Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2024, Issue-3, pp-89-97
https://bfszu.journals.ekb.eg/journal
Research Paper DOI: **10.21608/bfszu.2024.251144.1343**

==

Antibacterial activity of some natural pigments in Egypt

Ahmed Shindia[1], Seham Abdel-Shafi[1], Asmaa Atef[1,*] and Mahmoud Sitohy[2]
1 Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig 44519, Egypt
2Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
***Corresponding author:** Email: asmaakhidr1@gmail.com

ABSTRACT **: Crude phenolic-extracts were taken from the peels of different plants, including *Solanum melongena* (eggplant), *Beta vulgaris* (red beet), *Punica granatum* (Pomegranate), *Capsicum annuum* (paprika), *Daucus carota* (carrot), *Citrus reticulata* (tangerine), *Ipomoea batatas* (sweet potato) and *Actinidia deliciosa* (kiwi). These extracts showed remarkable antibacterial activities against *Salmonella typhi, Escherichia coli, Bacillus cereus, and Staphylococcus aureus*. The highest inhibition was observed with *Daucus carota* and *Solanum melongena* extracts. The pathogenic microorganisms showed avariable susceptibility to different antibiotics. Ciprofloxacin (cip) was the most effective one among the other tested antibiotics. The bactericidal effect of carrot extract and eggplant extract in combination with cip against *Staphylococcus aureus* P59 (VISA P59) and *Escherichia coli* O157 ATCC51659 was recognized by disc diffusion assay. The results demonstrated a considerable antibacterial effect when plant extracts and antibiotics were used to combat the tested microorganisms. When mixed with cip, the extract from *Solanum melongena* showed larger inhibitory zones than those of *Daucus carota.***

KEYWORDS: **Pigment; *Solanum melongena*; *Daucus carota*; Antibiotic.**

Date of Submission: 25-11-2023 Date of acceptance: 09-01-2024

I. INTRODUCTION

In the previous fifty years, there was an increase in the frequency of newly emerging infectious diseases **(Allen *et al*., 2017)**. Changes in lifestyle, such as rising migration, increasing antibiotherapy-resistant infections, expanding numbers of patients with immunosuppression, and a possible bioterrorism threats, all play a role in this **(Silva *et al*., 2012)**. The emergence of antibiotic-resistant microorganisms reduces the medicinal effectiveness of the medication in managing infections which are life-threatening in addition to increasing the total cost of therapeutic approaches **(Yang *et al*., 2021)**.

In recent years, different attempts have been made to find natural antimicrobials that can stop bacterial and fungal growth in order to improve food quality and shelf life **(Gyawali and Ibrahim, 2014)**. Recently, the interest in using plant extracts as green antimicrobials and environmentally acceptable alternatives to prevent adhesion and destroying biofilm has been increased **(Sadekuzzaman *et al*., 2015)**. Herbs and spices have most of the antimicrobials derived from plants **(Tajkarimi *et al*., 2010; Cueva *et al*., 2010 and Negi, 2012)**. Fruit phytochemicals, are widely employed for their therapeutic benefits. They are mixtures of many components and can be sorted into principal categories according to their chemical constitution, such as organic acids, terpenes, and polyphenols **(Barbieri *et al*., 2017)**.

Phenolic compounds originating from plants have been found to possess a range of antibacterial activities **(Bouarab Chibane *et al*., 2019; Efenberger-Szmechtyk *et al*., 2021; El Moussaoui *et al*., 2019; Lima *et al*., 2019 and Muniyandi *et al*., 2019).** Plant pigments are made up of several groups of constituents, such as betalains, carotenoids, anthocyanins, and chlorophylls **(Gandia- Herrero *et al*., 2010; Jensen *et al*., 2011)**. Pigments are thought to be natural, secure and may have antioxidant properties. They can be used as a culinary coloring ingredient **(Sagar *et al*., 2018)**.

Carotenoids pigments can be derived from a variety of vegetable and fruit waste products. For instance, paprika waste (lutein, 232.60 lg/g) **(Kang *et al*., 2016)**, tomato peel (carotenoids, 253.5 lg/g) **(de Andrade Lima *et al*., 2019)**, carrot peel (carotenoids, 82.66 lg/g) **(Tiwari *et al*., 2019).** Several kinds of carotenoids also have antibacterial activity **(Ibrahim, 2012; Karpiński and Adamczak, 2019)**. In the food sector, *Daucus carota* (*D. carota*) is commonly employed to stop the growth of spoilage microorganisms and thus increase the shelf life of foods because of its strong antioxidant and antibacterial activities **(Kiros *et al*., 2016; Hayashi *et al*., 2012)**.

