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Original Research Article Outline:

PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY OF LOWLAND BLACK POTATO PEELS (PLECTRANTHUS ROTUNDIFOLIUS) WITH COW MANURE USING ULTRASONIC BATH EXTRACTION METHOD

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ABSTRACT

Introduction: Black potato (Plectranthus rotundifolius) is one of the tubers in Indonesia and not well known by the public. It causes inadequate use of black potato than any other tubers. The parts that are currently used are the potato flesh. Meanwhile, the black potato peels are not used. Although the black potato peels contain polyphenol saponins, flavonoids, and antioxidant compounds. **Method:** Phytochemicals can be separated using extraction methods, one of the modern extraction methods used is the ultrasonic bath method. The ultrasonic bath method is a method that uses ultrasonic waves with a frequency greater than 16-20 KHz. The objective of this research is to determine the phytochemical content and antioxidant activity of lowland black potato peels with cow manure using ultrasonic bath extraction method. The research was conducted experimentally using RAL (Completely Randomized Design) with 3 factors and 3 replications, which included the difference in extraction temperature (55°C); extraction time (15 and 20 minutes); and the ratio of materials and solvents (1:5 and 1:10). **Results & Analysis:** The data obtained were yield, total phenolics, total tannins, flavonoids and antioxidant activity of black potato peels extracted using ultrasonic bath method with different temperature, extraction time and solvent ratio. The data obtained were analyzed using the Anova test with a significance of $\alpha = 0.05$. **Discussion:** The analysis indicated that the treatment of 5 kg/plot manure with an extraction time of 20 minutes, at a temperature of 55°C, and a ratio of ingredients and solvents is 1:10 provided the best quality at 4.13% moisture content, 17.90% yield, 0.177 g/g phenols total, 0.063 g/g tannins, 0.096 g/g flavonoids and 45.275 ppm antioxidant activity.

Keywords: Antioxidant Activity, Black Potato, Phytochemicals, Ultrasonic Bath.

INTRODUCTION

Black potato (Plectranthus rotundifolius) is one of the tubers that is less popular in the community, even though every 100 g of consuming black potatoes contains 21% carbohydrates; 1.4% protein; 0.2% fat; 0.7% fiber; 21% water; 0.1% ash; and vitamins and minerals (Nugraheni, Hamidah and Auliana, 2016; Dayu Ardani, Suminarti and Nugroho, 2017). Black potato has potential as a natural antioxidant and antiproliferation agent (Nugraheni, Hamidah and Auliana, 2016).

In general, people consume black potatoes by steaming or mixing in a dish and the peels are wasted. Therefore, the utilization of black potato peels need to be improved because they have high bioactive compounds compared to other types of potato peels (Nugraheni, Hamidah and Auliana, 2016). The compound content of black potato peels

is more than four times higher than the flesh of its tubers. There is a correlation of antioxidant and antiproliferation activity of 0.98 in the peels while only 0.93 in the tubers (Nugraheni, Hamidah and Auliana, 2016). Polyphenol compounds have potential as natural antioxidants, antioxidants are compounds that neutralize free radicals by accepting or donating one electron to eliminate the condition of "unpaired electrons" (Francenia Santos-Sánchez *et al.*, 2019). In addition to polyphenolic compounds, black potato peels also contain anthocyanin pigments, which are purple-black in color as a source of antioxidants. Anthocyanins are polyphenol derivative compounds. Soluble organic chemical compounds with potential as natural antioxidants (Du *et al.*, 2015). The antioxidant function of anthocyanins has various benefits in preventing various degenerative diseases, such as the prevention of cardiovascular disease due to atherosclerosis by inhibiting and reducing blood cholesterol levels caused by LDL oxidation or as an alternative, anthocyanins protect fat cell membranes from oxidation (Du *et al.*, 2015).

