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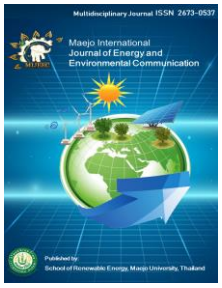

ARTICLE

Beneficiation of organic black onion and lemon peel by-products through extraction and investigation of bioactive compounds

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ABSTRACT

Much research on the shallot phytochemical is currently employed in various disciplines, such as medical reasons. Shallot (*Allium ascalonicum*) is a plant species belonging to the family Amaryllidaceae. It is a native plant grown in the North and Northeast region of Thailand. The present study aimed to determine the extracts' yield and antioxidant properties of black shallots made from fresh shallots. The black shallots were extracted with digestion by water, 47.5% and 95% ethanol at 50 °C for 1, 3 and 5 hours. The result discovered that black shallot extraction with digestion by water for 5 hours showed the highest weight of extracts (3.620 g). The determination of antioxidant activity by DPPH was observed, and extraction by water for 5 hours gave the highest percentage of inhibition. It was equal to 84.61%, which is not different from the digestion of 95% ethanol for 1 hour, with 83.48%. Similarly, the most outstanding value of IC_{50} was obtained from extraction with 95% ethanol for 1 and 5 hours with 21.30 mg/ml and 20.52 mg/ml. In terms of monitoring, the digestion method evaluated the productivity of pectin extracts from citrus peel. Hydrochloric acid (0.100, 0.050 and 0.025 M) was used as a solvent at 95 °C for 30, 60 and 90 minutes. The pectin extract by 0.1 M hydrochloric acid for 90 minutes gave the highest extract (5.237 g). The preliminary testing of cosmetic production was done by mixing black shallot extracts and pectin from citrus peels, and the two substances can be combined. Still, without adding preservatives, they could cause contamination in cosmetic samples.

1. Introduction

Phytochemicals are abundant in different parts of plants (Wongsa et al., 2022). It is essential to measure all phenylic compounds in this herbaceous plant to resist the antioxidant behavior

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in the group of herbal plants (Bhuyar et al., 2021a, b). Humans can cause damage to the immune system associated with diseases and deterioration of various bodily functions, such as blood vessels and ileus (cardiovascular disease). Due to these relationships' present health issues, phytochemicals are the best source of natural antioxidants (Chang et al., 2019). Antioxidants from natural products have received significant attention due to their role in preventing the automatic oxidation of fats, oil and wax (Bhuyar et al., 2020b, c). The primarily natural antioxidant derivatives are used in preparing food and medicine to replace synthetic antioxidants (Munir et al., 2021). Most of the antioxidants that can be extracted from plants are polysaccharides. However, according to the information reviewed from scientific journals, fresh shallots (*Allium ascalonicum*) are used in traditional medicine to benefit health-related problems (Charoenchai et al., 2017). From the previous reports, the Kulu family in Thailand planted shallots mainly in the northeastern regions.

In comparison, the famous red scallop shallot onion has efficient bioactive compounds which helps to expel balm, relieve gassiness, aid digestion, cure various inflammatory diseases, reduce oedema and parasites and help to keep the body warm (Bhuyar et al., 2020a; Črnivec et al., 2021). Shallots combined with curcumin and flavonoids (quercetin and flavonoid glycosides) may prevent cancer. As a medicine, it can be used to reduce fever and heal wounds. It is also used to mix with coconut oil and salt and boil for an hour, then mask the damage. In addition, shallots also help to reduce the level of blood sugar and prevent blood clots by consuming fresh food, cooking or consumer products. Pectin from lemon mainly offers moisture protection for cosmetic products (Chavan et al., 2018). Lemon is a fruit with a very sour taste and belongs to the Citrus family. The ripened fruit changes its color from green to yellow. The fruit's skin is thin, moist, and divided into several petals. In addition, it is considered that the nutritious medicine lemon is a native plant discovered in Southeast Asia. Lemons have been used as a natural antioxidant for a long time (Ramli et al., 2020). Lemon juice, besides being used to taste sour in many types of food. It is also a drink mixed with salt and sugar, like lemon juice, and is well known in Thailand and worldwide. In addition, some alcoholic beverages can be used to slice a lemon into thin slices by inserting them for seasoning into the rim of the beverage glass. This study aimed to gain additional knowledge about extracting the active ingredients of black onion. As per previous reports, different extraction creates the efficiency of the anti-allergic effect of the extracts. It can be said that the use of ethanol with different concentrations influences the extraction methods (Bettaieb Rebey et al., 2012) .