Anthocyanins are extensively and abundantly present in grains, vegetables, and fruit skins, and they are in charge of giving fruits, flowers, and vegetables their various hues **(Mazza *et al*., 2004)**. Anthocyanins exhibit antimicrobial activity via a variety of methods, including the induction of cell injury through the degradation of the intercellular matrix, membrane, and cell wall **(Pojer *et al*., 2013)**. Antimicrobial activity of *Solanum melongena* (*S. melongena*) against different pathogenic bacteria and fungi has been documented **(AL-Janabi *et al*., 2010)**. The eggplant possesses antibacterial activity that is sustained due to the presence of substances such nasunin, chlorogenic acid, and caffeic acid **(Salamatullah *et al*., 2021)**.

The aim of the present work was to determine the antimicrobial activity of pigment extracted from natural sources as a potential role in food and pharmaceutical industry.

II. Materials and methods

Plant material: *Solanum melongena*, *Beta vulgaris*, *Punica granatum*, red *Capsicum annuum, Daucus carota*, *Citrus reticulate*, *Ipomoea batatas* and *Actinidia deliciosa* totally ripe fruits were bought at the Egyptian local market.

Microorganisms: Gram-positive pathogenic bacteria *Staphylococcus aureus* P59 (VISA P59) (*S. aureus*) and *Bacillus cereus* ATCC 36621 (*B. cereus*) were used. Also, Gram-negative pathogenic bacteria including *Escherichia coli* O157 ATCC51659 (*E. coli*) and Salmonella overexpressed ramA serovar typhi (*S. typhi*) were used. The sources of all bacteria was the Botany and Microbiology Department, Faculty of Science, Zagazig University, Zagazig, Egypt. Stock bacterial cultures were preserved at 20 °C in glass beads and were sub-cultured and multiplied in Nutrient agar **(Monica, 1985)**.

Sample preparation and pigments extraction:

The peels of plant species were cleaned then let to dry at room temperature. The Mettler AE 200 blender was used to grind the dried materials. Until needed, the dry powder was stored in the freezer. Each plant species was extracted using a magnetic stirring method that involved agitating 5 g of dry powder in 100 mL of ethanol with 5% HCl for a full day. A vacuum rotary evaporator operating at 30°C was used to evaporate the ethanol extract after it had been pre-filtered using Whatman No. 4 filter paper.

Antibacterial activity of the plant pigments against Gram-positive and Gram-negative bacteria:

Using a disk diffusion assay, the antibacterial activity of the plant pigments was evaluated against the pathogenic microorganisms under test, as detailed by **(Bauer *et al*., 1966 and Ehinmidu, 2003)**.

The studied bacteria cultures were spread out over nutrient agar media (NA) for the night. Subsequently, 6-mm-diameter sterilized paper disks were immersed in solutions containing extracted pigments (3000 µg/mL) and placed on top of nutritional agar medium. After 24 hours of incubation at 37°C, the diameter (mm) of the clear zones on the nutrient agar plates was measured.

Quantitative inhibition of pathogenic bacteria by *S. melongena* and *D. carota* extracted pigments, and antibiotic-extracted pigments combinations (disc diffusion assay):

Ready antibiotic discs of ciprofloxacin (Cip 5 µg), Ceftazidime (CAZ 30 µg), Cefsulodin (CES 105 µg), Cefoxitin (Fox 30 µg) and Piperacillin (PRL 100 µg) were placed on the surface of nutrient agar medium that had been seeded with all of the tested bacteria, with the proper spacing between them. After 24 hours of incubation at 37°C on nutrient agar plates, the widths of the inhibitory zones (mm) were determined as previously mentioned. Antibiotic sensitivity results were obtained in compliance with **NCCLS (1999)** guidelines.

