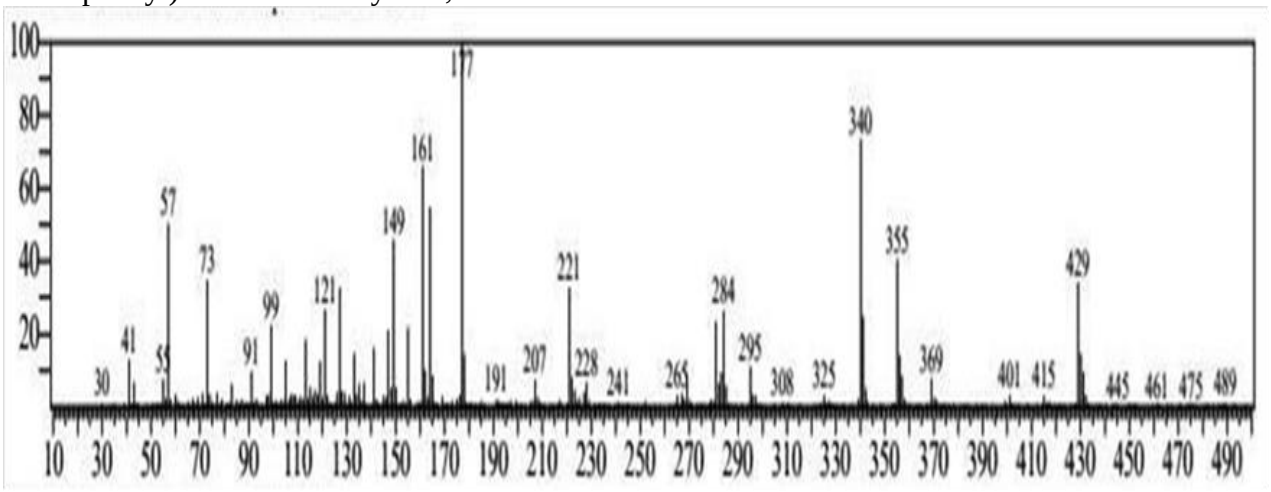


Fig.1. Mass spectra of compound [II].

The XRD crystallography approach enables the comprehensive determination of the whole structure of crystalline materials, encompassing both basic inorganic entities and complex macromolecules such as proteins. The amorphous character of the ribbons generated in this manner was verified by X-ray diffraction (4, 8, 13, 23). Thus, the current method was utilized to demonstrate the various forms of anthocyanin derivative compounds, specifically those classified as II, III, and IV. The figs. (3, 4, 5) showed clear peaks of compounds [II, III, and IV] at angles ɵ|r (18.4, 858), (23.6, 500), and (24.08, 600), respectively. This implies that the produced compounds are amorphous rather than crystalline, indicating a lack of regularity in these compounds (17,18 ,27,29). The molecules coated with [II] exhibit notable crystallization, but the ones coated with [III, IV] remain amorphous, suggesting enhanced stability.

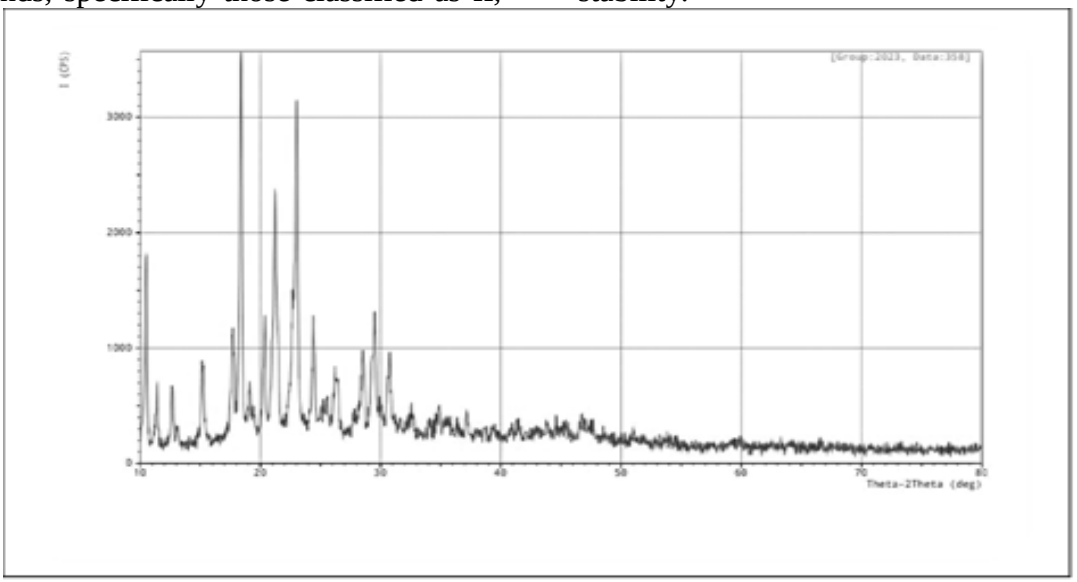


Fig.2: XRD of compound [II]

