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Total phenolic content, antioxidant, and antimicrobial activities of ripe and unripe Ghanaian habanero pepper (*Capsicum chinense*)

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ABSTRACT

This study compared the total phenolic content, antioxidant activity, and antimicrobial activity of ripe and unripe Ghanaian habanero pepper (*Capsicum chinense*) against some selected foodborne pathogens. The total phenolic content (TPC) of the pepper extracts was determined using the Folin-Ciocalteu method, while the radical scavenging potential of the pepper extracts was assessed using the DPPH and ABTS assays. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), combinatory effects with standard antimicrobial agents, and inhibition of biofilm formation were used to assess the antimicrobial activity of the pepper extracts. The results showed that the ripe pepper extract outperformed the unripe pepper extract in terms of TPC and radical scavenging potential. The TPC values of 1004.52 μgGAE/g and 1362.79 μgGAE/g were obtained for unripe pepper and ripe pepper extract, respectively. DPPH and ABTS free radical scavenging potential were 28.80 % and 91.32 % for unripe extracts and 34.27 % and 92.96 % for ripe pepper extract. In addition, varying degrees of MIC and MBC were obtained for both the unripe and ripe pepper extracts against test organisms. Combinatory effects with antimicrobial agents and antibiofilm formation activity varied for both pepper extracts. In conclusion, the use of pepper in food preparation could help reduce the menace of foodborne diseases.

1. Introduction

Peppers are one of the important members of the *Solanaceae* family. There are several varieties of peppers grown and consumed in fresh, dried, cooked, or fermented form (Pino et al., 2007). Pepper plays several roles in promoting healthy human nutrition and health. Consumption of pepper provides the body with nutrients such as vitamins and minerals for human nutrition (Hwang et al., 2012; Igbokwe et al., 2013; Martínez et al., 2009). Peppers are also employed in traditional medicine as a remedy for gastrointestinal disorders (Xia et al., 2021), respiratory diseases (Dumitrache et al., 2021), depression (Xia et al., 2021), and cardiovascular disease (Loizzo et al., 2015; Pham-Huy et al., 2008). In addition, pepper extracts are useful as pesticides (Chinn et al., 2011), flavoring agent (Loizzo et al., 2015), as well as colorant and seasoning agents in the food industry (Hwang et al., 2012; Loizzo et al., 2015; Palma-Orozco et al., 2021; Valková et al., 2021). Furthermore, consumption of pepper provides the body with bioactive compounds such as phenolic compounds (ferulic acid, rutin, gallic acid, quercetin, catechin, etc.) and alkaloids (capsaicin and dihydrocapsaicin) (Santos et al., 2022; Yap et al., 2021), carotenoid (Morales-Soriano et al., 2019), flavonoids (Bae et al., 2012) with antimicrobial properties. For instance, alkaloids function as antimicrobial agent by inhibiting synthesis of bacterial cell wall, damage to cell membrane, suppression of bacterial metabolism, and protein synthesis (Yan et al., 2021). The mechanism of action of flavonoids as antimicrobial agents includes suppressing nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism of the microbial cell (Ejueyitsi et al., 2023; Shamsudin et al., 2022). These bioactive compounds also give the pepper strong antioxidant (Loizzo et al., 2015; Oney-Montalvo et al., 2020; Santos et al., 2021), anti-inflammatory (Baenas et al., 2019), anticancer (Cao et al., 2015), antimicrobial properties (Aljaloud et al., 2012; Bacon et al., 2017; Careaga et al., 2003; Doğan et al., 2018; Keser et al., 2018; Koffi-Nevry et al., 2012; Valková et al., 2021).

One of the commonly consumed peppers in Ghana is 'Kpakpo shito'.

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Fig. 1. Whole and blended unripe and ripe pettie bell pepper.

Known commonly as pettie bell pepper, 'Kpakpo shito' is a Ghanaian indigenous habanero variety, but also common in some West African Countries (Specialty Produce, n.d.). It was introduced to Ghana and other West African Countries from South America through Spanish trade routes (Specialty Produce, n.d.). Through several years of cultivation and breeding, new varieties were developed leading to the creation of 'Kpakpo shito' (Specialty Produce, n.d.). The fruit is about 2.5 cm in diameter and has 3–4 lobes attached to the fruit stalk (Specialty Produce, n.d.). The immature fruit is green but undergoes colour transition to orange, then red when it reaches maturity. Apart from being a great source of nutrients for human nutrition such as vitamin C, potassium, calcium, vitamin A, and iron (Specialty Produce, n.d.), the pepper may possess antioxidant and antimicrobial activities. In Ghana and many West African countries, the pepper is sold in both unripe green or ripe red stages and utilized in making sauces or eaten raw (uncooked).

Despite the nutritional advantage of the ripe pepper over the unripe pepper (Igbokwe et al., 2013; Martínez et al., 2009), consumers still prefer the green unripe pepper (Frank et al., 2001). Furthermore, studies have shown that the ripening stage affects the type and concentration of the bioactive compound present in pepper (Barbero et al., 2014; Hwang et al., 2012; Martínez-Ispizua et al., 2021; Oney-Montalvo et al., 2020; Staveckienė et al., 2023). For consumers to obtain the maximum benefit from the consumption of pepper, it is essential to determine the effects of the ripening stage on the biological activities of the peppers. Thus, this study will highlight other possible benefits, other than nutritional benefits, derived from consuming both types of pepper. Moreover, since both ripe and unripe pepper is used as condiments to accompany meals it is important to determine the interaction between the pepper and any antimicrobial drugs taken in times of ill health. Therefore, this study aimed to compare the total phenolic content, antioxidant activity, and antimicrobial activity of *Capsicum chinense* pepper at different ripening stages against some selected pathogens. This study will highlight the use of pepper in food preservation and in getting rid of biofilm from food contact surfaces.

2. Materials and methods

2.1. Sample collection

Fresh healthy whole ripe and unripe peppers were bought from a local farmers market in Ho Central Market, Volta Region, and the stalks were removed by hand.

2.2. Preparation of crude pepper extract

The peppers were washed under running water and dried with a single-use paper towel to remove all water. About 100 g of both unripe and ripe pepper was blended for 3 min at constant blender speed (Fig. 1).

This blend was transferred into 50 mL tubes and centrifugated at 3000 rpm for 45 min. The resulting supernatant was collected and filtered using a 0.45 μm filter paper and stored at 4 °C for further analysis.

2.3. Chemicals and reagents

The chemicals and reagents used for the chemical analysis include Folin-Ciocalteu's reagent, sodium bicarbonate (20 % w/v), (1.0 M), methanol (100 %), distilled water, Iron (III) chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic) acid (ABTS), Mueller Hinton agar, phosphate buffered saline, tetracycline, fluconazole, ascorbic acid (vitamin C), and gallic acid. All chemicals used were of analytical grade.

2.4. Determination of total phenolic content

The total phenolic content (TPC) of the pepper extracts was determined using the Folin-Ciocalteu method according to Dzah (2022) with some modifications. Briefly, samples of pepper extracts (100 μL) were added to 500 μL of Folin Ciocalteu's reagent, followed by the addition of 1 mL sodium carbonate (20 % w/v). The mixture was kept at room temperature (25 °C) for 1 h after which the absorbance was measured at 765 nm using Drawell DNM-9602 microplate reader. A standard calibration curve for gallic acid (GA) was drawn using 0 to 0.3 mg/mL of gallic acid dissolved in distilled water. The values of the TPC of pepper extracts were interpolated using this standard calibration curve, quantified, and expressed as micrograms of GA per gram of sample (μgGAE/g). All experiments were performed in triplicates.

2.5. Determination of DPPH (2,2 diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity of pepper extracts was measured in triplicate according to the method of Dravie et al. (2020) with some modifications. A 40 μL of ripe and unripe pepper extract was added to 160 μL of 0.1 mM DPPH radical solution and incubated at room temperature (25 °C) for 30 min. The absorbance was then measured at 517 nm using Drawell DNM-9602 microplate reader. The DPPH radical scavenging ability was determined as a percentage of the control sample using Eq. (1):

$$\%\ inhibition = 1 - \frac{A_t}{A_c} \times\ 100 \tag{1}$$

where A_c is the absorbance of control (in which the same volume of methanol was used in place of the sample); A_t is the absorbance of the samples and DPPH radical solution.

2.6. Determination of ABTS (2, 2 -Azino-bis 3-ethylbenzthiazoline-6-sulphonic) radical scavenging activity

ABTS radical scavenging activity was determined according to the method of Dravie et al. (2020). A 40 μL of each sample was added to 160 μL of ABTS radical solution in microtiter plates. 200 μL of ABTS radical solution was pipetted in triplicates into microtiter plates to be used as a control. The mixtures were incubated for 30 min at room temperature (25 °C). Absorbance was subsequently read at 734 nm using Drawell DNM-9602 microplate reader. The radical scavenging capacity was compared with that of ascorbic acid. The radical scavenging activity was determined as a percentage of the control sample using Eq. (2):

$$\%\ inhibition = \frac{(A_c\ -\ A_t)}{A_c} \times\ 100 \tag{2}$$

where: A_c is the absorbance of control; A_t is the absorbance of the samples and ABTS radical solution

3. Determination of antimicrobial activity

3.1. Test organisms

The organisms used were *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 14028), *Klebsiella pneumoniae (NCTC 13440), Candida albicans* (ATCC 90028*), Staphylococcus aureus, Pseudomonas aeruginosa (ATCC 4853), Aspergillus niger (ATCC 6275*. These organisms were obtained from the Microbiology Laboratory of the Department of Basic Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Science (UHAS), Ghana. These organisms were selected based on their reputation as major causes of foodborne illnesses.

