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Evaluation of Antioxidant and Antibacterial Activity of Red Onion (*A.CEPA* L.) and Garlic (*A.SATIVUM* L.) Wastes on Homemade Tofu

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Abstract

In this research, waste of red onions (Allium cepa L.) and garlic (Allium sativum L.) were used to generate antioxidants of phenolic compound using Soxhlet extraction method. In order to evaluate the effectiveness of the phenolic compound produced from both allium waste, it was mixed into handmade tofu that had not previously included any antioxidants. The presence of antioxidants in tofu is widely recognized as a preservative that can extend the shelf life of the food and delay the time for the tofu to be spoiled. The two primary types of antioxidants that are present in food sources are natural antioxidants and synthetic antioxidants. According to studies, natural antioxidants derived from plant extracts, fruits, and vegetables are more effective than synthetic antioxidants. A synthetic antioxidant requires a high metabolism to break it down, therefore if it is consumed over an extended period, it may have negative effects on humans, such as cancer. Several published studies have found a relationship between long-term consumption of synthetic antioxidants and a variety of health concerns, including skin allergies, gastrointestinal disorders, and, in some circumstances, an increased risk of cancer. Excessive amounts of synthetic antioxidants have the potential to harm DNA and increase premature ageing. In animal experiments, it has previously been determined that BHA and BHT cause liver damage and carcinogenesis. Apart from that, synthetic antioxidants also pose some environmental impact. Synthetic antioxidants can find their way into the environment through various means, such as improper disposal or wastewater from manufacturing processes. These substances might have adverse effects on ecosystems or aquatic life, and some synthetic antioxidants might persist in the environment for extended periods, contributing to pollution concerns. Therefore, this study was carried out to extract natural antioxidants from allium waste, which was then applied to self-made tofu that was created without preservatives in order to investigate natural and synthetic antioxidants and their roles in self-made tofu.

1. Introduction

Based on studies in recent years, the onion peel and garlic husk are good sources of phenolic antioxidants [1]. Therefore, extracting phenolic compounds from onion peels and garlic husk are advantageous for society because it reduces environmental waste, and these phenolic compounds can be employed as natural antioxidants. This kind of trash disposal issue is a challenge for industries. These wastes are undesirable for the environment because they release unpleasant odors when they are dumped outside following industrial processing or residential use. Therefore, it's important to extract valuable bioactive chemicals from onion peels and garlic husk. Garlic (Allium sativum L.) has been applied for centuries for both culinary and medicinal uses. According Amagase, garlic is a particularly rich source of organosulfur compounds, which are response the onion (Allium cepa L.) is a multipurpose vegetable from the Allium family that is appreciated all over the world for both its flavor and its considerable supply of healthy ingredients [2]. Studies have shown that several onion types contain flavonoids as well as other bioactive chemicals, practically all of which are concentrated largely in the skin for some of the health benefits [2]. Antioxidant properties of garlic bioactive components are widely recognized.

In order to collect phenolic compounds from the matrix of waste garlic and onion, extraction is an essential step. It could take the form of an unusual tactic or a traditional one. Traditional techniques, such as Soxhlet, hydro distillation, boiling, maceration, and soaking, have been in use for a number of years. However, Soxhlet extraction is the most popular method for extracting phenolic compounds because it is less time-consuming, easier to use, suitable for both initial and bulk extraction, and good for total extract recovery when compared to other traditional methods like maceration or percolation. Its processing expenses are also reduced. Therefore, the main goal of this work was to recover phenolic chemicals from onion and garlic waste using the Soxhlet method.

2. Materials and Methods

2.1 Material and Sample Preparation

The waste of onion and garlic were collected from a nearby restaurant, which is located at Pagoh. The collected samples were weighed and washed under running water to remove any undesirable material and contaminants. Then, all the excess water were drained from the samples and the samples undergo drying process. The samples were dried in the oven at 50°C for 24 hours [3]. The oven-dried samples were grounded into coarse particles by using a grinder. These dried sample powders were kept in an airtight container for further use.

