[AMAI#117]

Antibacterial Activities and Efficacy of Carica Papaya Seed Extract on Fragaria x Ananassa (Strawberry)

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ABSTRACT

This study examines the antibacterial properties, chemical composition, and effectiveness of the extract Carica papaya seed (PS) var. Sekaki/Hongkong. The Carica papaya seed was extracted using two different solvents (water and ethanol), and different methods of extraction; maceration-filtration (F) and maceration-centrifuge-filtration (C). The extracts were tested for antibacterial properties against eight Gram-positive and four Gram-negative microorganisms. The most potent extract was subsequently subjected to phenolic and flavonoid assays and an efficacy evaluation on actual food, i.e.Fragaria x ananassa (Strawberry). Carica papaya seed extract using water (PSW) showed moderate antibacterial activities against L. monocytogenes and S. aureus, while ethanolic extract (PSE) exhibited high antibacterial activities against all Gram-positive bacteria. Therefore, the PSE was chosen as the most effective extract to inhibit the tested bacteria. Filtered PSE (PSEF) inhibited B. cereus, C. diphtheria, L. monocytogenes and S. aureus with a minimum inhibitory concentration (MIC) between 2.813 – 5.625 mg/mL. The PSE's total phenolic (TPC) and total flavonoid content (TFC) ranged between 32.183 to 42.725 mg GAE/g DW and 0.118 to 0.142 mg QE/g DW, respectively. The optical density measuring method was used in this study to assess the efficacy of the PSEF against C. diphtheria, L. monocytogenes and S. aureus on Fragaria x ananassa (Strawberry) on refrigerated temperatures. The outcome showed that the PSEF could efficiently inhibit the tested microorganisms.

Keywords: Carica papaya seed, Antibacterial activities, Bioactive compound, Food efficacy

1 Introduction

Every year, 1.3 billion tonnes of food are wasted, significantly contributing to the increase in recent years. Due to the gas emissions linked to its production, food waste raises home greenhouse gas emissions. Fruit industrial by-products are the most prevalent type of food waste and might comprise leaves, peels, seeds, pulp, or a combination. On the other hand, fruit by-products are abundant in beneficial bioactive chemicals, which justifies their usage as food additives and preservatives to maintain food quality over time (Nardella et al., 2022).

DOI: 10.21467/abstracts.155 ISBN: 978-81-961472-9-7 (eBook)

Papaya contains black in colour of papaya seeds that are embedded in it. Typically, the seed from ripe papaya constitutes around 16% of the weight of the fresh fruit and is regarded as a by-product (Sugiharto, 2020). According to a previous finding (Subandi & Nurowidah, 2019), several pathogenic bacteria, including *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were reported to be susceptible to the antibacterial properties of papaya seeds. Since there has no study has been done to investigate the effectiveness of *Carica papaya* seed extract on fresh fruit as it has the potential as a food preservative. Therefore, the purpose of this study was to examine the antibacterial activities and chemical components of *Carica papaya* seed extract and the effectiveness of *Carica papaya* seed extract on actual food, *Fragaria x ananassa* (Strawberry). This discovery is expected to make it easier to find antibacterial chemicals from plant leftovers that could be used as food preservatives.

2 Materials and Methods

2.1 Plant Material

Carica papaya seed (PS) var. Sekaki/Hongkong was bought from a fruit shop at International Islamic University Malaysia (IIUM). The fruit's seed was washed to remove the attached leftover pulps and underwent freeze-drying for 72 hours to ensure the seed was completely dried. The dried seed was ground into a fine powder and then kept in a tightly sealed container away from light until it was needed.

2.2 Disk Diffusion Test (DDT)

Carica papaya seed extracts (PSEs) were tested for antibacterial activity using DDT referred to Amanina et al. (2022) with slight modification. This approach was consistent with the approach that the Clinical and Laboratory Standards Institute has standardised. The 0.1g of PSEs were diluted in 0.1g/mL extract solution of dimethyl sulfoxide (DMSO) and then filtered through a 0.22m PVDF syringe filter. The antibacterial activity of PSEs was screened against eight bacteria which included *Bacillus cereus* (ATCC 10875), *Corynebacterium diphtheria* (ATCC 13812), *Listeria monocytogenes* (ATCC 19111), *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11229), *Proteus mirabilis* (ATCC 12453), *Salmonella typhimurium* (ATCC 13311), and *Vibrio vulnificus* (ATCC 27562).

2.3 Effect of incorporation of crude Carica papaya seed extract on Fragaria x ananassa

The effect of the incorporation of PSEF was determined by following the method of (O et al., 2019) with slight modification. The sample was tested on total bacteria count with different concentrations of ethanolic *Carica papaya* seed extract, 25.01, 1.563, and 0.098 mg/g.