3.2. Determination of minimum inhibitory concentration

The antimicrobial activities of the pepper extracts were evaluated using minimum inhibitory concentrations (MIC). This was done by using the micro broth dilution method involving 96 well microtiter plates according to the protocol previously reported in the literature (Ayisi et al., 2011; Clinical & Laboratory Standards Institute, 2020) with slight modifications. Dried pepper extracts were dissolved in 20 % DMSO to produce a 100 mg/mL stock solution. 100 μL of this stock solution was used to prepare 10 different well concentrations ranging from 50.0 to 0.049 mg/mL by mixing the stock solution with 100 μL of double-strength Mueller Hinton broth (Oxoid Limited, United Kingdom). Wells 11 and 12 in each column of the microtiter plates were used as the positive control (broth + organism only) and negative control (broth without organism), respectively, for each microbial strain. In separate plates, a similar procedure was used for antibiotics tetracycline, chloramphenicol, fluconazole, and nystatin, at concentrations ranging from 128.0 to 0.125 μg/mL for all microbial strains. This was followed by the addition of 100 μL of each of the 0.5 McFarland standardized test organisms, after which the plates were incubated at 37 °C for 24 h for 24 h for bacteria strains and 48 h for fungal strains. The MIC values were then evaluated by visual examination by adding tetrazolium chloride (TTC), 0.1 % (w/v) dye after 10 min and the MICs were determined as the lowest concentration which did not change colour from colourless/light yellow to red/pink.

3.3. Determination of minimum bactericidal (MBC) and minimum fungicidal concentration (MFC)

In order to determine the efficacy of the pepper extracts in killing different microorganisms, the MBC (in the case of bacterial strains) and MFC (in the case of fungal strains) against the test microbial strains were determined. Inoculum from individual wells of the susceptibility testing assays were picked with a sterile loop and streaked on sterile nutrient agar plates. These were then incubated for 24 h at 37 °C for bacteria strains and 48 h for fungal strains. The plates were subsequently checked for the presence or absence of microbial growth on the nutrient agar. The plates with the least concentration which did not show any growth were designated as MBCs/MFCs (Anderson et al., 2018).

3.4. Determination of combinatory effects of pepper extracts and antibiotics

The effects of combining pepper extract and drugs/antibiotics were assessed using the checkerboard test against the test microbial strains with slight modifications based on previous studies by Khodavandi et al. (2010), Odame et al. (2023), and Ankudze et al. (2023).

Briefly, solutions of varying proportions of each of the pepper extracts and the antibiotics (tetracycline, chloramphenicol, fluconazole, and nystatin) were prepared from twice MIC solutions of each pepper extract and different individual antibiotics' concentrations making final volumes of 200 μL. The antimicrobial activities of these solutions were determined, as described for MIC determination. The combinatory effect of the pepper extracts and individual antibiotics was then evaluated as a measure of the fractional inhibitory concentration (FIC) index which was calculated using Eq. (3) (Ankudze et al., 2023).

$$\text{FIC Index} = \frac{\text{MIC of antiobiotics} + \text{extract}}{\text{MIC of antibiotics only}} + \frac{\text{MIC of extract} + \text{antiobiotics}}{\text{MIC of extract only}} \tag{3}$$

The interaction between the antibiotics and the pepper extracts was classified as:

i. synergistic if the FIC Index $\leq$ 0.5;

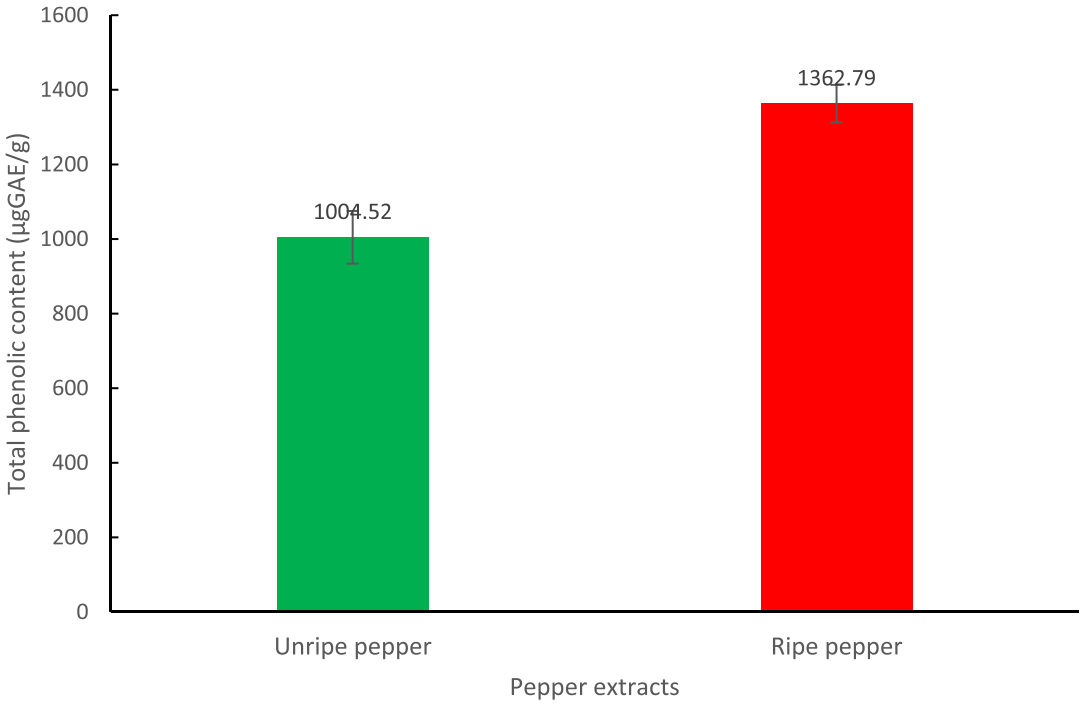

Fig. 2. Total phenolic content of ripe and unripe pettie bell pepper.

ii. partially synergistic if FIC index >0.5 and <1;
iii. additive if the FIC Index = 1;
iv. no difference if the FIC Index is >1 and ≤4; and
v. antagonistic if the FIC Index is >4.0 (Ankudze et al., 2023).

3.5. Determination of antibiofilm activity pepper extracts

The antibiofilm activity of pepper extracts against bacterial biofilms (inhibition of biofilm formation) was evaluated using a 96-well microtiter plate (Dickson et al., 2006; Odame et al., 2023; Pierce et al., 2010). In brief, double-strength Mueller Hinton broth (Oxoid Limited, United Kingdom) was pipetted into each well of the microplate (Citotest Labware Manufacturing Co. Ltd, Jiangsu, China). Each pepper extract (100 µL) was added to column 1 and serially diluted until column 10 at concentrations ranging from 1.0 to 0.001 mg/mL. Thereafter, 100 µL of microbial suspension of 1×10^6 cells/mL was pipetted into the wells of columns 1–11 to arrive at a final volume of 200 µL. 2 µL of 5 % (wt/vol) sterile-filtered tetrazolium chloride (TTC) was added to each of the solutions in the microplate wells, to reach a final TTC concentration of 0.05 % (w/v). The microtiter plates were subsequently incubated at 37 °C for 24 h. After incubation, the mixtures were carefully withdrawn from the wells and the biofilm was subsequently washed with phosphate-buffered saline (100 µL) two times to remove planktonic and non-adherent cells. The metabolic activity of the biofilm after the treatment with each pepper extract was analyzed by TTC (CDH) reduction assay (Dickson et al., 2006; Odame et al., 2023; Pierce et al., 2010). Finally, the optical density (OD) of microplates was measured at 492 nm using a microtiter plate reader and the percentage of biofilm inhibition was determined using Eq. (4).

$$\% \text{ biofilm inhibition} = \left(1 - \frac{\text{OD of treatment}}{\text{OD of control}}\right) \times 100 \quad (4)$$

The IC_{50} for each pepper extract was subsequently calculated by regression equation between pepper extract concentrations and microbial cell inhibition as follows: Y=mx+C; Y=concentration, m= coefficient, x= % inhibition, and C= constant.

4. Results and discussion

Both the ripe and unripe pepper samples produced different responses when subjected to TPC, antioxidant activity, and antimicrobial analyses.