2.2 Soxhlet extraction method

Red onion (*Allium cepa* L.) peel and garlic (*Allium sativum* L.) wastes were extracted by using Soxhlet extraction method. Firstly, 15 grams of red onion (*Allium cepa* L.) peel and garlic (*Allium sativum* L.) husk powder were extracted with 150 mL of ethanol (70%) by using the assembled Soxhlet apparatus. The temperature of the heating mantle was set to the heating stage of stage 3 for 18 hours. A rotary vacuum evaporator was used to condense the resulting dark-green extract up to 10 ml at 34 ^{o}C under reduced pressure (58 mbar) [4]. After that, the extracts were kept in refrigerator at 4 ^{o}C until it was needed.

2.3 DPPH radical scavenging assay

The antioxidant activity of the extracted red onion (*Allium cepa* L.) peel, garlic (*Allium sativum* L.) husk and butylated hydroxyanisole (BHA) as control were evaluated by using DPPH radical scavenging assay [5]. Firstly, 0.1 mM of ethanolic DPPH solution were prepared by weighing powder form DPPH at a particular mass, then mixing the DPPH with a specific amount of ethanol. 1 mL of extract solution was poured into a test tube. Then, 5 mL of the ethanolic solution of 0.1 mM DPPH were added into the test tube. Then, the solution was mixed using a vortex mixer. After that, it was incubated at room temperature in a dark room for 30 minutes [6]. Absorbance at 517 nm was measured spectrophotometrically after incubation. The ability to scavenge the DPPH radical was calculated by using the following equation (1).

$$\text{DPPH scavenging activity (\% inhibition)} = [1 - (\text{OD control} - \text{OD sample})] \times 100 \quad (2.1)$$

Where the OD control is the absorbance of DPPH + ethanol, while OD sample is the absorbance of DPPH + sample.

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2.4 Folin-Ciocalteu reagent method

The total phenolic content in the extracts of red onion (*Allium cepa* L.) peel, garlic (*Allium sativum* L.) husk and BHA were determined by the Folin– Ciocalteu method. Ten µL of the extract solution (1 mg/mL) was added, and then 0.5 mL of a 1:10 solution was added before Folin-Ciocalteu. The mixture was mixed for 5 minutes, and 0.4 ml of 75 mg/ml of sodium carbonate was added to the solution. The solution was incubated for two hours at room temperature. After two hours, the absorbance of the solution was determined using UV-Vis spectrophotometry at 765 nm absorbance, with methanol used as a blank [7]. The Total Phenolic Content (TPC) of the plant's extract samples was measured in milligrams of gallic acid equivalents (GAE) per 100 grams of the sample.The TPC was calculated by using the following equation:

$$C(GAE) = QE \times (v/m) \quad (2.2)$$

Where:
QE = concentration of gallic acid established from the calibration curve
v = volume of extract (mL)
m = weight (g) of the dry extract

2.5 Determination of antibacterial activity

The extracts of *A.sativum* and *A.cepa* wastes were tested for their antibacterial activities by using Kirby-Bauer disc diffusion method [8]. The antibacterial properties of *A.sativum* and *A.cepa* extracts were evaluated against gram-negative bacteria, namely *Escherichia coli* and gram-positive bacteria, *Staphylococcus epidermidis*. Both organisms were cultured in a nutrient broth and incubated for 24 hours before inoculated. Sterile paper discs 6mm in diameter were impregnated in extracts of *A.sativum* and *A.cepa* waste allowed to dry at room temperature for about 15 minutes. The media used for culturing the organisms were nutrient agar. Antibacterial activity was determined by the diameter of the clear inhibition zone around the discs.

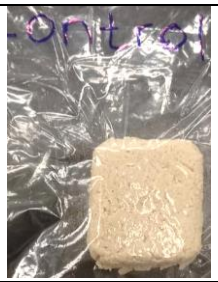
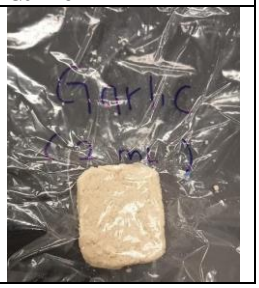
2.6 FTIR Spectroscopic analysis

The existence of bioactive compounds in the plant extract sample was determined by Fourier transform infrared (FTIR). All infrared (IR) spectra were acquired between 400 and 6000 cm^{-1} [9]. Approximately 0.05 ml of sample was placed onto the FTIR crystal, and after each analysis, the crystal was washed with deionized water and dried with a non-abrasive wipe.