This study's objectives are as follows: 1. To assess the efficacy of extracting bioactive substances from black onion with Ethanol and water, 2. To check the quantity and quality of the extracts in shallots (*Allium ascalonicum*) made black onion by digestion method, 3. To increase the richness of shallots (*Allium ascalonicum*), 4. To be a source of information on antioxidants in black onion to further study and research, 5. To assess the efficacy of extracting pectin from lemon peels with hydrochloric acid at different concentrations, 6. To test the making of the primary cosmetic product between antioxidants from shallot and pectin from the lemon. Future studies may gain additional knowledge about preparing black onions before introducing bioactive extraction.

2. Material and Methods

2.1 Preparation of the black onion

Onions are horses from the physiological laboratory of plants. There is a quick preparation method: the shallots were cleaned and incubated in the refrigerator at 60℃. The humidity was maintained at 70 % for 35 days, and the temperature was lowered to 50℃ and set for 35 days. Once the shallots turned dark black, the black onion was taken from the incubator to bake in the hot air oven at the temperature of 60℃ until dry and then followed by grinding with Lab Scale Grinder.

2.2 Experimental planning

The experimental planning was divided into two experiments: Extraction of black onion and extraction from lemon peel. All the experiments were conducted in triplicates. The practical plan was set up by a 3x3 factorial design in CRD (utterly randomized design) experiment was planned as shown in Table 1.

2.3 Extraction

2.3.1 Extraction of active substances from black onion

The samples were extracted using a digestion method of 95% ethanol and 47.5% water for 1, 3 and 5 hours in 3 samples at 50 ℃.

2.3.2 Digestion process

The black onion extraction was done by the method of braising with solvent (González-de-Peredo et al., 2021). In this experiment, a pre-prepared onion plant was used. Take 20 g of black onion samples into 250 ml bottles in the shape of rose apples (a total of 27 bottles, divided into 9 groups, 3 bottles each). All 3 types of solvent are poured into a 200 ml group of pomegranate-shaped bottles (water at 47.5% and 95% at ethanol). Cover the bottle and boil it at 50 ℃ for 1, 3 and 5 hours. The digested sample is strained with a white cloth, and the model is put in the bottle.

2.3.3 Distillation

Take the prepared sample for distillation using a rotary evaporator and ethanol as the polar solvent. Pour the sample into the round bottom flask and assemble it with the rotary evaporator. The temperature was maintained at 55 ℃, and the pressure was controlled for 155 lbs. The rotator was set up at 100 rpm speed, and the cycle was carried out for 1-2 hours. After the completion, the sample was collected from the evaporation and collected in 100 ml of the beaker. The sample was stored in the refrigerator.

2.3.4 Evaporation process

Place the prepared sample in the beaker and use a hot air oven to evaporate the ethanol. For 24 hours, the hot air oven was kept at 70 °C until the solvent had evaporated entirely. Then, collect the completely evaporated samples in a new bottle. Calculate the volatile compounds by dividing the initial and final weights. Those samples were stored in a refrigerator.

2.6 Extraction of pectin from lemon peel

Samples were extracted using HCL at different concentrations of 0.1M, 0.05 and 0.025M for 30, 60, and 90 minutes. The experiment was carried out 3 times replications with a total of 27 samples: Braised in 0.1 M H_2SO_4 for 30 min; 0.1 M H_2SO_4 for 60 min; 0.1 M H_2SO_4 for 90 min; 0.05 M H_2SO_4 for 30 min; 0.05 M H_2SO_4 for 60 min; 0.05 M H_2SO_4 for 90 min; 0.025 M H_2SO_4 for 30 min; 0.025 M H_2SO_4 for 60 min; 0.025 M H_2SO_4 for 90 min.