4.1. Total phenolic content

The Folin-Ciocalteu procedure was used to measure the TPC present in the extracts. This method is a simple, and reproducible colorimetric method developed in 1927 to measure proteins (Benavides et al., 2013; Echegaray et al., 2021). The reagent consists of sodium molybdate, sodium tungstate, and other reagents (Benavides et al., 2013; Walker, 2014). The application of the Folin-Ciocalteu method was later extended to measure phenols present in plant extracts. The principle of the Folin-Ciocalteu method is based on electron transfer between the Folin-Ciocalteu reagent and the phenols resulting in the change in colour of the reagent from yellow to blue proportional to the amount of phenols present, which is measured at 765 nm (Benavides et al., 2013). The search for natural antioxidants has led to interest in phenolic compounds (Aberoumand & Deokule, 2008). Phenolic compounds are the key plant compounds with high levels of antioxidant activity (Garg et al., 2012). In this study, the regression equation ($y = 1.9259x + 0.0654$) from the calibration curve was used to calculate the TPC of extracts of ripe and unripe pepper. The extract of the ripe pepper had a higher TPC (1362.79 µgGAE/g) compared to the unripe pepper (1004.52 µgGAE/g) (Fig. 2).

Generally, the TPC in fruit pulp reduces as the fruit ripens because the phenolic compounds are synthesized in the skin of fruits (Dzah et al., 2024; Mengyuan et al., 2021; Wang et al., 2018). As the fruit matures and ripens, genetic errors caused by reactive oxygen species (ROS) may reduce the synthesis of bioactive compounds. To add to this, degradation of the cell structure of the fruit pulp such as pectin as the fruit ripens may inhibit the synthesis of antioxidant compounds, leading to the reduction in phenolic content. That is, however, not the case for fruit seeds. The phenolic content of fruit seeds increases as the fruit matures and ripens (Dzah et al., 2024). Since the whole unripe and ripe pepper were blended and used in this study, the phenolic content of the ripe pepper seeds may have been greater than that of the unripe seeds to the extent

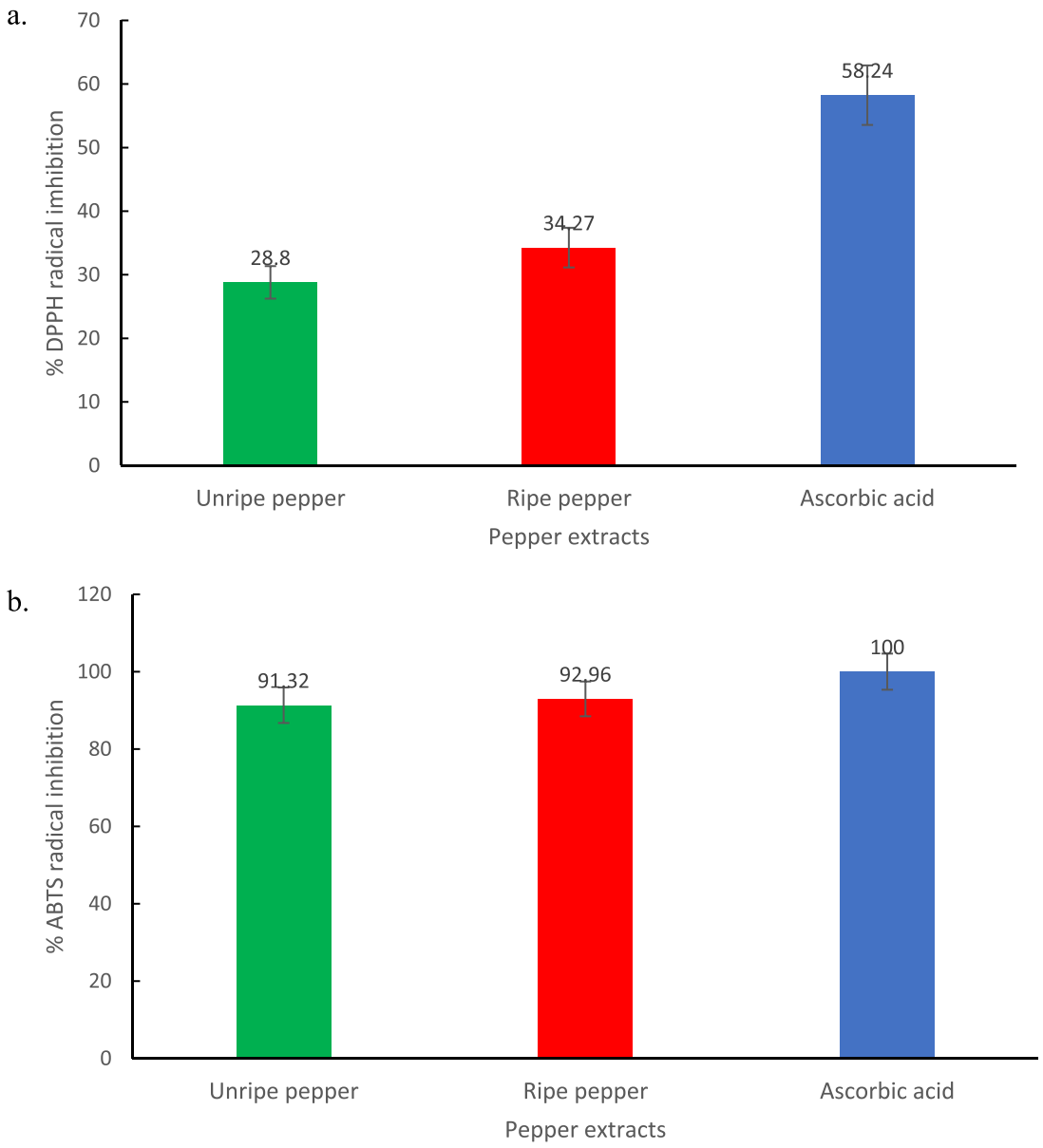

Fig. 3. a. DPPH; b. ABTS antioxidant activity of ripe and unripe pettie bell pepper.

that it greatly affected the TPC of the whole blend leading to higher TPC of ripe pepper than the unripe pepper (Fig. 2). The higher value of TPC recorded for the ripe pepper is in agreement with TPC results obtained for other pepper varieties. Sun et al. (2007) found that the TPC of ripe sweet pepper was higher (4.2 ± 0.9 9 μmol catechin equivalent/g) compared with the unripe sweet pepper (2.4 ± 0.9 μmol catechin equivalent/g) (Sun et al., 2007). Ozgur et al. (2011) also reported that the TPC of ripe pepper was higher (130.79±2.141 μgGAE/g dry weight) compared with green pepper (96.04±1.282 μgGAE/g dry weight).

It is important to state that the Folin-Ciocalteu method is not a specific test (Benavides et al., 2013) and is interfered with other compounds present in the extracts such as ascorbic acid and reducing sugars (Igbokwe et al., 2013; Martínez et al., 2009). The influence of these interferants can be reduced by (i) the partial purification of the extracts; (ii) the calculation of a corrected TPC by subtracting the ascorbic acid-reducing activity from the estimated TPC and (iii) the treatment of the extracts with oxidative agents before Folin-Ciocalteu addition, to oxidize interferants (Benavides et al., 2013).

4.2. *Radical scavenging activity*

Two of the commonly used antioxidant assays are the DPPH assay and the ABTS assay. DPPH antioxidant activity is a rapid, simple, inexpensive, and commonly used method to measure the ability of compounds to act as antioxidants in foods (Baliyan et al., 2022; Kedare & Singh, 2011). DPPH radical is a stable molecule that easily dissolves in methanol to produce a deep violet colour. Antioxidants react with the DPPH radical through electron or hydrogen transfer to reduce the DPPH radical to DPPH-H (2,2-diphenyl-1-hydrazine) characterized by pale-yellow or colorless which is detected by measuring the absorbance at 517 nm (Baliyan et al., 2022; Kedare & Singh, 2011). ABTS radical, on the other hand, is produced by the oxidation of ABTS solution in potassium persulfate to produce a deep-green colour. The ABTS radical reacts with hydrogen-donating antioxidants to convert colour of the ABTS radical to colourless which can be monitored by measuring the absorbance at 734 nm (Dong et al., 2015).

In this study, the DPPH radical scavenging activity of the ripe and unripe pepper extracts was estimated as percentage inhibition or scavenging of the DPPH free radical by each sample. The ripe pepper extract

Table 1
Correlation between total phenolic content and the antioxidant activities.

	Total Phenolic Content	ABTS	DPPH
Total Phenolic Content	1		
ABTS	1	1	
DPPH	1	1	1

Table 2
Minimum inhibitory concentration of pepper extracts against test organisms.

Test organism	MIC (mg/mL)	MBC (or MFC) (mg/mL)	MBC/MIC	Interpretation
Unripe				
E. coli	12.50	12.50	1	Bactericidal
K. pneumoniae	12.50	25.00	2	Bactericidal
S. aureus	6.25	25.00	4	Bactericidal
S. typhi	12.50	25.00	2	Bactericidal
P. aeruginosa	12.50	25.00	2	Bactericidal
C. albicans	25.00	25.00	1	Fungicidal
A. niger	12.50	12.50	1	Fungicidal
Ripe				
E. coli	12.50	12.50	1	Bactericidal
K. pneumoniae	1.56	12.50	8	Bacteriostatic
S. aureus	1.56	6.25	4	Bactericidal
S. typhi	0.02	3.13	156	Bacteriostatic
P. aeruginosa	0.08	3.13	40	Bacteriostatic
C. albicans	6.25	12.50	2	Fungicidal
A. niger	1.56	6.25	4	Fungicidal

recorded slightly higher DPPH radical scavenging activity (33.72 %) compared with the unripe pepper extract (28.2 %) (Fig. 3), although these values are not significantly different. These values are, however, significantly lower than the DPPH radical scavenging activity of 58.24 % obtained for ascorbic acid. In the case of ABTS radical scavenging activity, ripe pepper extract slightly outperformed (92.96 % radical scavenging activity) unripe pepper extract (91.32 % radical scavenging activity). Ascorbic acid (standard) recorded 100 % ABTS radical scavenging potential (Fig. 3).