ISBN: 978-81-961472-9-7

Series: AIJR Abstracts

DOI: 10.21467/abstracts.155

3 Results and Discussion

According to Shirwan et al. (2017), the inhibition zone classification was divided into three ranges: inhibition zone < 3mm indicated slight antibacterial activity, inhibition zone between ≤ 3mm and < 4mm expressed moderate antibacterial activity and inhibition zone ≥ 4 mm determined as strong antibacterial activity. As shown in Table 1 and Table 2, the outcomes showed PSEF as the best extract among others. It can inhibit six bacteria including *B. cereus, C. diphtheria*, *L. monocytogenes, S. aureus, E. coli,* and *S. typhimurium* followed by PSWCB and PSECA as second rank (five bacteria) and PSWF, PSWCA and PSECB (four bacteria).

Table 1: *Inhibition zone of Carica papaya seed extracts on gram-positive food microorganisms by different solvents and extractions methods.*

Method & Solvents	**Inhibition zone on gram-positive bacteria [1,2,3] (mm)**			
	B. cereus	*C. diphtheria*	*L. monocytogenes*	*S. aureus*
PSWF[4]	1.33 ± 0.5 ^{c} A	nd ^{c}	nd ^{e}	nd ^{e}
PSWCA[5]	0.33 ± 0.50 ^{cd} A	nd ^{c}	1.00 ± 0.00 ^{de} A	1.00 ± 0.00 ^{de} A
PSWCB[6]	0.67 ± 1.00 ^{cd} A	nd ^{c}	3.67 ± 1.80 ^{c} B	4.33 ± 0.5 ^{c} C
PSEF[7]	6.67 ± 2.00 ^{b} C	5.33 ± 1.00 ^{b} C	6.33 ± 1.00 ^{b} C	8.00 ± 0.87 ^{b} C
PSECA[8]	nd ^{d}	nd ^{c}	3.00 ± 0.00 ^{c} B	1.67 ± 0.5 ^{d} A
PSECB[9]	nd ^{d}	nd ^{c}	1.33 ± 0.50 ^{d} A	1.67 ± 0.50 ^{d} A
Positive[10]	16.67 ± 2.00 ^{a} C	22.22 ± 7.31 ^{a} C	24.78 ± 2.28 ^{a} C	28.44 ± 3.81 ^{a} C
Negative[11]	nd ^{d}	nd ^{c}	nd ^{e}	0.11 ± 0.33 ^{e} A

[1]Means ± SD were calculated from triplicate data. The various superscripts demonstrate a significant difference between extracts in the inhibitory zone ($p < 0.05$).

[2]na- No antibacterial activity (inhibition zone < 1 mm)

[3]Different capital letters denoted the ranges of antibacterial activity. A: slight antibacterial activity (inhibition zone < 3mm), B: moderate antibacterial activity (inhibition zone between ≤ 3mm and < 4mm), and C: strong antibacterial activity (inhibition zone ≥ 4 mm).

[4]PSWF: Papaya seed extract using water as solvent and filtration method

[5]PSWCA: Papaya seed extract using water as solvent and centrifuge method (upper part)

[6]PSWCB: Papaya seed extract using water as solvent and centrifuge method (lower part)

[7]PSEF: Papaya seed extract using ethanol as solvent and filtration method

[8]PSECA: Papaya seed extract using ethanol as solvent and centrifuge method (upper part)

[9]PSECB: Papaya seed extract using ethanol as solvent and centrifuge method (lower part)

[10]Tetracycline hydrochloride as the positive control.

[11]DMSO as the negative control

In this study, the inhibitory zone's diameter was not formed by the DMSO as a negative control. It demonstrates that the antibacterial activity is unaffected by the solvent used. Hence the antibacterial activity investigated is the potential of the *Carica papaya* seed extract. The density or viscosity of the culture medium, its rate of diffusion, the concentration of the extract in the filter paper disc, the sensitivity of the organism to the extract, and the medium itself may all have an impact on the inhibition zone size (Pohan & Rahmawati, 2022).

ISBN: 978-81-961472-9-7

Series: AIJR Abstracts

DOI: 10.21467/abstracts.155

Table 2: *Inhibition zone of Carica papaya seed extracts on gram-negative food microorganisms by different solvents and extractions methods.*