Bioactive compounds in black potato peels can be extracted through the extraction process. The decision of extraction method is important because the extraction result will determine the success rate of this method (Abubacker and Deepalakshmi, 2013). Conventional methods have the disadvantage that they require a long extraction time and a lot of solvent. Extraction optimization can use ultrasonic methods. The ultrasonic method uses ultrasonic waves, which are acoustic waves with frequencies greater than 16-20 KHz. The advantage of this method is that the extraction process does not need too much time (Zou *et al.*, 2014). Ultrasonic can also reduce the extraction temperature and can be used in the extraction of bioactive compounds that are not resistant to high temperatures (Zou *et al.*, 2014). The research of Rondang Tambun *et al* (2017) stated that the phenol content of red galangal extract using ultrasonic was highest at 9 hours with a temperature of 60°C at 45%. In addition, Handayani, Sriherfyna and Yunianta (2015) research stated that antioxidant extraction in soursop leaves was best treated at 45°C in 20 minutes with a yield of 11.72%, total phenol content of 15,213.33 ppm, 45,843 ppm flavonoid content, and antioxidant activity of 78.14%.

Agriculture has been using a lot of chemical fertilizers. Therefore, there is a solution in reducing chemical fertilizers, which can be harmful to the environment, and using organic fertilizers is recommended. One of the organic fertilizers that can be used is cow manure. Hafizah and Mukarramah (2017) stated that cow manure contains elements of Nitrogen, Phosphorus, Potassium and Carbon. This research was conducted to determine the phytochemical content and antioxidant activity of black potato plants grown in the lowlands with cow manure fertilizer using the ultrasonic bath method.

METHOD AND ANALYSIS

This research was conducted from June to October 2023 in the garden area of Wiyung and the laboratory of Biology Study Program, Universitas PGRI Adi Buana Surabaya. The tools used in this research are hoes, shovels, buckets, compost bags, spoons, balances, gauges, scales, ultrasonic baths, UV-Vis spectrophotometers, and glassware. The materials used were black potato, cow manure, EM4, anthracol 70 WP fungicide, kalebtin insecticide, top soil, absolute ethanol (Pa), DPPH solution, acrylic acid, Folin Ciocalteau reagent, 2% sodium carbonate, distilled water, gallic acid, 5% $NaNO_2$, 10% $AlCl_3$, Quercetin, Folin Phenol reagent, 35% Na_2CO_3.

Potato Tuber Planting and Treatments

There are several steps in planting potato tubers and its treatments, such as (1) preparation of fertilizer by fermentation with the addition of EM4. Cow manure is prepared as 20 kg of each. Mix each manure with 1 liter of EM4. Cover with a gunny sack and open every 3 days to remove the gas. If the fertilizer does not release water and does not smell after 4 weeks, then the fertilizer is ready to use; (2) the first cultivation is clearing the land of weeds and digging them in 20 cm in depth, then leaving them for 1 day. The second processing is loosening, then plotting; (3) the seedlings used were potato tubers with the same size and fresh. Seedlings were grown until 2 weeks old; (4) manure application was completed after land treatment and left for 1 week, then potato seedlings were planted with 4 levels of 0 kg/plot, 5 kg/plot, 10 kg/plot, 15 kg/plot, and NPK; (5) planting was made on research plots with a distance of 45x50 cm with a hole depth of 8 cm, with the position of the seedlings upright; (6) plant maintenance with watering and weed removal; and (7) harvesting is completed at 90 HST (Days After Planting).

Extraction of Black Potato Peels by Ultrasonic Bath Method

Extraction using ultrasonic bath method with different temperature and extraction time, with extraction temperature (55°C), extraction time (15 and 20 minutes) and solvent to material ratio (1:5 and 1:10).

Water Content Analysis

Analysis of water content in petri dishes was put in the oven (105°C) for 24 hours after that put in a desiccator for 15 minutes then weighed. The pulverized sample was weighed 2 grams in a container with a known constant weight and then oven at 100°C-105°C for 5 hours. After that, it was cooled in a desiccator for 15 minutes and weighed. Then heated again in the oven for 30 minutes then cooled in a desiccator and weighed. This treatment was repeated until a constant weight was reached.

$$\%\ Water\ Content = \frac{initial\ weight - final\ weight}{sample\ weight} \times 100\%$$