In a different experiment, a disc containing the most potent antibiotic against the most potent bacteria (*E. coli* and *S. aureus*) was chosen, and several combinations of *D. carota*-antibiotic and *S. melongena*-antiiotic were prepared at their respective minimum inhibitory concentrations as follow: (20µg/mL pigment + 80µg/mL antibiotic), (20 µg/mL pigment + 80 µg/mL antibiotic), (40µg/mL pigment + 60µg/mL antibiotic), (40 µg/mL pigment + 60 µg/mL antibiotic), (50µg/mL pigment + 50µg/mL antibiotic), (50 µg/mL pigment + 50 µg/mL antibiotic), (60µg/mL pigment + 40µg/mL antibiotic), (60 µg/mL pigment + 40 µg/mL antibiotic), (80µg/mL pigment + 20µg/mL antibiotic) and (80 µg/mL *D. carota* + 20 µg/mL antibiotic)**.**

Since *S. aureus* and *E. coli* were the most susceptible to pigments and antibiotics, they were utilized as indicator organisms. The experiment was conducted using the previously mentioned pigment-antibiotic combinations immersed in 6-mm-diameter paper discs.

III. Results

Antibacterial activity of natural pigments (3000 µg/ml) against Gram-positive and Gram-negative pathogenic bacteria using disc diffusion assay:

The antibacterial activity of the extracted pigments against pathogenic Gram-positive and Gram-negative bacteria was evaluated. **Table (1)** demonstrates that *D. carota* pigment had the biggest inhibition zones against all of the bacteria that were tested, while *Citrus reticulate* (*C. reticulate*) pigment showed the lowest inhibition zones against *S. typhi*.

Table (1): Antibacterial activity of some plant pigments (3000 µg/ml) against Gram-positive and Gram-negative pathogenic bacteria using disc diffusion assay:

	Gram-positive bacteria		Gram-negative bacteria	
	B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Extracts of pigment	**Inhibition zone (mm)**			
Actinidia deliciosa	59.00±1.00 **b**	53.50±1.32 **c**	54.50±0.5 **b**	39.00±2.00 **e**
Beta vulgaris	44.00±2.00 **d**	37.00±1.00 **f**	44.67±1.04 **d**	39.50±0.50 **e**
Capsicum annuum	55.50±0.50 **c**	55.50±0.50 **bc**	53.50±0.50 **b**	47.50±0.50 **c**
*Citrus reticulate**	44.00±1.00 **d**	41.50±0.50 **e**	41.67±1.53 **e**	34.50±1.32 **f**
*Daucus carota**	65.33±0.58 **a**	59.00±0.50 **a**	57.50±0.87 **a**	56.50±1.00 **a**
Ipomoea batatas	54.00±1.00 **c**	54.50±0.50 **c**	54.50±0.87 **b**	46.50±0.50 **cd**
Punica granatum	45.50±1.32 **d**	48.50±0.87 **d**	50.50±0.50 **c**	44.00±1.32 **d**
*Solanum melongena**	59.50±1.00 **b**	57.50±0.87 **ab**	55.50±0.50 **ab**	52.50±0.87 **b**
***P*-Value**	<0.001	<0.001	<0.001	<0.001

Means ± SD followed by different letters differ significantly by Tukey's HSD test (P < 0.01) at each column.

**Daucus carota*= Showed the highest inhibition zone against all pathogenic bacteria.

** *Citrus reticulate*= Showed the highest inhibition zone against *S. typhi*.

Antibiotic sensitivity test & mininmum inhibitory concentrations (MICs) of the most potent antibiotic:

Five antibiotics indicated in **(Table 2)** were used to conduct antibiotic sensitivity tests for all pathogenic microorganisms. The pathogenic microorganisms utilized varied in their sensitivity. It was discovered that every tested bacterium exhibited resistance against Ceftazidime (CAZ 30). The antibiotic with the highest efficacy was ciprofloxacin (CIP 5).

Table (2): Antibiotic sensitivity test:

Antibiotics	Amoun (µg/disc)	Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	***B. cereus***	***E. coli***	***S. typhi***
		Inhibition zone (mm)			
CIP *	5	31.0±1.0 **a**	30.0±1.0 **a**	29.0±1.0 **a**	31.0±1.7 **a**
FOX	30	19.0±1.0 **c**	13.0±2.0 **c**	0.0±0.0 **f**	14.0±2.0 **c**
CES	105	26.0±1.0 **b**	17.0±1.0 **d**	13.0±2.0 **d**	20.0±1.7 **b**
PRL	100	10.0±1.0 **d**	12.0±2.0 **c**	9.0±1.0 **e**	11.0±1.7 **c**

*CIP 5= The highest effective antibiotic against all bacteria.