However, this value is not significantly different from the values obtained for ripe and unripe pepper extracts. Thus, when compared with ascorbic acid which was used as the standard, both ripe and unripe pepper extracts are almost as powerful as ascorbic acid in scavenging ABTS radical. The slight differences observed for both DPPH and ABTS radical scavenging activities of ripe and unripe pepper extracts could be attributed to the differences in their TPC (Fig. 2). Phenolic compounds are one of the important plant compounds responsible for inhibition of radicals (antioxidant activity) (Garg et al., 2012). Thus, ripe pepper extracts with higher TPC recorded higher radical scavenging activity. Furthermore, it has been reported that during ripening unripe green fruit pulps possess a higher free radical scavenging activity compared with the ripe counterparts. However, the higher TPC in fruit seeds as the fruit ripens may have greatly influenced the TPC and consequently free radical scavenging activity (Dzah et al., 2024) of the ripe pepper blend.

4.3. Correlation between the TPC and antioxidant activity

Correlation analysis was used to establish the interrelationship between TPC and antioxidant activities (Table 1). It was found that there was a strong positive correlation between TPC and DPPH antioxidant activity ($r = 1$). This is justified as phenolic compounds function as antioxidants in biological systems. A similar relationship between TPC and antioxidant activity has been established for other fruits. For instance, a strong correlation ($r = 0.987$) was observed between TPC and DPPH antioxidant activity for noni fruit by Dzah et al. (2024) and soursop fruit ($r = 0.812$) (Osei et al., 2023). Furthermore, a strong positive correlation between ABTS and DPPH antioxidant activity ($r = 1$) was also observed.

5. Antimicrobial activity

Apart from the provision of nutrients, consumption of both unripe and ripe peppers may provide other benefits such as inhibition of microbial growth. This property was investigated by determining the MIC of both unripe and ripe pepper against some selected pathogens. Generally, there were varying levels of susceptibility of the test organisms against both unripe and ripe pepper extracts as demonstrated by different values of MIC as shown in Table 2. These values of MIC depend on the test organisms and the type of pepper (whether unripe or ripe). Specifically, the values of MIC for the test organisms are either the same for both pepper extracts or higher for unripe pepper extract (Table 2). For instance, the MIC value of 12.50 mg/mL was recorded for both unripe and ripe pepper extract against *E. coli*. When the pepper extracts were tested against *S. typhi* the MIC recorded was 0.02 mg/mL for ripe pepper and 12.50 mg/mL for unripe pepper extract (Table 2). Similarly, MIC recorded for ripe pepper extract against *P. aeruginosa* was 0.08 mg/mL, while unripe pepper extract against the same organism was 12.50 mg/mL respectively (Table 2). The differences in the MIC values may be attributed to the type and level of bioactive compounds present in both extracts. As a plant material, the pepper extracts may contain various bioactive compounds such as phenolics, flavonoids, quinones, alkaloids, terpenoids, and polystyrenes in varying concentrations (Alam et al., 2020; Alizadeh Behbahani & Imani Fooladi, 2018; Oney-Montalvo et al., 2020). These bioactive compounds have different mechanisms of action against test organisms (Alam et al., 2020). However, as the mature unripe pepper ripens the concentrations of these bioactive compounds change leading to differences in the MIC and MBC values (Oney-Montalvo et al., 2020) in Table 2. The action of the bioactive compounds depends also on the morphology of the test organisms. For instance, *S. typhi* is a Gram-negative bacterium with a protective outer membrane against antimicrobial agents (Alizadeh Behbahani & Imani Fooladi, 2018), hence its relatively higher MIC value. Furthermore, the varying values of MIC may also be attributed to extra polymeric compounds, flagella, extracellular structures, and surface hydrophobicity which are claimed to affect how different microorganisms react to various antimicrobial treatments (Ankudze et al., 2023). In addition, the treatment with the extracts may have distorted the shape and structure of the microorganisms. However, the exact effect also depends on the test organisms. For instance, *E. coli* and *S. aureus* were treated with an aqueous extract of *Calotropis procera,* and the treated organisms were examined using a scanning electron microscope (SEM) (Ahmad Nejhad et al., 2023). It was discovered that the normal rod structure of E. coli was distorted and the cell wall was damaged which increased cell permeability (Ahmad Nejhad et al., 2023). The cocci shape of *S. aureus* appeared as clusters and its cell structure was found to be damaged (Ahmad Nejhad et al., 2023). A similar reason could be attributed to the varying levels of susceptibility of fungal strains against the extracts.

To further understand the mechanism of action of the extracts against the test organisms, MBC/MIC ratio (for bacteria strains) or MFC/MIC ratio (for fungal strains) were calculated. The values obtained for these ratios showed that the mechanism of action of unripe pepper extract against the test organisms was bactericidal for all bacteria strains and fungicidal for all fungi strains (Table 2). In the case of the ripe pepper extract, the mechanism of action was bactericidal for *E. coli* and *S. aureus*; bacteriostatic against *K. pneumoniae, S. typhi,* and *P. aeruginosa* while the mode of action was fungicidal against all fungi strains (Table 2). Thus, while unripe pepper was able to kill the test organisms (bactericidal or fungicidal), ripe pepper extract was able to either kill the test organisms (bactericidal or fungicidal) or suppress their growth or reproduction (bacteriostatic).

5.1. The combinatory effect of pepper extracts with orthodox antimicrobial drugs

One of the major challenges facing human health is the prevalence of

Table 3
FIC index of unripe and ripe pepper extracts in combination with standard antibacterial agents.

Test Organism	FIC		FIC	
	Extract + Tetracycline	Interpretation	Extract + Chloramphenicol	Interpretation
	Unripe			
E. coli	0.01	Synergistic	8.10	Antagonistic
K. pneumoniae	0.07	Synergistic	4.13	No difference
S. aureus	0.02	Synergistic	4.13	No difference
S. typhi	0.07	Synergistic	2.12	No difference
P. aeruginosa	0.02	Synergistic	1.12	Additive
	Ripe			
E. coli	0.02	Synergistic	4.11	No difference
K. pneumoniae	0.13	Synergistic	1.13	Additive
S. aureus	0.04	Synergistic	1.13	Additive
S. typhi	2.56	No difference	3.50	No difference
P. aeruginosa	1.34	No difference	0.13	Synergistic

Table 4
FIC index of unripe and ripe pepper extracts in combination with standard antifungal agents.

Test Organism	FIC		FIC	
	Extract+ Nystatin	Interpretation	Extract + Fluconazole	Interpretation
Unripe				
C. albicans	4.5	Antagonistic	8.5	Antagonistic
A. niger	1.0	Additive	2.5	No difference
Ripe				
C. albicans	8.5	Antagonistic	8.5	Antagonistic
A. niger	1.0	Additive	2.3	No difference

antimicrobial resistance by microorganisms against orthodox antibiotics. This, consequently, leads to the inability of orthodox medicine to fight life-threatening infections. One of the sustainable ways to increase the efficacy of orthodox antimicrobial agents is through combination with plant extracts. Plant extracts contain several bioactive compounds with different mechanisms of action which reduce the ability of microorganisms to develop resistance against them (Alizadeh Behbahani et al., 2024; Monte et al., 2014). In this study, ripe and unripe pepper extracts were combined with standard antimicrobial agents such as tetracycline and chloramphenicol (for bacteria) and fluconazole and nystatin (for fungi) to evaluate their effects on the test organisms. The results of this study as presented in Table 3 revealed that combining unripe pepper extract with tetracycline had a synergistic effect for all test organisms (Table 3). Combining ripe pepper extract with tetracycline, on the other hand, had a synergistic effect for *E. coli, K. pneumoniae*, and *S. aureus* while no effect was observed for *S. typhi* and