2.7 Application of extracted herbs and BHA on homemade tofu

The tofu was prepared in 4 types. The first tofu was a controlled sample and prepared without addition of any antioxidant. Then, the other three tofu were added with red onion peel, garlic, and BHA. The stability of the cakes was identified according to the set time of total 14 days interval. For the antimicrobial activity, the tofu was observed by how much mould and fungal were developed and grown throughout the 14 days of storage in the airtight container. Then, the container store in the refrigerator at 4°C Table 2.1 shows the four types of samples on the 1st day of storage.

Table 2.1 *Types of samples on the 1st day of storage*

Type of Extracts	Blank	BHA	Red onion	Garlic
Sample				

3. Result and Discussions

3.1 DPPH radical scavenging assay

The antioxidant activity for antioxidant which was extracted from garlic peel was higher than the antioxidant that extracted from red onion peel. The calculated antioxidant activity value obtained was 49.891 ± 0.143% for antioxidant extracted from garlic peel and 20.087 ± 0.04 % for antioxidant that extracted from red onion peel respectively. However, synthetic antioxidant (BHA) that was used in this experiment tends to obtain much higher in antioxidant activity compared to both natural antioxidants which were extracted from red onion and garlic peel. The percentage of antioxidant activity of synthetic antioxidants was 64.654 ± 0.079 %. The outcomes of the research were shown in Table 3.1 below. The antioxidant activity of these antioxidants was calculated in percentage.

Table 3.1 *Comparison of antioxidant activity of each sample in percentage form (%)*

Sample	DPPH scavenging activity (%inhibition)	Concentration (µg/mL)
BHA (control)	64.654 ± 0.079	74.585
A.sativum extract	49.891 ± 0.143	59.035
A. cepa extract	20.087 ± 0.041	27.643

The DPPH assay is a fundamental test for antioxidant activity that assesses the ability of each sample to scavenge free radicals. Primary antioxidant involves the mechanism, whereby it blocks the oxidation reaction by combining it with the free radicals or reacting hydrogen peroxides. When a DPPH solution is combined with a material that can contribute a hydrogen atom, which results in the DPPH compound's reduced form and a decrease in the violet colour [10]. In this study, mechanism of the radical scavenging activity was observed based on the reducing purple colour of DPPH solution.

3.2 Folin-Ciocalteu reagent method

This method was applied to determine the total phenolic content of three different antioxidants, which were previously mentioned antioxidants extracted from red onion and garlic peel, as well as synthetic antioxidant (BHA). The amount of total phenolic content of the antioxidants were calculated from the standard curve of gallic acid (y = 0.0064x+0.0029) which was shown in figure 3.1 and the result obtained was tabled in table 3.2.

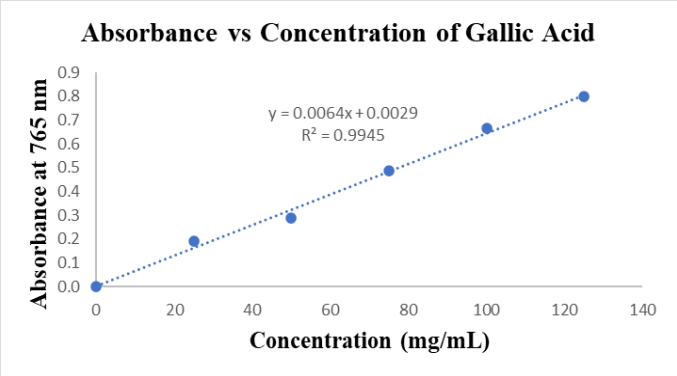


Fig. 3.1 *The standard curve of gallic acid to determine TPC at 765 nm.*

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Table 3.2 *Amount of total phenolic content obtained for different antioxidants*.