Antibacterial activity of *D. carota*-ciprofloxacin (cip.) and *S. melongena*- ciprofloxacin combinations against *S. aureus* and *E. coli* (the most sensitive bacterium) by disc diffusion assay:

D. carota and *S. melongena* demonstrated a stronger antibacterial activity than other pigments, according to the disc diffusion experiment. *S. aureus* and *E. coli* were selected as the indicator organisms to examine the antibacterial activity of the ciprofloxacin antibiotic and pigment combination since they were the most susceptible bacteria.

The diameter of the inhibition zones (mm) that *S. melongena* produced against the growth of *S. aureus* and *E. coli* was greater than the diameters produced by the cip. antibiotic alone. The diameter of the inhibitory zones (mm) against the studied bacteria when *S. melongena* was combined with cip at varying concentrations was directly correlated with the concentrations of *S. melongena*. By raising *S. melongena* concentrations and lowering cip concentrations, inhibition zones grew.

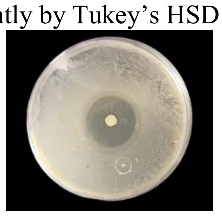
The diameter of the inhibition zones against *S. aureus* in bacterial cultures treated with *D. carota* was less than in those treated with cip alone. However, *D. carota* exhibited larger inhibition zones against *E. coli* than did cip alone. When cip was combined with *D. carota*. The diameter of the inhibition zones (mm) against the tested microorganisms was nearly constant throughout a range of doses. The highest inhibition was observed by mixing equal concentrations of pigments and cip. **(Table 3).**

Table (3): Antibacterial activity of mixed combinations of *S. melongena*, *D. carota* pigments and ciprofloxacin (cip.) against *S. aureus* and *E. coli* by disc diffusion assay:

Treatment 1	***S. aureus***	***E. coli***	**Treatment 2**	***S. aureus***	***E. coli***
cip (100%)	21.00±1.73 **a-c**	25.00±1.73 **bc**	cip (100%)	21.00±1.73 **a-c**	25.00±1.73 **bc**
S. melongena (100%)	23.00±1.73 **ab**	30.00±1.00 **a**	*D. carota* (100%)	19.00±1.00 **b-c**	26.00±1.00 **ab**
S. melongena (20%) + cip (80%)	18.00±1.00 **cd**	18.00±1.73 **d**	*D. carota* (20%) + cip (80%)	17.00±1.73 **d**	17.00±1.00 **d**
S. melongena (40%) + cip (60%)	20.00±1.73 **a-c**	19.00±1.00 **d**	*D. carota* (40%) + cip (60%)	18.00±2.65 **cd**	19.00±1.73 **d**
S. *melongena* (50%) + cip (50%)	24.00±1.00 **a**	30.00±1.00 **a**	*D. carota* (50%) + cip (50%)	21.00±1.00 **a-c**	26.00±1.73 **ab**
S. melongena (60%) + cip (40%)	21.00±1.00 **a-c**	28.00±1.73 **ab**	*D. carota* (60%) + cip (40%)	18.00±1.00 **cd**	19.00±1.73 **d**
S. melongena (80%) + cip (20%)	22.00±1.73 **a-c**	29.00±1.73 **ab**	*D. carota* (80%) + cip (20%)	20.00±1.73 **a-c**	21.00±1.00 **cd**
***P*-Value**	<0.001	<0.001	***P*-Value**	<0.001	<0.001

Means ± SD followed by different letters differ significantly by Tukey's HSD test ($P < 0.01$) at each column.

cip antibiotic



S. melongena

cip antibiotic

D. Carota

Fig. (1): Antibacterial activity of MICs of *S. melongena* and *D. carota* extracts compared to CIP antibiotic against *S. aureus* by disc diffusion assay.

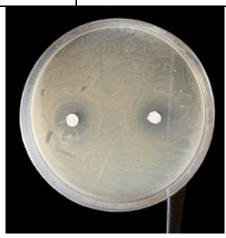
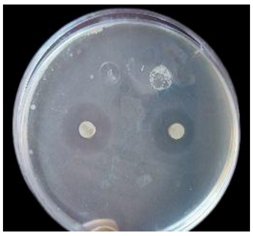
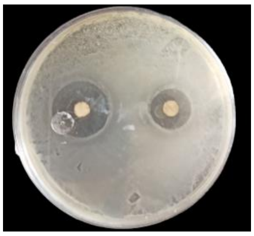
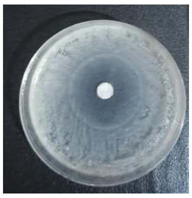
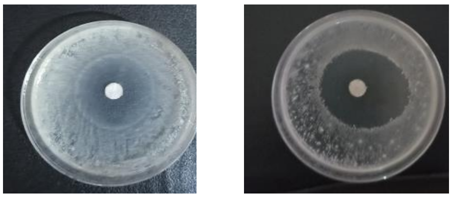
Concentrations	**A**	**B**
20% pigments + 80% CIP		
40% pigments + 60% CIP		
50% pigments + 50% CIP		
60% pigments + 40% CIP		
80% pigments + 20% CIP		