2.6.1 Sample preparation

The fresh sample was thoroughly washed and finely ground up by a blender after extracted from the inner part of the lemon peel. Collect the boiled samples and place them in a 1000 ml beaker, then add 95% ethanol at a 1: 1 (w/v) ratio. Braise the beaker for 10 min at 80 °C and cover it with aluminium foil. Pour out 95% ethanol and rinse three with water several times before drying in an incubator at 65 °C and storing in the refrigerator.

2.6.2 Digestion process

In a 500ml beaker, place 40g of the prepared lemon peel (a total of 27 bottles, divided into 9 groups, 3 bottles per group). Fill all bottles with 200ml of HCL at 0.100 M, 0.050 M, and 0.025 M concentrations, and boil at 95 ° C for 30, 60, and 90 minutes. Obtain boiled samples, filter them through the filtering cloth and store them in 250 mL bottles. All samples from the three replications and stored in the refrigerator.

2.6.3 Pectin precipitation

Fill the filtered samples with 95% ethanol at a 1: 1 (v/v) ratio and leave them at high temperatures for 15 hours before filtering with filter papers. Take the filtered samples and place them on a plate with an accurate weight. In the hot air incubator, place the plates unsuitable for baking. The sample is heated to 35 ° C until it is dry. The dry pectin is weighed and thoroughly ground. The sample is extracted and placed in the storage bag.

2.7 Antioxidants assay

Weigh 2.0 g of pectin and mix in 20 ml of water in the different ratios for standard preparation, such as 0.1: 10.0: 100.0 (w/w/v) and vortex them. The DPPH solution (Susawaengsup et al., 2022; Bhuyar et al., 2021b) is thoroughly mixed into the first 0.3 ml bottle. The preservative composition of cosmetics used on the face must not exceed 0.3% antioxidative nature. Set it aside and look at the outcomes where P is positive, and N is negative. Add 20 µl of 47.5% ethanol and 180 µl of DPPH solution to the microplate. When S is the number of samples to be tested, add 200 µl of DPPH solution. 20 µl of sample and 180 µl of DPPH solution. To adjust blank (B = blank). 20 µl of sample and 180 µl of 50% ethanol were mixed. The number of samples to be analyzed ranges from 1 to 14. The microplate is stored in a dark place for 30 minutes at a high temperature. The absorbance value at 517 nm was therefore determined by calculating. The graph analyzed the result for each sample's percentage inhibition ability (% inhibition).

2.8 Calculation of percentage of inhibition (% inhibition)

The equation calculated the percent DPPH inhibition:

$$\% \text{ inhibition} = A0 - A1 / A0 \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance of the reaction mixture.

2.9 Analysing % inhibition by responsive surface method (RSM)

The trend of optimal response conditions in antioxidants was studied using the RSM program and plotted using CCD (central composite design), independent variables for time and solvent, as shown in Table 1 and 2.

Table 1 Determination of ideal conditions for % inhibition of black onion active ingredient with time and solvent as independent variables

Factor	Range and level		
	-1	**0**	**+1**
Time (hr)	1	3	5
Ethanol concentration (% v/v)	0	47.5	95

Table 2 Experimental plan in RSM program per % inhibition

Run	Factor 1 A: Time (hrs)	Factor 2 B: Ethanol concentration (% v/v)	Response % Inhibition
1	1	0	70.585
2	1	0	84.069
3	1	0	56.740

4	3	0	72.275
5	3	0	69.637
6	3	0	70.956
7	5	0	87.153
8	5	0	82.148
9	5	0	80.283
10	1	47.5	73.040
11	1	47.5	61.947
Run	**Factor 1 A: Time (hr)**	**Factor 2 B: Ethanol concentration (% v/v)**	**Response % Inhibition**
12	1	47.5	56.940
13	3	47.5	60.247
14	3	47.5	63.854
15	3	47.5	56.537
16	5	47.5	58.193
17	5	47.5	60.873
18	5	47.5	57.618
19	1	95	84.669
20	1	95	90.423
21	1	95	82.288
22	3	95	79.772
23	3	95	78.484
24	3	95	78.523
25	5	95	86.332
26	5	95	80.687
27	5	95	81.140

2.10 Statistical analysis

To compare the mean difference using Duncan's Multiple Range Test (DMRT) and the SPSS Statistics V26 program were employed.