Type of antioxidants	Mean OD ± SD	Concentration (mg/mL)	TPC ± SD (mgGAE/g)
BHA	0.431 ± 0.004	66.891	0.654 ± 0.415
A.cepa extract	0.295 ± 0.018	45.641	0.446 ± 0.321
A.sativum extract	0.154 ± 0.022	23.609	0.231± 0.043

Based on table 3.2, synthetic antioxidant (BHA) has the highest total phenolic content with 0.654 ± 0.415. The highest total phenolic content that extracted antioxidant from garlic and red onion peel were 0.231± 0.043 and 0.446 ± 0.321 respectively. Both of natural antioxidants have lower total phenolic content when compared to BHA, but red onion peel extracts have slightly higher than garlic peel. This shows that synthetic antioxidant (BHA) obtained the highest total phenolic content compared to garlic and red onion peel. The primary class of molecules that gives vegetables, fruit, grains, and other plant-based materials their antioxidant properties is composed of phenolic compounds. The antioxidant activity of the compounds is attributable in part to one electron reduction potential, which is the ability to act as hydrogen or electron donors [11]. According to a study, phenolics' primary source of antioxidant activity is their redox characteristics, which enable them to function as singlet oxygen quenchers, hydrogen donors, and reducing agents [12]. The identification of these substances is typically carried out by using Folin-Ciocalteu's reagent to react phenolic compounds.

3.3 Fourier-transform infrared spectroscopy (FTIR) analysis

The extracts of red onion and garlic peel were analysed to determine the main component that may be present in the extract that contributes to the antioxidant activity. Figure 3.2 shows the IR spectra of the ethanolic extract of red onion (*A.cepa*) peel, while Figure 3.3 shows the IR spectra for ethanolic extract of garlic (*A.sativum*) peel, which exhibits prominent absorbance bands in the broad region of 400 - 6000cm^{-1} that provide details on the characteristics for various classes of compounds [13].

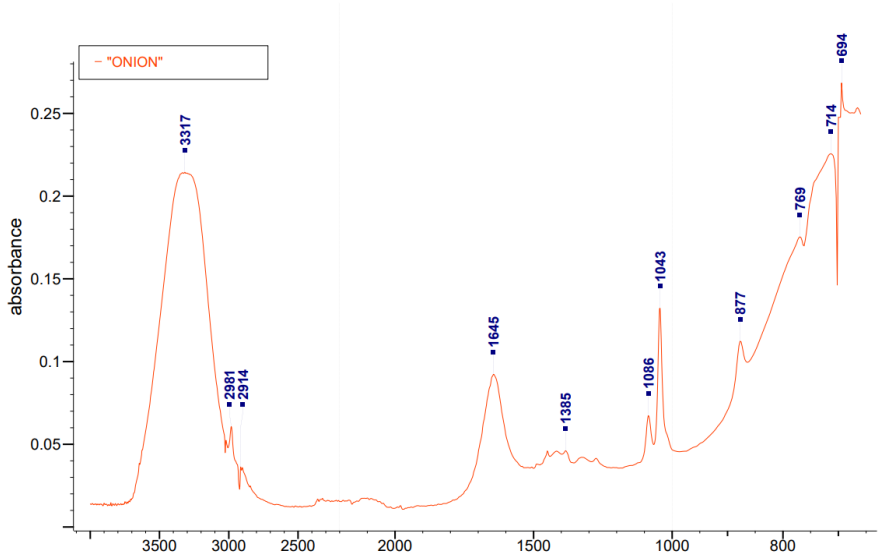

Fig. 3.2 *Fourier-transform infrared spectroscopy (FTIR) spectra of red onion (A.cepa) waste extract*

The frequency range from 3350-3200 cm represents the OH stretching, presence of alcohol. Therefore, peaks at 3317 cm could be attributed to ethanol. The bands at 2981 cm and 2914 cm are for the chitin and chitosan respectively due to the C-O groups stretching vibrations. The C-O groups in chitosan were depressed due to deacetylation. The absorption band at 1645 cm characterized stretching vibration of amino group in chitosan. Besides that, the peaks between 1385-1043 cm and 877- 694 cm indicated the presence of saccharide structure of chitin and chitosan respectively due to the varying C-O groups [14].