Method & Solvents	**Inhibition zone on gram-negative bacteria [1,2,3] (mm)**			
	E. coli	*P. mirabilis*	*S. typhimurium*	*V. vulnificus*
PSWF[4]	2.33 ± 1.00 [b]	0.33 ± 0.50 [c]	nd [c]	2.67 ± 4.00 [b]
PSWCA[5]	nd [b]	1.67 ± 0.50 [b]	nd [c]	nd [b]
PSWCB[6]	1.00 ± 0.00 [b]	nd [c]	0.67 ± 1.00 [c]	nd [b]
PSEF[7]	2.33 ± 1.80 [b]	nd [c]	3.00 ± 0.87 [b]	nd [b]
PSECA[8]	0.33 ± 0.50 [b]	1.00 ± 0.00 [bc]	nd [c]	1.67 ± 0.50 [b]
PSECB[9]	0.67 ± 1.00 [b]	nd [c]	nd [c]	2.33 ± 1.32 [b]
Positive[10]	25.67 ± 7.19 [a]	21.89 ± 3.82 [a]	21.89 ± 2.76 [a]	27.56 ± 6.80 [a]
Negative[11]	0.33 ± 1.00 [b]	nd [c]	nd [c]	nd [b]

[1-11] Refer Table 1

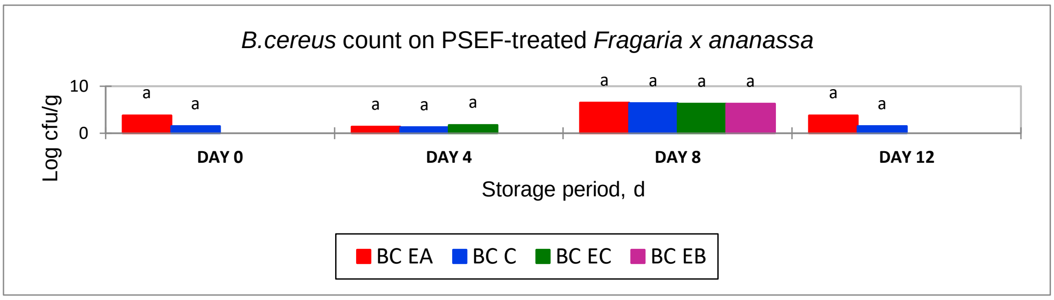

Figure 1: *B.cereus* count on PSEF-treated *Fragaria x ananassa*

BC C: B.cereus count on untreated Fragaria x ananassa

BC EA: B.cereus count on PSEF (25.01 mg/g)-treated Fragaria x ananassa

BC EB: B.cereus count on PSEF (1.563 mg/g)-treated Fragaria x ananassa

BC EC: B.cereus count on PSEF (0.098 mg/g)-treated Fragaria x ananassa

The impact of *Carica papaya* seed extract on the *B cereus* growth on *Fragaria x ananassa* (Strawberry) was investigated at concentrations of as stated in subheading 1.3 and Figure. The strawberry was treated with PSEF and stored at 4^{o}C for several days. Referring to Figure 1, the *B.cereus* count on untreated *Fragaria x ananassa* was examined as 6.34 log CFU/g on day eight of storage but decreased to 1.54 log CFU/g on day twelve of storage. This trend was similar to the treated *Fragaria x ananassa* that showed the increased *B.cereus* count value on day eight of storage and then the values decreased on day twelve of storage. *Fragaria x ananassa* started to spoil at 6 log CFU/g which is on day eight of storage. The Institute of Food Science and Technology (IFST) determined the maximum acceptable viable count limit for a shelf life study at 6 log CFU/g for fruit-based products (Bierhals et al., 2011). According to the finding, the highest concentration of PSEF (BC EA) that treated the *Fragaria x ananassa* showed a longer shelf life.

Previous research by Shahbazi (2018) stated that the control group (untreated) of *Fragaria x ananassa* increased from an initial value of 2.11 log CFU/g on day first to 6.29 log CFU/g on day twelve. Another research by Pinzon et al. (2020) stated that the mesophilic microorganism count for uncoated strawberries was near 4.49 ± 0.19 log CFU g^{-1} at day seven while the total

ISBN: 978-81-961472-9-7

Series: AIJR Abstracts

DOI: 10.21467/abstracts.155

count for coated strawberries with aloe vera gel remained below 4 log CFU g^{-1} even after twelve days of storage. Fruit shelf life is affected by mass loss, which is also referred to as weight loss, water loss, or moisture loss. This physiological and commercial deterioration manifests as wilting, shriveling, and a loss of stiffness, turgidity, and juiciness in fresh produce (Jalali et al., 2020). All of these may be can be some factors for *B.cereus* to grow.

4 Conclusions

The *Carica papaya* seed extracted with ethanol displayed high antibacterial activity against all Gram-positive bacteria, *B. cereus*, *C. diphtheria, L. monocytogenes,* and *S. aureus*. The PSEF at a concentration 25.01 mg/g extract exhibited the potential to preserve *Fragaria x ananassa* at refrigerated temperature. With proper knowledge of the possible advantages of the *Carica papaya* seed, we can anticipate creating an all-natural food substitution that will benefit society.

Acknowledgement

The present work was financially supported by the Innovation Fund (MYS1013) awarded by the Islamic Development Bank.

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