Yield Analysis

The resulting evaporated concentrated extract was weighed in a container of known weight then the weight of the concentrated extract was compared to the initial weight of the powder.

$$\%\ Yield = \frac{final\ sample\ weight}{initial\ sample\ weight} \times 100\%$$

Phytochemical Test

Total Phenol Test

The sample extract was dissolved into methanol to obtain a concentration of 1 mg/ml. A total of 100 µl of the mixture was added with 100 µl of 50% Folin Ciocalteau reagent. The mixture was incubated for 3 minutes at room temperature and 2 ml of 2% sodium carbonate was added. The volume of the mixture was made to 3 ml by adding distilled water. After that, the mixture was kept for 1 minute in a 100°C water bath and allowed to cool in a dark place. Samples were absorbed with a UV-Vis spectrophotometer at a wavelength of 720 nm. Total phenolics were calculated using a standard curve of 1 mg/ml gallic acid.

Calculation of total phenolics as follows:

$$TTC\ (\mu gGAE/mg) = \frac{c.v}{g} \times fp$$

Description:

c : Phenolic Concentration (x-value)
v : Volume (ml)
fp : Dilution factor
g : Weight of used sample (g)

Total Tannin Test

The sample extract was extracted as much as 0.1 ml, added to a volumetric flask containing 7.5 ml of distilled water and 0.5 ml of folin reagent, as well as 1 ml of 35% Na_2CO_3 dissolved in 10 ml of distilled water. The mixture was then shaken and kept at room temperature for 30 minutes. Gallic acid was used as a standard of 1 mg/ml. The sample and standard solutions were measured for absorbance using a UV-vis spectrophotometer at a wavelength of 725 nm. Calculation of total tannins as follows:

$$TTC\ (\mu gGAE/mg) = \frac{c.v}{g} \times fp$$

Description:
c : Tannin Concentration (x-value)
v : Volume (ml)
fp : Dilution factor
g : Weight of used sample (g)

Flavonoid Test

A sample extract of 1 mg/ml was taken as 20 µl, added with 150 µl of 5% $NaNO_2$ and incubated at room temperature for 5 min. Then, the sample was added with 2.5 ml of $AlCl_3$10% and incubated at room temperature for 6 minutes. The sample was absorbance at 510 nm wavelength. Total flavonoids were calculated using the standard curve of Quercetin from the preparation of 1 mg/ml Quercetin standard. Calculation of total flavonoids as follows:

$$TFC\ (\mu gQE/mg) = \frac{c.v}{g} \times fp$$

Description:
c : Flavonoid Concentration (x-value)
v : Volume (ml)
fp : Dilution factor
g : Weight of used sample (g)

Antioxidant Activity Test

10 mg of sample extract was dissolved into 10 ml of methanol. Stock solution of extract in methanol was made in series. 2 ml of extract solution was then added with 1 ml of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. The sample solution was then measured for absorbance using UV-Vis spectrophotometer with λ 517 nm. The decrease in absorbance value of DPPH solution indicates an increase in antioxidant activity against DPPH. The activity was measured by calculating the amount of reduction in purple light intensity of DPPH which is proportional to the reduction in DPPH concentration. The immersion is produced by the reaction of diphenyl picryl hydrazine molecules with hydrogen atoms released by the sample component molecules so that diphenyl picryl hydrazine compounds are formed and cause the decay of DPPH color from purple to yellow. Antioxidant activity (AOA) can be calculated with the following equation.

$$AOA\ (\%) = \frac{Ao - As}{Ao} \times 100$$

Description:
Ao : DPPH solution without sample (control)
As : DPPH solution with sample (control)

The percent inhibition of each sample concentration was plotted on the x and y axes of the linear regression equation, respectively. The linear regression equation obtained in the form of the equation y = a + bx was used to find the IC50 (Inhibition Concentration 50%) value of each sample by expressing the y value as 50 and the x value to be obtained as IC50. The compound is specifically categorized as a very strong antioxidant if the IC50 value is less than 50 ppm, strong for IC50 is 50-100 ppm, moderate if IC50 is 100-150 ppm, and

weak if IC50 is 151-200 ppm (Purwanto, Bahri and Ridhay, 2017).