Fig. (2): Antibacterial activity of *D. carota*-cip. and *S. melongena*-cip. Combinations aganist *S. aureus* by disc diffusion assay where **(A)** *S. melongena* pigment + cip and **(B)** *D. carota* pigment + cip.



cip antibiotic

S. melongena



cip antibiotic ***D. Carota***

Fig. (3): Antibacterial activity of MICs of *S. melongena* and *D. carota* extracts compared to CIP antibiotic against *E. coli* by disc diffusion assay.

A **B**

Concentr tions

20% pigments + 80% CI

40% pigments + 60% CI

50% pigments + 50% CI

60% pigments + 40% CI

80% pigments + 20% CI

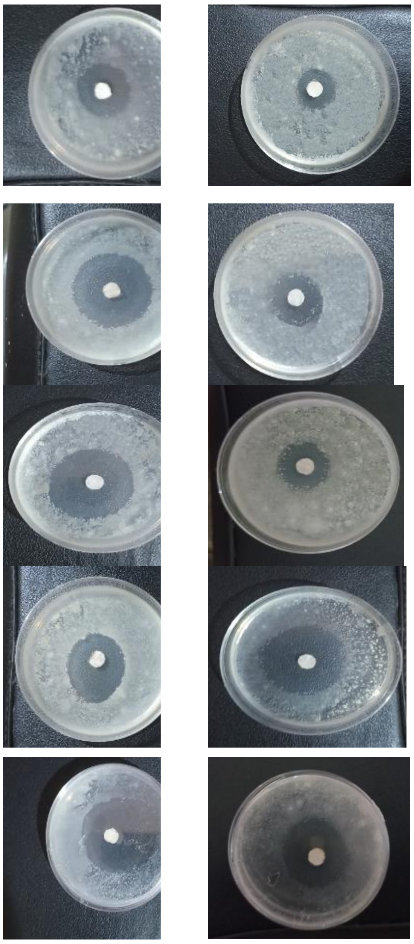


Fig. (4): Antibacterial activity of *D. carota*-cip. And *S. melongena*-cip. Combinations aganist *E. coli* by disc diffusion assay where (A) *S. melongena* pigment + cip and **(B)** *D. carota* pigment + cip.

IV. **Discussion**

Natural pigments are mostly found in plants and microorganisms, such as bacteria and fungi **(Azman *et al*., 2018)**. Due to their carcinogenic precursor products and the environmental implications of their disposal, some synthetic dyes and colorants are restricted from usage **(Dufossé 2006).** If natural colors are non-toxic, non-allergic, non-carcinogenic, and biodegradable, they are considered safe, there is no longer any environmental threat as a result **(Aberoumand, 2011; Wrolstad *et al*., 2012)**.

Due to the presence of a significant amount of tannins, *punica granatum* and many natural pigments have been shown to be effective antibacterial agents **(Siva, 2007).** At doses exceeding 25 mg/mL, a 70% water-ethanol extract of the flavonoid-rich plant *Equisetum arvense* L. showed antibacterial action against Gram-positive cocci, including *S. aureus* ATCC 29213 and clinical isolates of the same pathogen **(Pallag *et al*., 2018)**.

Strong antibiotic efficacy against both Gram positive and negative bacteria (*S. aureus*, *Bacillus subtilis*, *Salmonella sp*., and *E. coli*) is exhibited by piperine derived from the Piper species (*Piper nigrum*, *Piper longum*) **(Hikal, 2018).** *Bacillus cereus* and *Salmonella Typhimurium* were found to be inhibited by the sweet potato leaf extract. This antimicrobial action may be related to the presence of phenolic acids such gallic acid, 3, 4-dihydroxybenzoic acid, and sinapic acid **(Costa *et al*., 2022)**.