3. Results and discussion

3.1 The black onion extract

Various methodologies of extracting the substance from the technique of steaming were applied. A 3.620 g sample was suspended in water for 5 hours, and the extract weight as compared to other methods (as shown in Table 3). The properties of black onion extract were studied after ethanol volatilization. It has a sticky appearance with reddish black color in the example using ethanol extract. Most of the extracts that use water are black because there continues to be no evaporation. When all of the samples have evaporated, it appears vicious and viscous. It has a pleasant reddish colour because there isn't enough blending. According to the experimental results of antioxidant extraction by boiling water, 47.5%, and 95% ethanol, the percentage of antioxidant properties in each item of the experimental unit has a small volume.

Table 3 Average weight of black onion extract

No.	Extraction method	Extract weight (gms)
1	Extraction of sample in water for 1 hour	1.226 ^{z}
2	Extraction of sample in water for 3 hours	2.604
3	Extraction of sample water for 5 hours	3.620
4	Extraction of sample 47.5% ethanol for 1 hour	2.219
5	Extraction of sample 47.5% ethanol for 3 hours	2.718
6	Extraction of sample 47.5% ethanol for 5 hours	3.532
7	Extraction of sample 95% ethanol for 1 hour	1.749
8	Extraction of sample in 95% ethanol for 3 hours	3.338
9	Extraction of sample in 95% ethanol for 5 hours	2.299

Note: ^{z}; There is no statistically significant difference between the characters within the same column at the significance level of 0.05.

3.2 Analysis of the % inhibition and IC_{50} values of shallot

This section compares the inhibition percentage (% inhibition) and the concentration of 50% antioxidant (IC_{50}). The IC50 value indicates the amount of antioxidant potential in a sample (Brighente et al., 2007). The concentration of DPPH is reduced by half due to oxidation. DPPH was employed to evaluate the antioxidant activity of black onion extract. The percentage of free radical inhibitory activity and the IC50 were determined using

optical absorption at 517 nm. After 5 hours of treatment in water, the percentage of inhibition reached 84.612%, which is significant compared to braising. At the same time, the sample in 95% ethanol for 1 hour had the same probability as 83.484%. IC_{50} analysis of compounds obtained by boiling 95% ethanol for 5 hours and boiling with 95% ethanol for 1 hour yields a good value. Compared to other methods, the totals are 20.52 and 21.30 mg/ml, respectively, as shown in Table 4. In light of the lack of accuracy, the experiment to analyze % inhibition using the DPPH method yielded different and inconsistent results (Ramli et al., 2021a, b, c). The above error significantly impacts the analysis result, and the % inhibition analysis example reveals that there is no error also compute IC_{50} values too.

Table 4 The percentage inhibition and IC_{50} of black onion

No.	Extraction method	% Inhibition	IC_{50} (mg/ml)
1	Extraction of sample in water for 1 hour	72.409 z	101.231 z
2	Extraction of sample in water for 3 hours	73.686	37.219
3	Extraction of sample in water for 5 hours	84.612	46.428
4	Extraction of sample in 47.5% ethanol for 1 hour	60.696	62.208
5	Extraction of sample in 47.5% ethanol for 3 hours	62.952	102.349
6	Extraction of sample in 47.5% ethanol for 5 hours	60.285	108.909
7	Extraction of sample in 95% ethanol for 1 hour	83.484	21.300
8	Extraction of sample in 95% ethanol for 3 hours	78.926	25.911
9	Extraction of sample in 95% ethanol for 5 hours	80.914	20.520

Note:

- z; The characters are the same in the same column; there is no statistical difference at the level of significance of 0.05.
- % Inhibition; Inhibitory concentration, IC_{50} (concentration of the substance that can suppress 50% of free radical).