Data Analysis

The research data were analyzed using the Anova test with a significance of $\alpha = 0.05$, if there is a difference, the Duncan test is used.

RESULT

Water Content

The results of the research on phytochemical content and antioxidant activity of black potato peels (Plectranthus rotundifolius) in the lowland with organic fertilizer for 90 HST and extraction method using ultrasonic bath are presented in the table below:

Table 1. Water Content (%) Black Potato Peel Extract 90 HST

Cow Manure (kg/plot)	Extraction Time (minutes)	Ingredient & Solvent Comparison	Water Content (%)
0	15	1:5	**4.13**
		1:10	
	20	1:5	
		1:10	
5	15	1:5	
		1:10	
	20	1:5	
		1:10	
10	15	1:5	
		1:10	
	20	1:5	
		1:10	
15	15	1:5	
		1:10	
	20	1:5	
		1:10	
NPK	15	1:5	
		1:10	
	20	1:5	
		1:10	

Based on Table 1, the results of black potato peels extraction process optimization response to the treatment of manure, extraction time and the ratio of ingredients and solvents indicated that the 5 kg/plot manure treatment with an extraction time of 20 minutes, a temperature of 55°C, and a ratio of ingredients and solvents of 1:10 was best than the other treatments at 4.13%.

Yield of Black Potato Peels

The yield results on black potato peel extract treated with cow manure after 90 HST with ultrasonic bath

extraction method are presented in Table 2.

Table 2. Yield (%) Black Potato Peel Extract 90 HST

Cow Manure (kg/plot)	Extraction Time (minutes)	Ingredient & Solvent Comparison	Yield (%)
0	15	1:5	9.31
		1:10	9.53
	20	1:5	9.87
		1:10	9.90
5	15	1:5	14.09
		1:10	16.21
	20	1:5	17.15
		1:10	**17.90**
10	15	1:5	15.17
		1:10	16.05
	20	1:5	16.21
		1:10	16.54
15	15	1:5	16.02
		1:10	16.10
	20	1:5	16.23
		1:10	16.36
NPK	15	1:5	16.45
		1:10	16.51
	20	1:5	16.73
		1:10	17.02

Based on Table 2, the yield results of the black potato peels extraction process optimization response to the treatment of manure, extraction time and the ratio of ingredients and solvents indicated that the 5 kg/plot manure treatment with an extraction time of 20 minutes, a temperature of 550°C, and a ratio of ingredients and solvents of 1:10 showed the best yield of 17.90%.

Phytochemical Content (Phenols, Tannins, and Flavonoids) of Black Potato Peels

The test results of phenol, tannin, and flavonoid levels in black potato peel extract treated with cow manure after 90 HST with ultrasonic bath extraction method are presented in Table 3.

Table 3. Phytochemical Content (Phenols, Tannins, and Flavonoids) of Black Potato Peel Extract 90 HST

Cow Manure (kg/plot)	Extraction Time (minutes)	Ingredient & Solvent Comparison	Average Total Phenol (g/g)	Average Total Tannin (g/g)	Average Total Flavonoid (g/g)
0	15	1:5	0,075	0,046	0,067
		1:10	0,096	0,048	0,070
	20	1:5	0,077	0,050	0,072
		1:10	0,076	0,052	0,075
5	15	1:5	0,164	0,054	0,087

		1:10	0,167	0,055	0,090
	20	1:5	0,165	0,058	0,087
		1:10	**0,177**	**0,063**	**0,096**
10	15	1:5	0,150	0,061	0,087
		1:10	0,154	0,060	0,091
	20	1:5	0,152	0,061	0,092
		1:10	0,150	0,060	0,094
15	15	1:5	0,138	0,060	0,081
		1:10	0,144	0,060	0,082
	20	1:5	0,143	0,060	0,083
		1:10	0,144	0,060	0,082
NPK	15	1:5	0,159	0,055	0,090
		1:10	0,159	0,061	0,090
	20	1:5	0,158	0,056	0,091
		1:10	0,154	0,056	0,093

Based on table 3, the best phenol, tannin and flavonoid levels in the 5 kg/plot manure treatment with an extraction time of 20 minutes, a temperature of 55°C, and a ratio of ingredients and solvents of 1:10, which are phenol 0.177 g/g, tannin 0.063 g/g, and flavonoids 0.096 g/g, compared to other treatments.