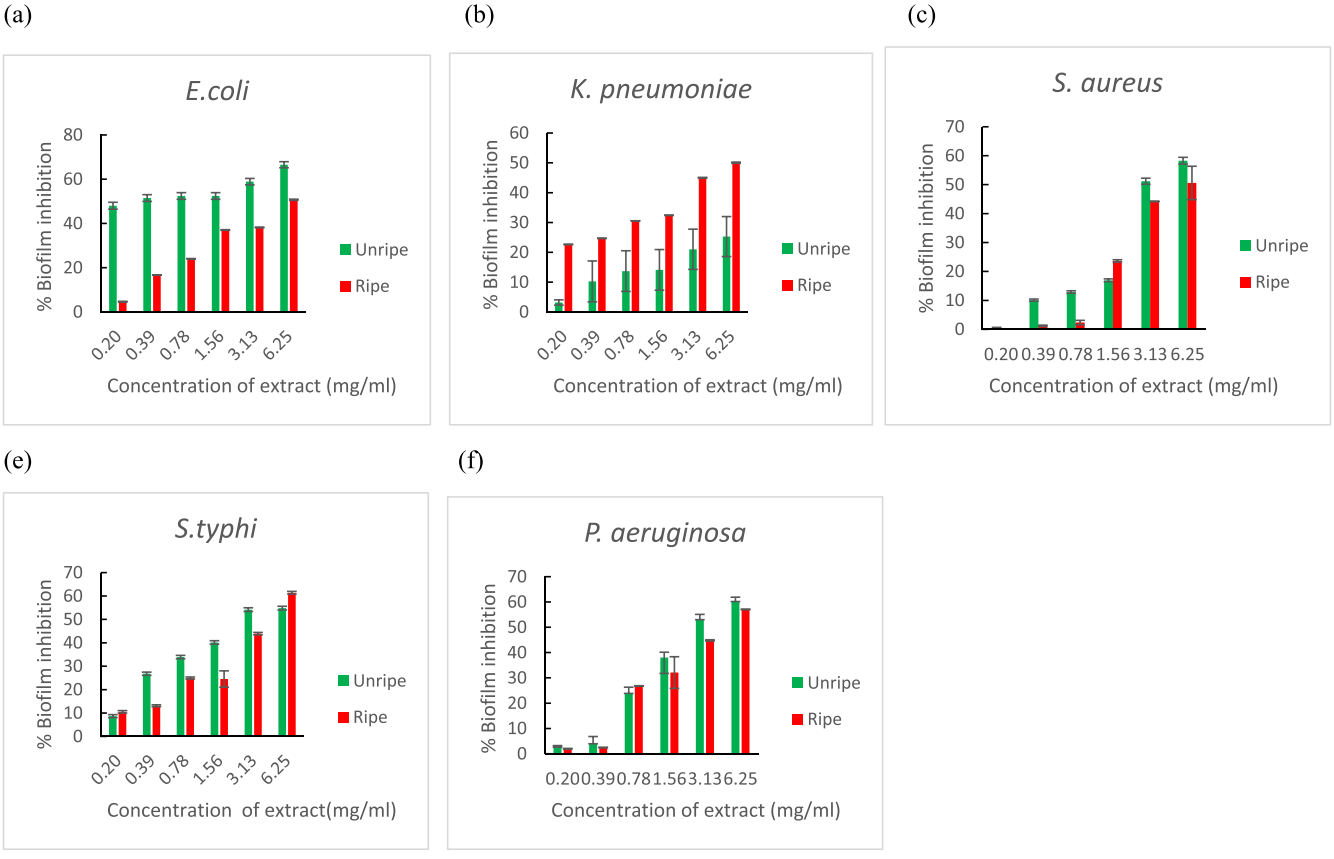

Fig. 4. Biofilm inhibition properties (a-f) of unripe and ripe pettie bell pepper against test bacteria.

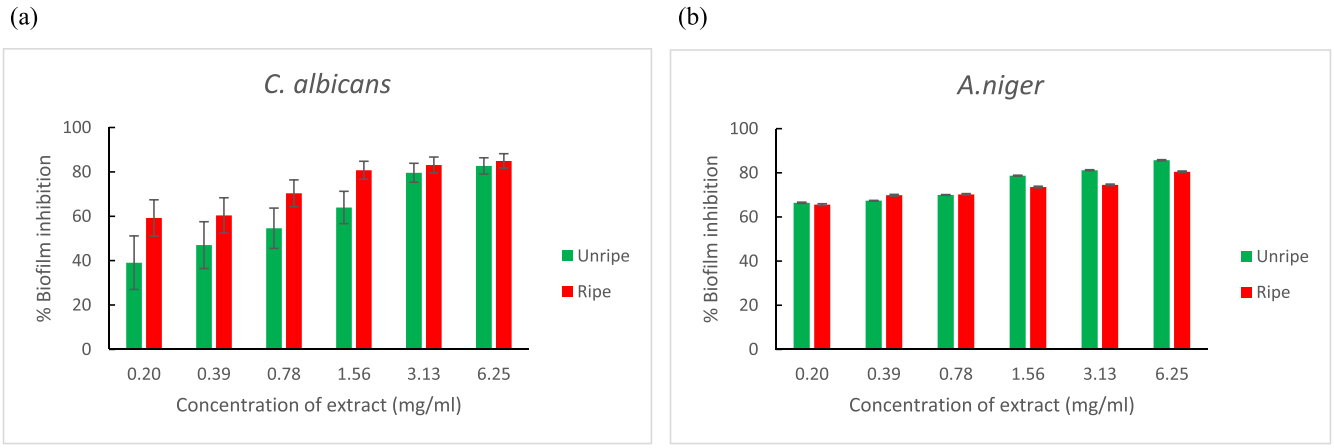

Fig. 5. Biofilm inhibition properties (a-b) of unripe and ripe pettie bell pepper against test fungi.

P. aeruginosa (Table 3).

Combining chloramphenicol with unripe pepper extract had different effects on different organisms. For instance, combining chloramphenicol with unripe pepper extract had an antagonistic effect with *E. coli*, an additive effect with *P. aeruginosa,* and no difference was observed with *K. pneumoniae, S. aureus,* and *S. typhi* (Table 3). A combination of chloramphenicol with ripe pepper extract had an additive effect with *K. pneumoniae*, and *S. aureus*, a synergistic effect with *P. aeruginosa*, and no effect was observed with *S. typhi* and *E. coli* (Table 3).

For the combinatory effect against fungi, nystatin combined with unripe pepper extract had an antagonistic effect with *C. albicans*, and an additive effect with *A. niger,* On the other hand, ripe pepper extract combined with nystatin had an antagonistic effect with C. albicans and an additive effect with *A. niger* (Table 4). Furthermore, when unripe and ripe pepper extract was combined with fluconazole, an antagonistic effect was observed with *C. albicans*, while no effect was observed with *A. niger* (Table 4).

5.2. Antibiofilm property of the extracts

Microbial biofilms are microcolonies of microbial cells attached to the surfaces with the help of extracellular polymeric substances (Kumar & Anand, 1998). The formation of biofilm is a survival strategy adopted by microorganisms. Thus, bacterial biofilms exhibit an increased resistance to antimicrobial agents than the individual actively growing cells (Carrascosa et al., 2021; Kumar & Anand, 1998). This biofilm resistance is attributed to the combination of the following mechanisms: reduced diffusion, physiological changes due to reduced growth rates, and the production of enzymes degrading antimicrobial substances (Kumar & Anand, 1998). Therefore, it is important to determine to what extent the pepper extract can inhibit the growth of microbial biofilms. As shown in Figs. 4 & 5, biofilm inhibition of both unripe and ripe pepper extracts increased with increased concentration of the extracts. However, the degree of inhibition varies with the type of test organism and the type of pepper extract (whether ripe or unripe). For instance, unripe pepper extract significantly outperformed the ripe pepper extract in inhibition of *E. coli* biofilm formation (Fig. 4). The percentage *E. coli* biofilm inhibition increased from 5 % for 0.2 mg/mL extract to 51 % for 6.25 mg/mL extract and 48 % for 0.2 mg/mL extract to 67 % mg/mL for 6.25 mg/mL extract, respectively for ripe and unripe pepper extract (Fig. 4). Similar observation could be made for *S. aureus, S. typhi*, and *P. aeruginosa* biofilms for all levels of concentration (Fig. 4). This is, however, not the case for *K. pneumoniae* biofilm inhibition where the ripe pepper extract performed better in inhibiting *K. pneumoniae* biofilm compared with the unripe pepper extract. The percentage *K. pneumoniae* biofilm inhibition ranged from 23 % for 0.2 mg/mL extract to 50 % for 6.25 mg/mL extract and 3 % for 0.2 mg/mL extract to 23 % mg/mL for 6.25 mg/mL extract, respectively for ripe and unripe pepper extract (Fig. 4). Several factors could account for variations in biofilm inhibition by the pepper extracts against the test bacteria strains (Borges et al., 2016). The morphology of the biofilms affects their susceptibility to inhibition by the phytochemicals (Monte et al., 2014). The denser the biofilm the more difficult it is for the phytochemicals to penetrate the biofilm to inhibit or destroy it. For instance, *S. aureus* biofilms are denser than *E. coli* biofilm, hence lower percentage inhibition was obtained for S. aureus compared with E. coli (Fig. 4). Furthermore, phytochemicals showed higher potential to prevent and control biofilms formed by Gram-negative bacteria compared to Gram-positive (Monte et al., 2014). This is because Gram-negative bacteria have an outer membrane containing lipopolysaccharide that makes them less susceptible to antimicrobials than Gram-positive bacteria (Monte et al., 2014). In addition, the type of phytochemicals in both unripe and ripe pepper extract may also explain why unripe pepper extract performed better in inhibiting *E. coli, S. aureus, S. typhi*, and *P. aeruginosa* biofilms. In the case of *K. pneumoniae* biofilm, the transition of the pepper fruit from unripe to ripe may have caused some biochemical changes in the fruit leading to the production of some bioactive compounds in levels that are higher than the unripe pepper to inhibit *K. pneumoniae* biofilm (Oney-Montalvo et al., 2020). Another factor that may have contributed to the variation in biofilm inhibition of the test bacteria strains is the variation in the ability of each test bacteria strain to disseminate resistant genes against phytochemicals (Olawuwo et al., 2022). Consequently, different levels of biofilm inhibition were observed for each bacteria strain.