Combination therapy lowers the chance of developing cross-resistance and offers possible adjuvant targets of non-overlapping signaling pathways, making it an appealing and optional treatment **(Bozic *et al*., 2013).** Several studies have demonstrated that combination antimicrobial therapy is more effective at preventing infections than using an antibiotic alone **(Pletz *et al*., 2017; Kuo *et al*., 2020; Dodou *et al*., 2017 and Subramaniam *et al*., 2014)**. Synergistic effects may be occur because microbial metabolic pathway may be successively blocked by crude extract and antibiotics or One of two medications may have an impact on the cell membrane, making the second antibiotic more easily absorbed. **(Adwan & Mhanna, 2008)**.

Numerous in vitro investigations have discovered noteworthy synergistic effects when antibiotic interacts with various plant extracts against *S. aureus* strains, resulting in a considerable decrease in the minimum inhibitory concentration **(Adwan & Mhanna, 2008; Ahmed *et al*., 2009; Rakholiya and Chanda, 2011).** Many plants have antibacterial components that may work in synergy by making the pathogen more susceptible to the antibiotic **(Betoni *et al*., 2006)**.

Punica granatum rind ethanol extract demonstrated excellent synergistic efficacy with ciprofloxacin, leading to a 34-fold decrease in minimum inhibitory concentration (MIC) and subsequent re-sensitization of *Klebsiella pneumoniae* **(Rafiq *et al*., 2017).**

V. Conclusions

The results obtained from this study showed that carrot and eggplant grown in Egypt have antibacterial potential, as well as a synergistic effect with ciprofloxacin.

References

Aberoumand, A. (2011). A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World J Dairy Food Sci*., *6*, 71–8.

Adwan, G. & Mhanna, M. (2008). Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. *Middle-East Journal of Scientific Research*, *3*(3), 134-139.

Ahmed, Z., Khan, S. S., Khan, M., Tanveer, A. and Lone, Z. A. (2009). Synergistic Effect of Salvadora persica extracts tetracycline and penicillin against Staphylococcus aureus. *Afr J Basic Appl Sci*, *2*(1-2), 25-29.

AL-Janabi, A. A. H. S. & AL-Rubeey, S. A. H. (2010). Detection of antimicrobial activity of *Solanum melogena* L. (Egg plant) against pathogenic microorganisms. *Pharmacogn. J.*, *2*, 35–39.

Allen, T., Murray, K. A., Zambrana-Torrelio, C., Morse, S. S., Rondinini, C., Di Marco, M., Breit, N., Olival, K. J. & Daszak, P (2017). Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun*, *8*(1), 1124.

Azman, A. S., Mawang, C. I. & Abubakar, S. (2018). Bacterial Pigments: The Bioactivities and as an Alternative for Therapeutic Applications. *Natural Product Communications*, *13*(12), 1747-1754.

Bauer, A. W., Kirby, W. M. H., Sherris, J. C. and Truck, M. (1966): Antibiotic susceptibility testing by a standard single disk method. American Journal of Clinical Pathology. 45: 493-496.

Barbieri R., Coppo E., Marchese A., Daglia M., Sobarzo-Sánchez E., Nabavi S.F., Nabavi S.M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol. Res.*, *196*, 44–68.

Betoni, J. E. C., Mantovani, R. P., Barbosa, L. N., Di Stasi, L. C. & Fernandes Junior, A. (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memórias do Instituto Oswaldo Cruz*, *101*, 387-390.

Bouarab Chibane, L., Degraeve, P., Ferhout, H., Bouajila, J. & Oulahal, N. (2019). Plant antimicrobial polyphenols as potential natural food preservatives. *Journal of the Science of Food and Agriculture*, *99*(4), 1457–1474.

Bozic I., Reiter J.G., Allen B., Antal T., Chatterjee K., Shah P., Moon Y. S., Yaqubie A., Kelly N., Le D. T. (2013). Evolutionary dynamics of cancer in response to targeted combination therapy. *Elife.*, *2*, e00747.

Cueva, C., Moreno-Arribas, M. V., Martín-Álvarez, P. J., Bills, G., Vicente, M. F., Basilio, A., Rivas, C. L., Requena, T., Rodríguez, J. M. & Bartolomé, B. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Res. Microbiol*, *161*, 372–382.

de Andrade Lima, M., Kestekoglou, I., Charalampopoulos, D., & Chatzifragkou, A. (2019). Supercritical Fluid Extraction of Carotenoids from Vegetable Waste Matrices. *Molecules (Basel, Switzerland)*, *24*(3), 466.