Antioxidant Activity IC50 of Black Potato Peel Extract

The results of antioxidant activity test IC50 of black potato peel extract treated with kendang fertilizer after 90 HST with ultrasonic bath extraction method are presented in Table 4.

Table 4. Antioxidant Activity IC50 (ppm) Black Potato Peel Extract 90 HST

Cow Manure (kg/plot)	Extraction Time (minutes)	Ingredient & Solvent Comparison	Antioxidant Activity IC50 (ppm)
0	15	1:5	68,055
		1:10	65,120
	20	1:5	63,918
		1:10	63,574
5	15	1:5	48,711
		1:10	47,852
	20	1:5	45,447
		1:10	**45,275**
10	15	1:5	65,378
		1:10	65,292
	20	1:5	64,235
		1:10	64,003
15	15	1:5	66,667
		1:10	66,451
	20	1:5	65,349
		1:10	65,152
NPK	15	1:5	62,615
		1:10	57,241

	20	1:5	57,454
		1:10	57,161

Based on Table 4, the antioxidant activity of black potato peels extract indicated the best antioxidant results in the 5 kg/plot manure treatment with an extraction time of 20 minutes, temperature 55°C with an IC50 value of 45.275 with a very strong category.

DISCUSSION

The use of ultrasonic bath extraction using ultrasonic waves, is an extraction by energy propagation through waves, using liquid as a propagation medium that can increase the intensity of energy transfer so that the extraction process is maximized, safer, shorter and suitable for the extraction of bioactive compounds that cannot withstand heat (Carreira-Casais *et al.*, 2021). Meanwhile, conventional extraction generally takes a long time, is less environmentally friendly, and has the potential to trigger damage to compounds, requires a lot of solvents and the extract obtained is less than optimal (Noor *et al.*, 2021).

The results of the analysis of raw material characteristics in the table indicated that the water content of black potato peels powder was 5.13%, because the black potato peels powder had been dried and reduced in size which caused the water content of black potato peels to decrease. Preliminary treatment of black potato peels can facilitate the next process, that is extraction, where the size reduction will cause microwaves to be well absorbed. According to Ratnayani *et al* (2021), potato peels flour (Solanum tuberosum) is a flour that has the potential to be developed as a food source of potassium. Potato peel flour with various soakings has characteristics of water content (7.84 - 9.03%). The result of checking the water content is 4.13%, this is to find out the percentage of water content in simplisia which is determined to maintain the quality of simplisia is ≤10% (Utami *et al.*, 2017). Water content that is too high (>10%) will cause the growth of various microbes such as mold and fungus. This can reduce the level of stability of the extract (Pamungkas, Dewi and Tapilouw, 2022).

The yield increases with the extraction time of natural materials, the more the extraction time is conducted, the more the yield will increase because the longer the extraction, the longer the contact between the material and the solvent (Supriwanti, Warsidah and Prayitno, 2023). In addition, table 2 indicates that the yield produced from black potato peels extract is different and the difference in yield produced from the extraction process is a response of materials and solvents to the treatment that has been applied, such as temperature and length of extraction time. Low temperatures can cause the transfer of a mass to occur slowly so that it takes longer to make a component out of the material (Scher, Brandelli and Noreña, 2015). Therefore, the longer the extraction time, the greater the opportunity for the material to contact the solvent and the results will also increase until the solution saturation point (Handayani, Sriherfyna and Yunianta, 2015).

The increase in yield can be caused by the amount of solute (antioxidant) diffusion ester substances from the solid matrix to the surface, the solute mass transfer rate increases the solubility of the solute in the solvent. The high and low yield of food ingredients is strongly affected by the water content of food ingredients. Temperature is one of the determining factors in the heating process, in addition to the nature of the heated material such as the initial water content and product size will affect the heating process (Sukma, Harsojuwono

and Arnata, 2017). The increase in temperature is able to soften the tissue of a plant, able to increase the extraction coefficient increases the diffusion rate and provides a higher extraction rate (Thirugnanasambandham, Sivakumar and Prakash Maran, 2014).