Like the inhibition of bacterial biofilm formation, inhibition fungal biofilm formation increased with the concentration of the pepper extracts. However, the ability of the extract to inhibit the formation of fungal biofilm is higher compared with the percentage inhibition of bacterial biofilm (Fig. 4 & 5). The inhibition of fungal biofilm also varies with the type of fungal strain and the ripening stage. For instance, the unripe pepper extract recorded between 66 % (for 0.2 mg/mL extract) and 86 % (for 6.25 mg/mL extract), while ripe pepper extract recorded between 66 for 0.2 mg/mL extract and 80 % (for 6.25 mg/mL extract) *A. niger* biofilm inhibition. The performance of unripe pepper extract against *C. albicans* is, however, lower compared with the other fungal strains. The unripe pepper extract inhibited the formation of between 39 % (for 0.2 mg/mL extract) and 83 % (for 6.25 mg/mL extract) *C. albicans* biofilm while the ripe pepper extract inhibited between 59 % (for 0.2 mg/mL extract) and 85 % (for 6.25 mg/mL extract) *C. albicans* biofilm formation. The variation in values of antifungal biofilm formation may be due to the morphology of the fungal strains involved and the levels of bioactive compounds in each pepper extract (Monte et al., 2014; Oney-Montalvo et al., 2020). Thus, both pepper extracts demonstrated a higher potential to inhibit fungal biofilm compared with bacterial

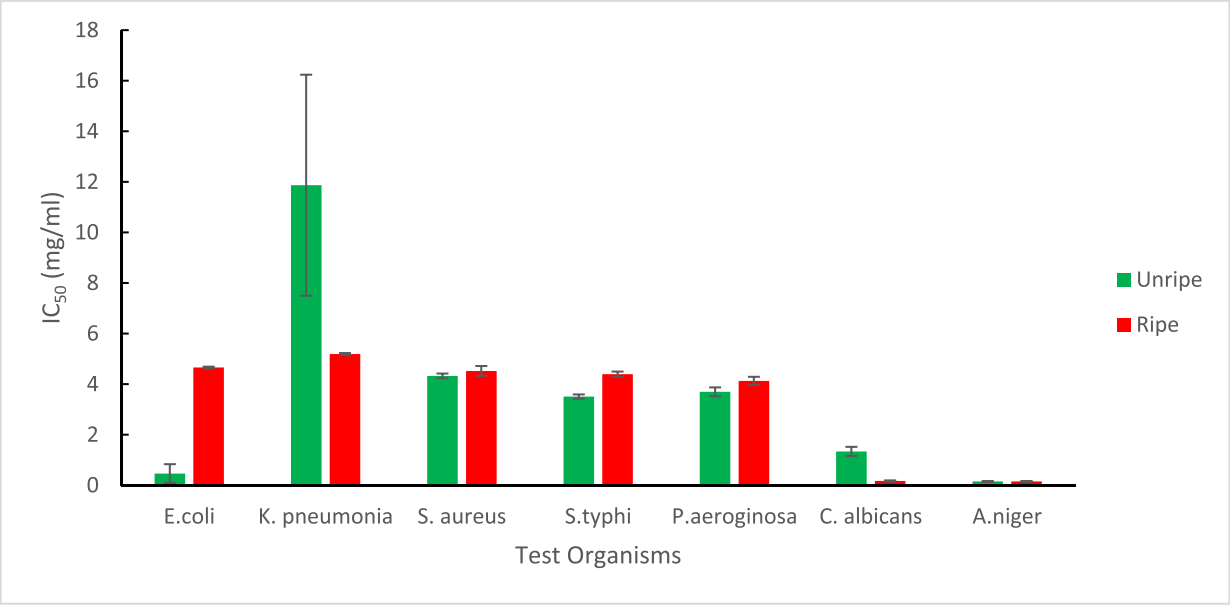

Fig. 6. IC_{50} for unripe and ripe pepper extracts against test organisms.

biofilm (Figs. 4 & 5). In conclusion, the result of the antibiofilm study showed that the use of pepper in food preparation could confer antimicrobial properties on the food since pepper possesses antimicrobial activities with the ability to inhibit or kill the pathogens that may be with biofilm-producing ability thereby helping to reduce the menace of foodborne diseases in most local, underprivileged communities

To determine the potency of the pepper extract to inhibit the formation of biofilm, half-maximal inhibitory concentration (IC_{50}) was calculated using a linear regression model. The lower the IC_{50} value more potent the antimicrobial agent (Berrouet et al., 2020). As shown in Fig. 6, both unripe and ripe pepper extracts showed more potency in inhibiting fungal biofilms than bacteria biofilms. Furthermore, among the bacteria strains, unripe pepper extract was more potent in *E. coli* biofilm inhibition due to its lower IC_{50} value compared with the ripe pepper extract (Fig. 6). On the other hand, among the test organisms unripe pepper extract showed less potency to inhibit *K. pneumonia* biofilm due to its higher IC_{50} value compared with the ripe pepper extract (Fig. 5).

6. Conclusion

Both ripe and unripe pettie bell peppers (*Capsicum chinense*) are utilized in dishes in Ghana and other African countries. The ripening stage may affect the biological properties derived from their consumption. This study sought to find the effect of the ripening stage (ripe or unripe) on the TPC, and antioxidant activity derived from the consumption of pettie bell pepper. This study has shown that beyond the nutritional considerations in choosing between ripe and unripe peppers, their antimicrobial and antioxidant properties must also be considered. The TPC and antioxidant activity are higher in ripe peppers compared with unripe peppers. In the case of antioxidants, extracts from ripe pepper would be the best choice due to its high radical scavenging activity compared with the unripe pepper. Furthermore, the choice between ripe and unripe for use as an antimicrobial agent depends on the application. For food preservation purposes, the ripe pepper extract may be more applicable as a food preservative due to its low MIC values. In the case of getting rid of biofilm on food surfaces, the choice of ripe or unripe pepper extract depends on the target microorganism. For instance, unripe pepper extract would be more appropriate for *E. coli* biofilm while ripe pepper extract would be the best choice for *K. pneumoniae* biofilm. Findings from this study would also benefit the pharmaceutical industry as it can help develop a new pepper-based cure for *candida* infections, as well as other antimicrobial drugs against other pathogens.

Recommendation for future research

The peppers used in this study were not subjected to thermal treatment. Studies have proven that thermal treatment could influence the content of bioactive compounds present in the pepper. Since peppers are one of the main ingredients utilized in making sauces, it would be important to determine the effect of the thermal treatment of both ripe and unripe peppers on their biological activities. Therefore, it is recommended that future research should explore the effects of various thermal treatment methods on the biological activities of both the ripe and unripe pepper. In addition, studies should be done with more food pathogens to determine the extent to which the extracts could function as food preservatives.

CRediT authorship contribution statement

Aaron Dzigbor: Writing – original draft, Supervision, Conceptualization. **David Neglo:** Writing – review & editing, Methodology, Investigation. **Clement O. Tettey:** Writing – review & editing, Investigation, Formal analysis. **Frank Nsaful:** Writing – review & editing, Formal analysis, Data curation. **Francisca Adzaho:** Methodology, Investigation. **Esther Mawusey Botchway:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Aberoumand, A., & Deokule, S.S. (2008). Comparison of phenolic compounds of some edible plants of Iran and India. 7(4), 582–585.

Ahmad Nejhad, A., Alizadeh Behbahani, B., Hojjati, M., Vasiee, A., & Mehrnia, M. A. (2023). Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of Calotropis procera leaf aqueous extract. *Scientific Reports, 13*(1), 1–13. https://doi.org/10.1038/s41598-023-42086-1

Alam, K., Farraj, D. A. A., Mah-e-Fatima, S., Yameen, M. A., Elshikh, M. S., Alkufeidy, R. M., Mustafa, A. E. Z. M. A., Bhasme, P., Alshammari, M. K., Alkubaisi, N. A., Abbasi, A. M., & Naqvi, T. A. (2020). Anti-biofilm activity of plant derived extracts against infectious pathogen-Pseudomonas aeruginosa PAO1. *Journal of Infection and Public Health, 13*(11), 1734–1741. https://doi.org/10.1016/j.jiph.2020.07.007

Alizadeh Behbahani, B., & Imani Fooladi, A. A. (2018). Antibacterial activities, phytochemical analysis and chemical composition Makhlaseh extracts against the growth of some pathogenic strain causing poisoning and infection. *Microbial Pathogenesis, 114*, 204–208. https://doi.org/10.1016/j.micpath.2017.12.002 (December 2017).