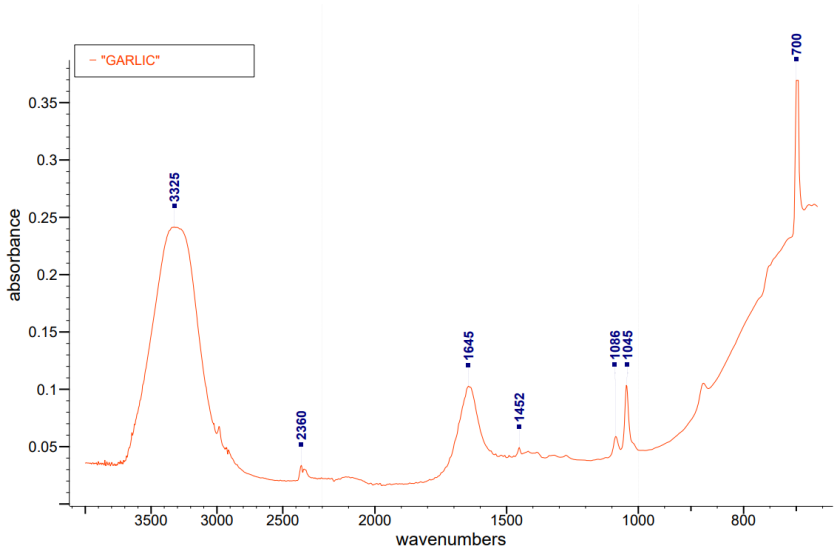

Fig. 3.3 *Fourier-transform infrared spectroscopy (FTIR) spectra of garlic (A.sativum) waste extract*

The frequency ranges from 3325 cm peaks are represents the C-N stretching mainly: amino acids and symmetric C-H stretching vibration which represent the presence of antioxidant enzymes. The frequency ranges from 2360 cm peaks represent the SO symmetric stretching vibration, which indicate the presence of acid and RSO ionic sulphonates. Then frequency ranges from 1645cm peaks represents the C-N stretching mainly: aminoacids. The frequency of 1452 cm peaks represent the SO symmetric stretching mainly: sulphur compounds and the frequency from 1086 cm peaks are represents the N-Hstretching of proteins. The 1045 cm peaks represent the C-H bending mainly: glycogen. The frequency ranges from 700 cm peaks represents the N-H stretching of proteins [15].

3.4 Antibacterial activity test (disk diffusion method)

Based on the analysis, the extract of garlic (*A.cepa*) waste showed an effective antibacterial activity with clear zone for both E. coli followed by red onion (*A.cepa*). Based on the results that were obtained, there is no growth of microorganism in gram positive *S. epidermidis.* The most common factor for this problem is that the source of the culture was dead. Normally, the dead microbes look the same as live bacteria. Therefore, we cannot identify if the cells on an agar surface were alive or dead. Another possible problem is the medium or growth conditions for the strain might be not correct. Apart from that, we also need to consider several things while conducting this analysis. For example, selecting the appropriate inoculum density, maintaining the proper incubation conditions, adjusting the pH, agar depth, and moisture content. Diffusion range, evaporation, and solubility of the tested drug may all have an impact on the inhibition zone's diameter. Some of other reason of the low inhibition zone from the plant sample is because of the overgrown bacterial lawn that cause high concentration of microbe. This will cause the microbe to negate the plant extract action on them. Thus, the antioxidant activities, and total phenolic content were not directly proportional to antibacterial properties. Furthermore, E. coli and S. epidermidis were sensitive to the antibacterial effects of Penicillin, which were used as a positive control with a zone of inhibition of 11.00mm and 8.00mm, respectively. However, the antibacterial effect of positive control gives a smaller inhibition zone compared to extracts. This might be due to the improper spreading of bacteria culture during susceptibility test or resistivity of both microorganism species to the antibiotic control. Meanwhile, there is no inhibition zone that can be observed on the negative control for both types of microorganisms. The diameter of the inhibition zone (IZ) around the discs was shown in table 3.3.