When preventing black onion extract, DPPH analysis, where P is positive, is the sample containing DPPH solution and ethanol. Change the colour to yellow, as shown in Figure 1 and 2.

3.3 Time and solvents affecting the % inhibition

The results of percentage inhibition were investigated by boiling the sample in water for 5 hours. The percentage of inhibition is low for the sample in 95% ethanol stirred for 1 hour and the sample in 95% ethanol for 5 hours. As shown in Table 5, scam in 47.5% ethanol for 5 hours, sample in 47.5% ethanol for 1 hour, and sample in 47.5% ethanol for 3 hours all have very high sugar content.

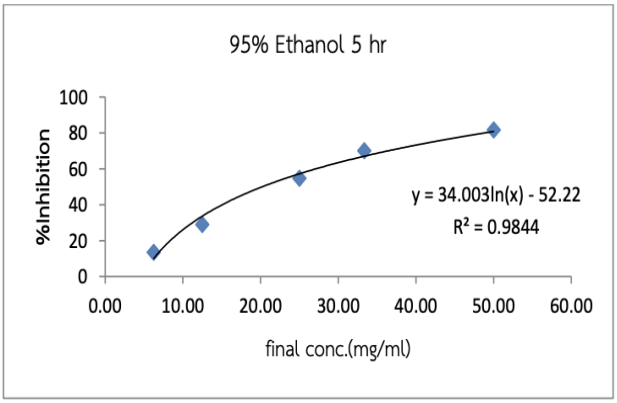

Figure 1 The graph depicts the percentage inhibition and black onion extract concentration. stewing 95% ethanol for 5 hours with samples containing 20.520 mg/ml.

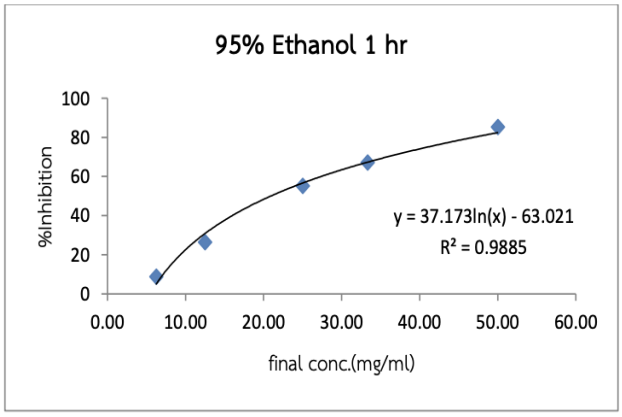

Figure 2 The graph depicts the percentage inhibition and concentration of black onion extract. Using the method of stewing 95% ethanol for one hour and the same as braising with 95% ethanol for 5 hours in fresh samples with equal odds of 21.300 mg/ml.

Table 5 The relationship between time and solvent to sample inhibition percentage

Time	% Inhibition
1 hour	72.810 z
3 hours	65.278
5 hours	70.621

Solvent	% Inhibition
Water	75.199 [z]
47.5 % ethanol	53.794
95 % ethanol	79.716

Note: [z]; There is no statistically significant difference between the characters within the same column at the significance level of 0.05.

3.4 Influence of time and temperature factors on % Inhibition

A statistically analyzed study of the typical efficacy of time and solvent factors in the 3 x 3 Factorial in CRD experimental plan revealed that only factor B (solvent) gave different% inhibition and factor A (time) had no shared effect due to a significance of 0.05.

3.5 Effects of the Responsive Surface Method (RSM) analysis

Analysis of the % inhibition response surface methodology (RSM) from Figure 3 (b) and (c) presents a tendency towards pathogens by threatening the test results in the solvent, which is a one-component substance, water, and 95% ethanol, which tends to neutralize radicals. 47.5% ethanol has a low tendency, meaning the active ingredient is ineffective. As shown in Figure 3 (a), the analysis of a percentage inhibition response method was chosen, as was the method of boiling the sample in water for 5 hours and the method of boiling in 95% ethanol for 1 hour. A period and a suitable solvent are frequently used to extract active ingredients from black onions. IC_{50} response surface methodology (RSM) analysis from Figure 4 (a) and (b). The anti-ulcer tendency of the one-component compounds, water and 95% ethanol, is higher than that of the samples. For the time being, the colored solvent, 47.5% ethanol, tends to remain below. The active ingredient is ineffective in employing response surface analysis using the IC50 method (Alam et al., 2022). As a result, a method was chosen to boil the sample in 95% ethanol for 5 hours and 1 hour, which is the appropriate timing and solvent for the extraction of black onion active ingredients.