Alizadeh Behbahani, B., Noshad, M., Falah, F., Zargari, F., Nikfarjam, Z., & Vasiee, A (2024). Synergistic activity of Satureja intermedia and Ducrosia anethifolia essential oils and their interaction against foodborne pathogens: A multi-ligand molecular docking simulation. *LWT, 205*, Article 116487. https://doi.org/10.1016/j.lwt.2024.116487 (July).

Aljaloud, S. O., Gyawali, R., Reddy, M. R., & Ibrahim, S. A. (2012). Antibacterial activity of red bell pepper against Escherichia coli O157:H7 in ground beef. *Internet Journal of Food Safety, 14*, 44–47.

Anderson, D. G., Salm, S. N., Allen, D. P., & Nester, E. W. (2018). *Nester's microbiology a human perspective* (9th Editio). McGraw-Hill Education.

Ankudze, B., Neglo, D., & Nsiah, F. (2023). Green synthesis of silver nanoparticles from discarded shells of velvet tamarind (Dialium cochinchinense) and their antimicrobial synergistic potentials and biofilm inhibition properties. *BioMetals*. https://doi.org/10.1007/s10534-023-00534-5, 0123456789.

Ayisi, N. K., Appiah-Opong, R., Gyan, B., Bugyei, K., & Ekuban, F. (2011). Plasmodium falciparum : Assessment of selectivity of action of chloroquine, Alchornea cordifolia, Ficus polita, and other drugs by a tetrazolium-based colorimetric assay. *Malaria Research and Treatment, 2011*, 1–7. https://doi.org/10.4061/2011/816250

Bacon, K., Boyer, R., Denbow, C., O'Keefe, S., Neilson, A., & Williams, R. (2017). Evaluation of different solvents to extract antibacterial compounds from jalapeño peppers. *Food Science and Nutrition, 5*(3), 497–503. https://doi.org/10.1002/fsn3.423

Bae, H., Jayaprakasha, G. K., Jifon, J., & Patil, B. S. (2012). Extraction efficiency and validation of an HPLC method for flavonoid analysis in peppers. *Food Chemistry, 130*(3), 751–758. https://doi.org/10.1016/j.foodchem.2011.07.041

Baenas, N., Belović, M., Ilic, N., Moreno, D. A., & García-Viguera, C. (2019). Industrial use of pepper (Capsicum annum L.) derived products: Technological benefits and biological advantages. *Food Chemistry, 274*, 872–885. https://doi.org/10.1016/j.foodchem.2018.09.047 (April 2018).

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules, 27*(4). https://doi.org/10.3390/molecules27041326

Barbero, G. F., Ruiz, A. G., Liazid, A., Palma, M., Vera, J. C., & Barroso, C. G. (2014). Evolution of total and individual capsaicinoids in peppers during ripening of the Cayenne pepper plant (Capsicum annuum L.). *Food Chemistry, 153*, 200–206. https://doi.org/10.1016/j.foodchem.2013.12.068

Benavides, J., Heredia, J. B., Cisneros-, L., & S, J. C (2013). The Folin – Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods, 5*, 5990–5999. https://doi.org/10.1039/c3ay41125g

Berrouet, C., Dorilas, N., Rejniak, K. A., & Tuncer, N. (2020). Comparison of drug inhibitory effects (IC 50) in monolayer and spheroid cultures. *Bulletin of Mathematical Biology, 82*(6). https://doi.org/10.1007/s11538-020-00746-7

Borges, A., Abreu, A. C., Dias, C., Saavedra, M. J., Borges, F., & Simões, M. (2016). New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms. *Molecules, 21*, 877. https://doi.org/10.3390/molecules21070877

Cao, S., Chen, H., Xiang, S., Hong, J., Weng, L., & Zhu, H. (2015). Anti-cancer effects and mechanisms of capsaicin in chili peppers. December, 3075–3081.

Careaga, M.ónica, Fernández, E., Dorantes, L., Mota, L., Jaramillo, M. E., & Hernandez-Sanchez, H. (2003). Antibacterial activity of Capsicum extract against Salmonella typhimurium and Pseudomonas aeruginosa inoculated in raw beef meat. *International Journal of Food Microbiology, 83*(3), 331–335. https://doi.org/10.1016/S0168-1605(02)00382-3

Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., & Raposo, A. (2021). Microbial biofilms in the food industry—A comprehensive review. *International Journal of Environmental Research and Public Health, 18*(4), 1–31. https://doi.org/10.3390/ijerph18042014

Chinn, M. S., Sharma-Shivappa, R. R., & Cotter, J. L. (2011). Solvent extraction and quantification of capsaicinoids from Capsicum chinense. *Food and Bioproducts Processing, 89*(4), 340–345. https://doi.org/10.1016/j.fbp.2010.08.003

Clinical and Laboratory Standards Institute. (2020). *Standards for antimicrobial susceptibility testing* (30th ed.). Clinical and Laboratory Standards Institute.

Dickson, R. A., Houghton, P. J., Hylands, P. J., & Gibbons, S. (2006). Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of Mezoneuron benthamianum Baill., Securinega virosa Roxb. & Wlld. and Microglossa pyrifolia Lam. *Phytotherapy Research, 20*(1), 41–45. https://doi.org/10.1002/ptr.1799

Doğan, A. N. C., Çelik, E., Kiliçle, P. A., Atalay, E., Sağlam, A. G., Doğan, A., & Otlu, S. (2018). Antibacterial effect of hot peppers (capsicum annuum, capsicum annuum var globriusculum, capsicum frutescens) on some arcobacter, campylobacter and helicobacter species. *Pakistan Veterinary Journal, 38*(3), 266–270. https://doi.org/10.29261/pakvetj/2018.057

Dong, J. W., Cai, L., Xing, Y., Yu, J., & Ding, Z. T. (2015). Re-evaluation of ABTS·+ assay for total antioxidant capacity of natural products. *Natural Product Communications, 10*(12), 2169–2172. https://doi.org/10.1177/1934578x1501001239

Dravie, E. E., Kortei, N. K., Essuman, E. K., Tettey, C. O., Boakye, A. A., & Hunkpe, G. (2020). Antioxidant, phytochemical and physicochemical properties of sesame seed (Sesamum indicum L). *Scientific African, 8*(March), e00349. https://doi.org/10.1016/j.sciaf.2020.e00349

Dumitrache, M.-D., Jieanu, A., Scheau, C., Badarau, I., Popescu, G., Caruntu, A., Costache, D., Costache, R., Constantin, C., Neagu, M., & Caruntu, C. (2021). Comparative effects of capsaicin in chronic obstructive pulmonary disease and asthma (Review). *Experimental and Therapeutic Medicine, 22*(3), 1–12. https://doi.org/10.3892/etm.2021.10349

Dzah, C. S. (2022). Optimized ultrasound-assisted recovery, HPLC/LC-MS identification and biological activities of Tetrapleura tetraptera L. dry fruit polyphenols. *Food Chemistry Advances, 1*(July), Article 100093. https://doi.org/10.1016/j.focha.2022.100093

Dzah, C. S., Kpodo, F. M. K., Asante-Donyinah, D., & Boateng, N. A. S (2024). The influence of Morinda citrifolia fruit maturity level, parts and storage length on total phenols, ascorbic acid, antioxidant activity and ethylene gas emission. *Food Chemistry Advances, 4*, Article 100599. https://doi.org/10.1016/j.focha.2023.100599 (December 2023).

Echegaray, N., Pateiro, M., Munekata, P. E., & Lorenzo, J. M. (2021). Measurement of antioxidant capacity of meat and meat products: Methods and applications. *Molecules*. https://doi.org/10.3390/molecules26133880. June.

Frank, C. A., Nelson, R. G., Simonne, E. H., Behe, B. K., & Simonne, A. H. (2001). Consumer preferences for color, price, and vitamin C content of bell peppers. *HortScience, 36*(4), 795–800. https://doi.org/10.21273/hortsci.36.4.795

Garg, D., Shaikh, A., Muley, A., & Marar, T. (2012). Original Article In-vitro antioxidant activity and phytochemical analysis in extracts of Hibiscus rosa-sinensis stem and leaves. 2(3), 41–46. https://doi.org/10.5530/ax.2012.3.6.