Dodou, H. V., de Morais Batista, A. H., Sales, G. W. P., de Medeiros, S. C., Rodrigues, M. L., Nogueira, P. C. N., Silveira, E. R. & Nogueira, N. A. P. (2017). Violacein antimicrobial activity on *Staphylococcus epidermidis* and synergistic effect on commercially available antibiotics. *Journal of applied microbiology*, 123(4), 853–860.

Dufossé L. (2006). Microbial production of food grade pigments. *Food Technology and Biotechnology*, *44*(3), 313–323.

Efenberger-Szmechtyk, M., Nowak, A. & Czyzowska, A. (2021). Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. *Critical Reviews in Food Science and Nutrition*, *61*(1), 149–178.

Ehinmidu, J. O. (2003): Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Tropical Journal of Pharmaceutical Research. 2: 223-228.

El Moussaoui, A., Jawhari, F. Z., Almehdi, A. M., Elmsellem, H., Fikri Benbrahim, K., Bousta, D & Bari, A. (2019). Antibacterial, antifungal and antioxidant activity of total polyphenols of Withania frutescens. *L. Bioorganic Chemistry*, *93*, 103337.

Gandia-Herrero, F., Escribano, J., Garcia-carmona, F. (2010). Structural implications on color, fluorescence, and antiradical activity in betalains. *Planta, 232,* 449-460.

Gilmar Freire da Costa, Cristiani Viegas Brandão Grisi, Bruno Raniere Lins de Albuquerque Meireles, Solange de Sousa & Angela Maria Tribuzy de Magalhães Cordeiro. (2021). Extracts of Sweet Potato leaf (*Ipomoea Batatas*) and Taioba (*Xanthosoma Sagittifolium*): New Sources of Natural Bioactives for the Food Industry. *Acta Scientific Nutritional Health*, *6*(4) 23-31.

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food control*, *46*, 412-429.

Hayashi, M., Naknukool, S., Hayakawa, S., Ogawa, M., & Ni'matulah, A.-B. A. (2012). Enhancement of antimicrobial activity of a lactoperoxidase system by carrot extract and β-carotene. *Food Chemistry*, *130*(3), 541–546.

Hikal D. M. (2018). Antibacterial activity of piperine and black pepper oil. *Biosci Biotech Res Asia.*, *15*(4), 877–880.

Ibrahim H. A. H. (2012). Antibacterial carotenoids of three *Holothuria* species in Hurghada, Egypt. *Egypt. J. Aquat. Res.*, *38*, 185–194.

Jensen, M. B., Lopez-de-dicastillo Bergamo, C. A., Payet, R. M., Liu, X., Konczak, I. (2011). Influence of copigment derived from Tasmannia pepper leaf on Davidson's plum anthocyanins. *J. Food Sci*., *76*, C447eC453.

Kang J-H, Kim S. & Moon B. (2016). Optimization by response surface methodology of lutein recovery from paprika leaves using accelerated s olvent extraction. *Food Chem*., *205*, 140–145.

Karpiński T. M. &Adamczak A. (2019). Fucoxanthin-An Antibacterial Carotenoid .*Antioxidants*, *8*, 239.

Kiros E., Seifu E., Bultosa G. & Solomon, W. K. (2016). Effect of carrot juice and stabilizer on the physicochemical and microbiological properties of yoghurt. *LWT-Food Sci Technol*., *69*, 191-196.

Kuo, S. C., Liu, C. E., Lu, P. L., Chen, Y. S., Lu, M. C., Ko, W. C., Hsueh, P. R., Chuang, Y. C., Wang, F. D., & SMART Asia-Pacific Group (2020). Activity of ceftolozane-tazobactam against Gram-negative pathogens isolated from lower respiratory tract infections in the Asia-Pacific region: SMART 2015-2016. *International journal of antimicrobial agents*, *55*(3), 105883.

Lima, M. C., Paiva de Sousa, C., Fernandez-Prada, C., Harel, J., Dubreuil, J. D. & de Souza, E. L. (2019). A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. *Microbial Pathogenesis*, *130*, 259–270.

Mazza, G., Cacace, J. E., & Kay, C. D. (2004). Methods of analysis for anthocyanins in plants and biological fluids. *Journal of AOAC International*, *87*(1), 129–145.