3.6 Amount of pectin from lemon peel

When comparing different extraction methods, the boiling method with 0.1 M hydrochloric acid for 90 min in the sample yielded 5.237 g (Table 6). The average weight of pectinate from lemon peel is displayed in Table 6. Using a total of 540 g of dried lemon peel from 6 kg of prepared fresh lemon peel, the average value obtained was 33.604 g and the dry lime weight per pectin content was 6.22%. Furthermore, the previous work's strategy of avoiding dead times in the hydrolysis step by using parallel precipitation tanks resulted in the implementation of continuous distillation to recover a concentrated ethanol stream (85.2 wt.%) that can be recycled to the precipitation stage without changing the precipitation times, reducing ethanol consumption by 76% (Casas-Orozco et al., 2015).

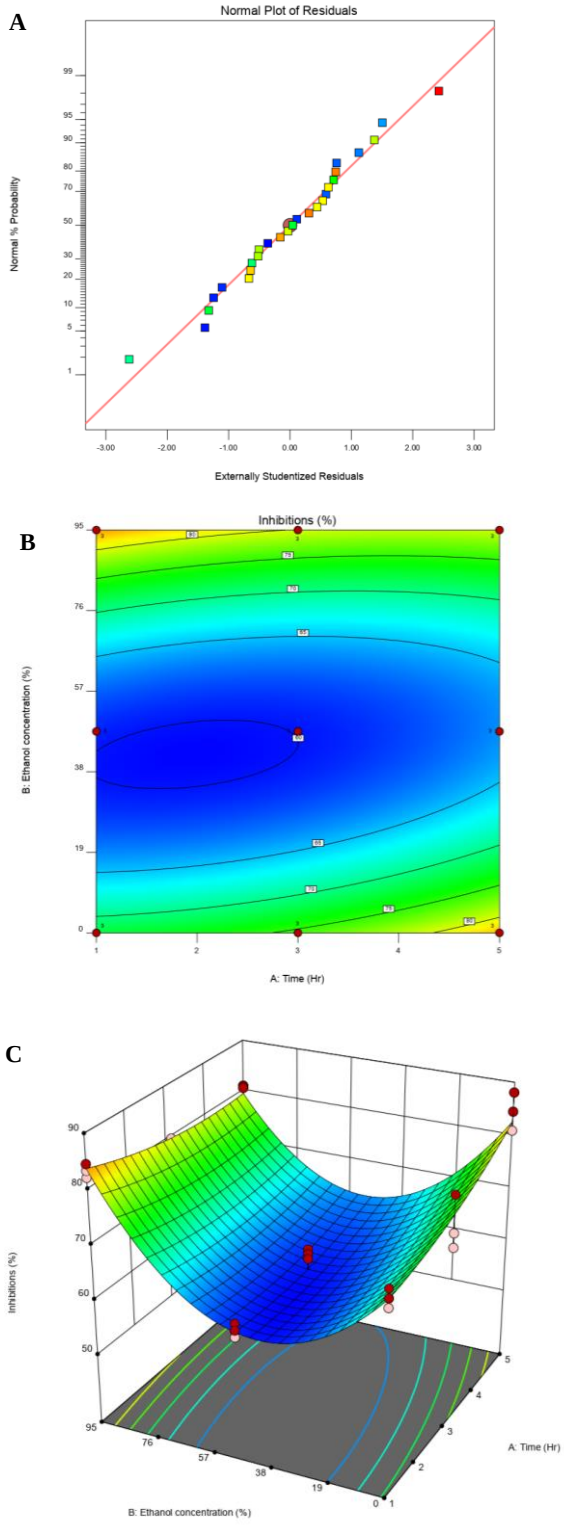

Figure 3 The effect of cooking time and solvent on antioxidant inhibition.