Table 3.3 *Antibacterial activity of BHA, onion, and garlic extract (zone of inhibition in mm)*

	Inhibition Zone (mm)				
Sample	BHA	*A.cepa*	*A.sativum*	*Positive control*	*Negative control*
S. epidermidis	NIL	NIL	NIL	8	NIL
E. coli	NIL	28	50	11	NIL

* Key: NIL = No growth

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3.5 Application of herbs extract and BHA on homemade tofu.

This examination revealed the fungus's growth on the tofu. There doesn't seem any fungal growth on the tofu's surface from day 0 to day 9. On days 10 to 14, several fungal colonies were able to multiply on the surface of garlic, and blank tofu. Tofu without any addition of antioxidant contains many mould growth. The tofu with garlic extract developed a few mould spores. However, after being exposed to BHA and red onion extracts for 14 days, the tofu's surface did not support the growth of any mould or fungi. This demonstrates that the red onion extract was effective in keeping the tofu from spoiling and becoming mould infested.

In conclusion, when compared to the garlic extract, the onion extract demonstrated superior defense against microbial development in the tofu. The two extracts demonstrated comparable efficacy to butylated hydroxyl toluene (BHT), the most widely used artificial preservative in food goods. By adding extracts of onions and/or garlic to tofu, consumers may be able to purchase that, in contrast to those derived from artificial sources, contain healthy preservatives. Once these extracts have been optimized and validated for usage in industrial settings, they may also be utilized to extend the shelf life of the tofu. Figure 3.4 shows the growth of microorganisms on the four types of tofu within the 14 days interval in refrigerator at 4°C.

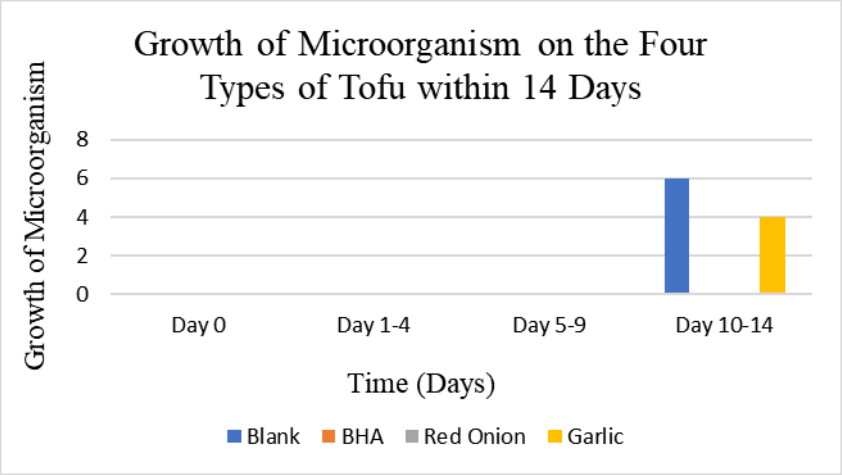

Fig. 3.4 *Growth of microorganisms on the four types of tofu within 14 days*

4.0 Conclusion

In conclusion, this study shows that the addition of both herbs on tofu has potential to act as preservative. It could be considered to replace BHA as a natural preservative in the future. As we all know, antimicrobial and antioxidant agents are frequently utilized in the food sector when preparing food. Many producers prepared their food using synthetic preservatives that are chemicals that can be harmful to people's health. According to this problem, it is necessary to investigate safer food preservation options, such as herbs phenolic compounds. The herb's phenolic compound allowed the study to investigate its advantageous qualities, including its antioxidant and antibacterial activities. One of the many bioactive substances found in every section of the herbs are phenolic compounds. Recent studies have shown that the waste from red onions (A. cepa) and garlic (A. sativum) has a high concentration of phenolic content, which makes it a promising natural preservative. In summary, this study demonstrated that the herbs sample's phenolic content was able to be extracted by using ethanol and the Soxhlet extraction method. More research is needed to fully understand the composition of possible natural herbs, discover antioxidant components, and assess the potential usage of natural antioxidants products and food supplements. Peroxide value and sensory testing can be included in future research to obtain more precise results when using these herbs as preservatives.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

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Journal

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