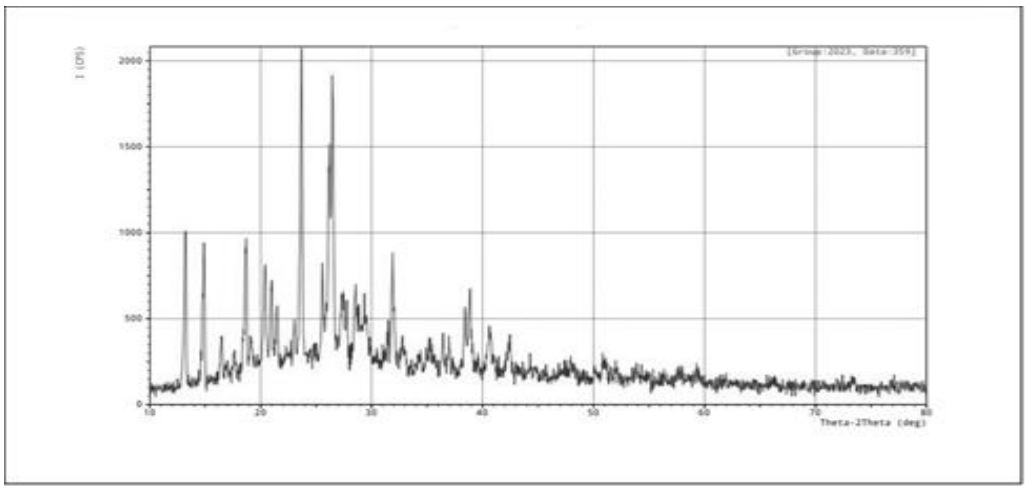


Fig.3. XRD of compound [III]

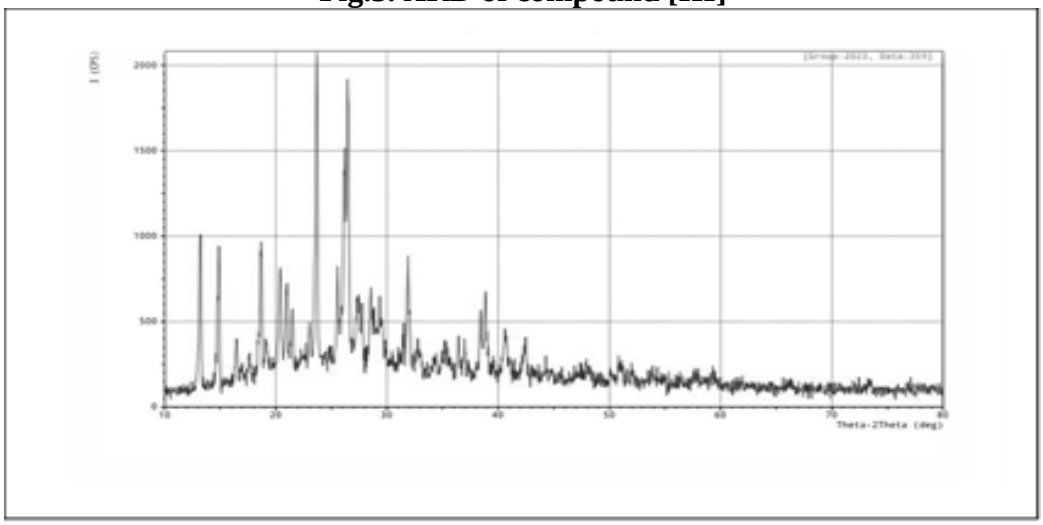


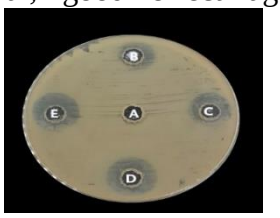
Fig.4. XRD of compound [IV]

This research involved the testing of two types of genetically modified (GM) bacteria and fungus. Specifically, they were evaluated for their effectiveness against antibiotic-resistant gram-positive *Staphylococcus aureus* bacteria on Mueller-Hahn agar plates. The experiment was conducted using sterile cotton swabs, and the concentration of the GM organisms used was 4mg/mol (18,27,40). The antifungal activity of *Candida albicans* was assessed by applying sterile cotton swabs to Sabouraud Dextrose agar plates, at a concentration of 4mg/ml. DMSO is employed as a solvent (5). The antimicrobial analysis of the synthesized compounds (I, II, III, and IV) and anthocyanin, as illustrated in figs. (5, 6, 7, 8, 9 and 10), revealed favorable to moderate efficacy against at varying concentrations. Notably, these compounds exhibited a significant inhibitory effect on *Staphylococcus aureus* and moderate activity against *Candida albicans* fungi (18,27). Furthermore, the findings indicated that anthocyanin exhibited remarkable antibacterial activities when compared to different concentrations of bacteria and fungi, as shown in (table 3).