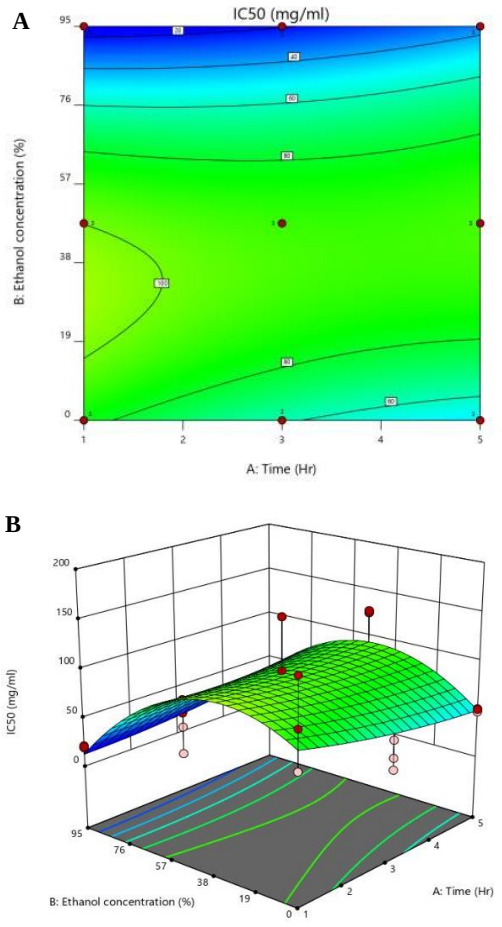

Figure 4 The effect of cooking time and solvent on IC_{50} values against the antioxidant effect.

Table 6 Average weight of pectin from lemon peel

No.	Extraction method	Extract weight (g)
1	0.100 M HCl for 30 min	2.300z
2	0.100 M HCl for 60 min	4.593
3	0.100 M HCl for 90 min	5.237
4	0.050 M HCl for 30 min	2.759
5	0.050 M HCl for 60 min	3.886
6	0.050 M HCl for 90 min	4.956
7	0.025 M HCl for 30 min	2.619
8	0.025 M HCl for 60 min	3.261
9	0.025 M HCl for 90 min	3.993

Note: z; The characters are the same in the same column; there is no statistical difference of 0.05.

4. Conclusion

The experiment to find the extraction method and to evaluate the activity of the antioxidant substance from black onion revealed that in the determination of the content of black onion extract. Samples were taken with water 47.5% and 95.0% ethanol for 1, 3, and 5 hours to obtain weights. The solution was used to extract the substance in the most significant quantity possible using the extraction method. Compared to other methods, the sample was removed in water for 5 hours, yielding as much extract as 12.621 g. The percentage of inhibition (% inhibition) by the DPPH method and the concentration of substances capable of inhibiting antioxidant substances was 50% based on the test results (IC_{50}).

After 5 hours in water and 1 hour in 95% ethanol, the difference was 84.612% and 83.484%, respectively. The IC_{50} analysis revealed that the sample was harvested in 95% ethanol for 5 hours and 1 hour, with IC50s of 20.520 and 21.300 mg/ml, respectively. The pectin content of Lemon peel was determined by boiling hydrochloric acid at concentrations of 0.100, 0.050, and 0.025 M for 30, 60, and 90 minutes to obtain the wet sample weight from the experiment for extracting pectin from lemon peel. They differ depending on the extraction concentration. The weight of the sample varies according to the extraction method used.

Compared to other methods, the 0.1 M hydrochloric acid extraction method for 90 minutes in the sample yielded the highest pectin content of 5.237 g. An experiment was created to create a simple cosmetic product by combining an antioxidant from black onion with pectin. The results can be mixed from the peel, and pectin can help with water retention by keeping it at the same temperature for three days. Although the temperature was clean, the undiluted cauliflower was kept for some time. One discovered that the contamination. Keep an eye on the container's lid. According to the findings of this experiment, only both substances were tested for further processing into an exfoliating product.

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