Monica, C. (1985): Medical laboratory manual for tropical countries. English language book society editions, Tropical health technology. **42:** 250-290.

Muniyandi, K., George, E., Sathyanarayanan, S., George, B. P., Abrahamse, H., Thamburaj, S. & Thangaraj, P. (2019). Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of Rubus fruits from Western Ghats, India. *Food Science and Human Wellness*, *8*, 71–83.

National Committee for Clinical Laboratory Standards (NCCLS), (1999): Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: 4th ed.; Approved standard M7-A4: Wayne, Pa.

Negi P.S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *Int. J. Food Microbiol*, *156*, 7–17.

Pallag, A., Filip, G. A., Olteanu, D., Clichici, S., Baldea, I., Jurca, T., Micle, O., Vicaş, L., Marian, E., Soriţău, O., Cenariu, M. & Mureşan, M. (2018). *Equisetum arvense* L. Extract Induces Antibacterial Activity and Modulates Oxidative Stress, Inflammation, and Apoptosis in Endothelial Vascular Cells Exposed to Hyperosmotic Stress. *Oxidative medicine and cellular longevity*, *2018*, 3060525.

Pletz M. W., Hagel S., Forstner C. (2017). Who benefits from antimicrobial combination therapy?. *Lancet Infect. Dis.*, *17*, 677–678.

Pojer, E., Mattivi, F., Johnson, D., & Stockley, C. S. (2013). The Case for Anthocyanin Consumption to Promote Human Health: A Review. *Comprehensive reviews in food science and food safety*, *12*(5), 483–508.

Rafiq Z, Narasimhan S, Haridoss M, Vennila R, Vaidyanathan R. (2017). *Punica granatum* rind extract: Antibiotic potentiator and efflux pump inhibitor of multidrug resistant *Klebsiella pneumoniae* clinical isolates. *Asian Journal of Pharmaceutical and Clinical Research*, *10*, 1-5.

Rakholiya, K. & Chanda, S. (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Microbiol Book Series, 520529.

Sadekuzzaman, M., Yang, S. M.,Mizan, F. R. & Ha, S.D. (2015). Current and recent advanced strategies for combating biofilms. *Compr Rev Food Sci Food Saf, 14*, 491-509.

Sagar NA, Pareek S, Sharma S, Yahia, E. M., & Lobo, M. G. (2018). Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. *Compr Rev Food Sci Food Saf.*, *17*(3):512–531.

Salamatullah, A. M., Alkaltham, M. S., Hayat, K., Ahmed, M. A., Arzoo, S., Husain, F. M., Alzahrani, A. (2021). Bioactive and Antimicrobial Properties of Eggplant (*Solanum melongena* L.) under Microwave Cooking. *Sustainability*, *13*, 1519.

Silva M. T. (2012). Classical labeling of bacterial pathogens according to their lifestyle in the host: inconsistencies and alternatives. *Frontiers in microbiology*, *3*, 71.

Singh, T., Pandey, V. K., Dash, K. K., Zanwar, S. & Singh, R. (2023). Natural bio-colorant and pigments: Sources and applications in food processing. *Journal of Agriculture and Food Research*, *12*, 100628.

Siva, R. (2007). Status of natural dyes and dye yielding plants in India, Current science, *92*(7), 10.

Subramaniam S., Ravi V. & Sivasubramanian A. (2014). Synergistic antimicrobial profiling of violacein with commercial antibiotics against pathogenic micro-organisms. *Pharm. Biol.*, *52*:86–90.

Tajkarimi M. M., Ibrahim S. A., Cliver D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control.*, *21*:1199–1218.

Tiwari, S., Upadhyay, N., Singh, A. K., Meena, G. S. & Arora, S. (2019). Organic solvent-free extraction of carotenoids from carrot bio-waste and its physico-chemical properties. *Journal of food science and technology*, *56*(10), 4678–4687.

Wrolstad, R. E., & Culver, C. A. (2012). Alternatives to those artificial FD&C food colorants. *Annual review of food science and technology*, *3*, 59–77.

Yang, X., Ye, W., Qi, Y., Ying, Y., & Xia, Z. (2021). Overcoming Multidrug Resistance in Bacteria Through Antibiotics Delivery in Surface-Engineered Nano-Cargos: Recent Development for Future Nano-Antibiotics. *Frontiers in bioengineering and biotechnology*, *9*, 696514.