Table 3. Antimicrobial activity of anthocyanin derivate

Antibacterial analysis (Zone of inhibition)						
Sample		**A**	**B**	**C**	**D**	**E**
Staphylococcus. aureus	**[II]**	**6**	**8**	**11**	**12**	**13**
	[III]	**6**	**6**	**7**	**11.5**	**18**
	[IV]	**6**	**6**	**7**	**11.5**	**18**
Candida	**[II]**	**6**	**8**	**9**	**11**	**17**
	[III]	**6**	**8**	**11**	**14**	**17**
	[IV]	**6**	**6**	**6**	**7**	**11**

DMSO used as a solvent (5). Antimicrobial examination of synthesized compounds [I, II, III and IV and anthocyanin] figs. (5, 6, 7, 8, 9 and 10), showed good to moderate activities against two types of bacteria in different concentration, special, good effect against *Staphylococcus aurous* moderate activities against, and *Candida albicans* fungi (5). Additionally, the results demonstrated that anthocyanin had excellent antibacterial properties in contrast to various concentrations of bacteria and fungi, Table (3).



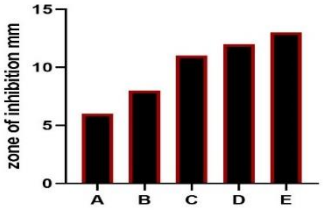
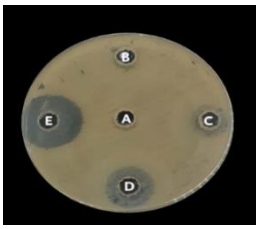


Fig.5. Antibacterial activity of compound [II], against *S. aureus.* A, Control. B, 50 µg/ml. C, 100 µg/ml. D, 150 µg/ml. F, 200 µg/ml.



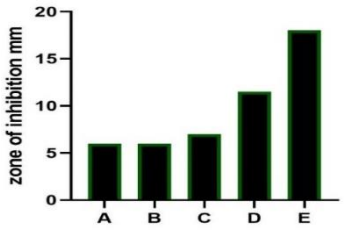
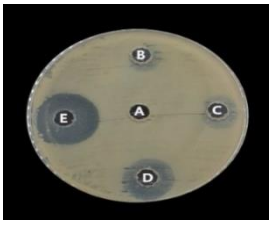


Fig.6. Antibacterial activity of compound [III] against *S. aureus.* A, Control. B, 50µg /ml. C, 100 µg/ml. D, 150 µg/ml. F, 200 µg /ml.



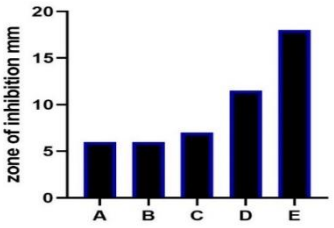
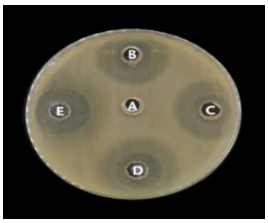


Fig.7. Antibacterial activity of compound [IV], against *S. aureus.* A, Control. B, 50µg /ml. C, 100 µg/ml. D, 150 µg/ml. F, 200 µg/ml.



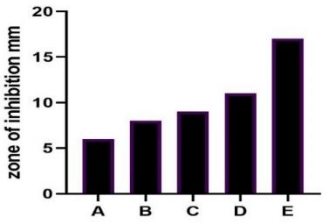
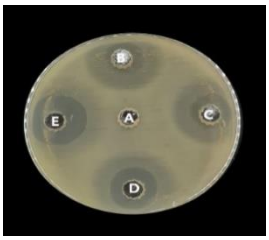


Fig.8. Antibacterial activity of compound [II], against *Candida.* A, Control. B, 50 µg /ml. C, 100 µg/ml. D, 150 µg/ml. F, 200 µg /ml.



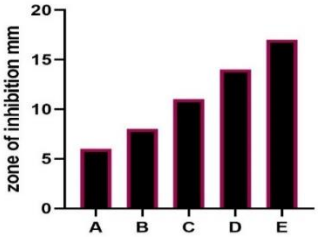


Fig.9. Antibacterial activity of compound [III], against *Candida.* A, Control. B, 50 µg /ml. C, 100 µg/ml. D, 150 µg/ml. F, 200 µg/ml.

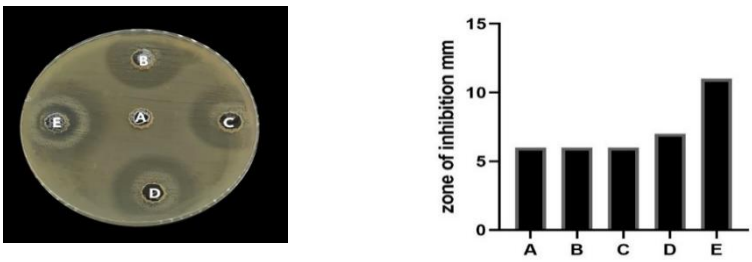


Fig.10. Antibacterial activity of compound [IV], against *Candida.* A, Control. B, 50 µg/ml. C, 100 µg/ml. D, 150 µg /ml. F, 200 µg/ml.

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