Hwang, I. G., Shin, Y. J., Lee, S., Lee, J., & Yoo, S. M. (2012). Effects of different cooking methods on the antioxidant properties of red pepper (Capsicum annuum L.). *Preventive Nutrition and Food Science, 17*(4), 286–292. https://doi.org/10.3746/pnf.2012.17.4.286

Igbokwe, G., Aniakor, G., & Anagonye, C. (2013). Determination of β–carotene & vitamin C content of fresh green pepper (Capsicum annnum), fresh red pepper (Capsicum annum) and fresh tomatoes (Solanumly copersicum) fruits. *The Bioscientist, 1*(1), 89–93.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology, 48*(August), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Keser, S., Kaygili, O., Keser, F., Tekin, S., Yilmaz, Ö., Demir, E., Kirbag, S., & Sandal, S. (2018). Phytochemical composition, antiradical, antiproliferative and antimicrobial activities of Capsicum frutescens L. *Analytical Chemistry Letters, 8*(5), 642–652. https://doi.org/10.1080/22297928.2018.1485511

Khodavandi, A., Alizadeh, F., Aala, F., Sekawi, Z., & Chong, P. P. (2010). In vitro investigation of antifungal activity of allicin alone and in combination with azoles against Candida species. *Mycopathologia, 169*(4), 287–295. https://doi.org/10.1007/s11046-009-9251-3

Koffi-Nevry, R., Kouassi, K. C., Nanga, Z. Y., Koussémon, M., & Loukou, G. Y. (2012). Antibacterial activity of two bell pepper extracts: Capsicum annuum L. and Capsicum frutescens. *International Journal of Food Properties, 15*(5), 961–971. https://doi.org/10.1080/10942912.2010.509896

Kumar, C. G., & Anand, S. K. (1998). Significance of microbial biofilms in food industry: A review. *International Journal of Food Microbiology, 42*(1–2), 9–27. https://doi.org/10.1016/S0168-1605(98)00060-9

Loizzo, M. R., Pugliese, A., Bonesi, M., Menichini, F., & Tundis, R. (2015). Evaluation of chemical profile and antioxidant activity of twenty cultivars from Capsicum annuum, Capsicum baccatum, Capsicum chacoense and Capsicum chinense: A comparison between fresh and processed peppers. *LWT, 64*(2), 623–631. https://doi.org/10.1016/j.lwt.2015.06.042

Martínez-Ispizua, E., Martínez-Cuenca, M. R., Marsal, J. I., Díez, M. J., Soler, S., Valcárcel, J. V., & Calatayud, Á. (2021). Bioactive compounds and antioxidant capacity of Valencian pepper landraces. *Molecules, 26*(4), 1–23. https://doi.org/10.3390/molecules26041031

Martínez, S., López, M., González-Raurich, M., & Alvarez, B. (2009). The effects of ripening stage and processing systems on vitamin C content in sweet peppers (Capsicum annuum L .). *International Journal of Food Sciences and Nutrition ISSN, 56*(1), 45–51. https://doi.org/10.1080/09637480500081936

Mengyuan, W., Haoli, W., Tingting, M., Qian, G., Yulin, F., & Xiangyu, S. (2021). Comprehensive utilization of thinned unripe fruits from horticultural crops. *Foods, 10*(2043).

Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial activity of selected phytochemicals against Escherichia coli and Staphylococcus aureus and their biofilms. *Pathogens, 3*, 473–498. https://doi.org/10.3390/pathogens3020473

Morales-Soriano, E., Panozzo, A., Ugás, R., Grauwet, T., Van Loey, A., & Hendrickx, M. (2019). Carotenoid profile and basic structural indicators of native Peruvian chili peppers. *European Food Research and Technology, 245*(3), 717–732. https://doi.org/10.1007/s00217-018-3193-2

Odame, F., Madanhire, T., Tettey, C., Neglo, D., Adzaho, F., Sedohia, D., & Hosten, E. C. (2023). Anticancer and antimicrobial activity of some new 2,3-dihydro-1,5-benzodiazepine derivatives. *Heteroatom Chemistry, 2023*. https://doi.org/10.1155/2023/3390122

Olawuwo, O. S., Famuyide, I. M., & Mcgaw, L. J. (2022). Antibacterial and antibiofilm activity of selected medicinal plant leaf extracts against pathogens implicated in poultry diseases. *Frontiers in Vetinary Science, 9*(March). https://doi.org/10.3389/fvets.2022.820304

Oney-Montalvo, J. E., Avilés-Betanzos, K. A., Ramírez-Rivera, Jesús, De, E., Ramírez-Sucre, M. O., & Rodríguez-Buenl, I. M. (2020). Polyphenols content in capsicum chinense fruits at different harvest times and their correlation with the antioxidant activity. *Plants, 9*(10), 1–15. https://doi.org/10.3390/plants9101394

Osei, P. A., Kpodo, F. M., Tettey, C. O., Dzah, C. S., Agbenorhevi, J. K., Hunkpe, G., & Nanga, S. (2023). Proximate composition, phenolic, and antioxidant properties of soursop fruit parts. *CYTA - Journal of Food, 21*(1), 475–480. https://doi.org/10.1080/19476337.2023.2227250

Ozgur, M., Ozcan, T., Akpinar-Bayizit, A., & Yilmaz-Ersan, L. (2011). Functional compounds and antioxidant properties of dried green and red peppers. *African Journal of Agricultural Research, 6*(25), 5638–5644. https://doi.org/10.5897/AJAR11.709

Palma-Orozco, G., Orozco-Álvarez, C., Chávez-Villeda, A. A., Mixtega-Martínez, A., & Castro-Muñoz, R. (2021). Capsaicin content in red habanero chilli (Capsicum chinense Jacq.) and its preservation after drying process. *Future Foods, 4*(August), Article 100070. https://doi.org/10.1016/j.fufo.2021.100070

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science, 4*(2), 89–96.

Pierce, C. G., Uppuluri, P., Tummala, S., & Lopez-Ribot, J. L. (2010). A 96 well microtiter plate-based method for monitoring formation and antifungal susceptibility testing of Candida albicans biofilms. *Journal of Visualized Experiments, 44*, 1–4. https://doi.org/10.3791/2287

Pino, J., González, M., Ceballos, L., Centurión-Yah, A. R., Trujillo-Aguirre, J., Latournerie-Moreno, L., & Sauri-Duch, E. (2007). Characterization of total capsaicinoids, colour and volatile compounds of Habanero chilli pepper (Capsicum chinense Jack.) cultivars grown in Yucatan. *Food Chemistry, 104*(4), 1682–1686. https://doi.org/10.1016/j.foodchem.2006.12.067

Santos, L. S., Fernandes, C. C., Santos, L. S., Candido, A., Magalhães, L. G., Andrade, G., Pires, R. H., & Miranda, M. L. D. (2022). Phenolic compounds and biological activities of ethanolic extract from Capsicum chinense unripe fruit (var. bode pepper). *Mediterranean Journal of Chemistry, 12*(1), 31–37. https://doi.org/10.13171/mjc02205121623miranda

Santos, L. S., Fernandes, C. C., Santos, L. S., de DEUS, I. P. B., de SOUSA, T. L., & Miranda, M. L. D (2021). Ethanolic extract from capsicum chinense jacq. Ripe fruits: Phenolic compounds, antioxidant activity and development of biodegradable films. *Food Science and Technology, 41*(2), 497–504. https://doi.org/10.1590/fst.08220

Specialty Produce. (n.d.). Green Kpakpo Chile peppers information, Recipes and Facts. Retrieved February 3, 2024, from https://specialtyproduce.com/produce/Green_Kpakpo_Chile_Peppers_17555.php#:~:text=Green Kpakpo Shito peppers are,and then red with maturity.

Staveckienė, J., Kulaitienė, J., Levickienė, D., Vaitkevičienė, N., & Vaštakaitė-Kairienė, V. (2023). The effect of ripening stages on the accumulation of polyphenols and antioxidant activity of the fruit extracts of solanum species. *Plants, 12*(14). https://doi.org/10.3390/plants12142672

Sun, T., Xu, Z., Wu, C. T., Janes, M., Prinyawiwatkul, W., & No, H. K. (2007). Antioxidant activities of different colored sweet bell peppers (Capsicum annuum L.). *Journal of Food Science, 72*(2), 98–102. https://doi.org/10.1111/j.1750-3841.2006.00245.x

Valková, V., Ďúranová, H., Ivanišová, E., Galovičová, L., Godočíková, L., Borotová, P., Kunová, S., Miklášová, K., Lopašovský, Ľ., Mňahončáková, E., & Kačániová, M. (2021). Antioxidant and antimicrobial activities of fruit extracts from different fresh chili peppers. *Acta Scientiarum Polonorum, Technologia Alimentaria, 20*(4), 465–472. https://doi.org/10.17306/J.AFS.2021.0997

Walker, R. B. (2014). A thorough study of reactivity of various compound classes towards the Folin-Ciocalteu. *Journal of Agricultural and Food Chemistry, 58*(14), 8139–8144. https://doi.org/10.1021/jf1005935.A

Wang, Y., Qi, D., Wang, S., Cao, X., Ye, Y., & Suo, Y. (2018). Comparison of phenols content and antioxidant activity of fruits from different maturity stages of Ribes stenocarpum maxim. *Molecules, 23*(12). https://doi.org/10.3390/molecules23123148

Xia, J., Gu, L., Guo, Y., Feng, H., Chen, S., Jurat, J., Fu, W., & Zhang, D. (2021). Gut microbiota mediates the preventive effects of dietary capsaicin against depression-like behavior induced by lipopolysaccharide in mice. *Frontiers in Cellular and Infection Microbiology, 11*(April), 1–13. https://doi.org/10.3389/fcimb.2021.627608

Yap, E. S. P., Uthairatanakij, A., Laohakunjit, N., Jitareerat, P., Vaswani, A., Magana, A. A., Morre, J., & Maier, C. S. (2021). Plant growth and metabolic changes in 'Super Hot' chili fruit (Capsicum annuum) exposed to supplemental LED lights. *Plant Science, 305*(January), Article 110826. https://doi.org/10.1016/j.plantsci.2021.110826