The length of extraction time can determine the amount of yield that can be extracted from the material. Based on research of Chairunnisa, Wartini and Suhendra (2019) stated that extraction using ultrasonic for 30 minutes with hexane solvent resulted in a total yield of 4%. Therefore, the longer the extraction will allow the material to contact with the solvent longer, so that the yield produced is greater. Appropriate and proper extraction time will produce the maximum quantity of yield as well. The amount of extracted yield will depend on the nature of the compound, extraction method, particle size, conditions, temperature and time, sample comparison as well as the type of solvent used (Mujipradhana, 2018).

Based on Table 3, the higher the ratio of ingredients and solvents causes the average total phenols, tannins and flavonoids to be higher and there is an effect between parameters. The more solvent added, the greater the ability of the solvent to dissolve the material so that more material components can be extracted by the solvent (Zhang, Lin and Ye, 2018). The extracted material components will continue to increase until the solution becomes saturated, after passing the saturation point of the solution, there will be no increase in extraction results with the addition of solvents (Handayani, Sriherfyna and Yunianta, 2015). According to Rondang Tambun *et al* (2017), temperature and time are two of several factors that affect extraction results, while other factors are the ratio of solvents and materials.

Phenol, tannin and flavonoid compounds are unstable in heat. The phenol content will be damaged at temperatures of 60°C and 80°C (S. Teixeira *et al.*, 2017). Phenol compounds are antioxidant compounds and these antioxidant properties will be oxidized in the presence of light, heat and oxygen (Kruk *et al.*, 2022). The levels of phenols, tannins, flavonoids and antioxidant activity are well correlated where the levels of phenol compounds, tannins and flavonoids are high, the stronger the antioxidant activity. The IC50 value of black potato peels extract was 45.275 with a very strong category. The category is very strong if the IC50 value is less than 50 ppm, strong for IC50 worth 50-100 ppm, moderate if IC50 worth 100-150 ppm, and weak if IC50 worth 151-200 ppm (Purwanto, Bahri and Ridhay, 2017). Tannin and flavonoid compounds are phenolic group compounds that have antioxidant activity properties. Phenolic compounds have the ability as antioxidants because of their ability in redox reactions that allow these compounds to act as reducing agents, hydrogen donors, free radical catchers. Some types of phenolics can have different antioxidant activities depending on their structure (B. Prabawati *et al.*, 2021).

Tannins are phenolic compounds that have a high molecular weight. The mechanism of tannin performance in preventing free radicals by capturing free radicals in a complex kinetic manner (Riedl *et al.*, 2002). According to Pietta (2000), flavonoids are phenolic compounds that are widely distributed in plants with a C6-C5-C6 (C15) skeleton that has high antioxidant activity. Flavonoids have high antioxidant activity due to the presence of hydroxyl groups in their chemical structure that can be donated to neutralize free radicals. The content of flavonoids, hydroxybenzoic and hydroxycinnamic acids in food can increase cellular antioxidant activity (Khanam and Oba, 2013).

The application of 5 kg/plot manure gave good test results for phenol, tannin,

and flavonoid levels compared to the 10-15 kg/plot manure treatment which presented in table 1. According to Hawkesford *et al* (2012), manure contains nitrogen, mineral nutrients, and especially nitrogen. Nitrogen is one of the main environmental factors that affect plant development, physiology, and metabolism because nitrogen is the nutrient most consumed by plants, and is needed for most metabolic processes, one of the important factors for plant development and function, and is known as a major limiting factor in many agricultural systems (Saloner and Bernstein, 2022). Therefore, there is much evidence on the impact of nitrogen supply on crop yield (Lin *et al.*, 2019) and on plant secondary metabolism (Rioba *et al.*, 2015).

CONCLUSION

It is found that progressive muscle relaxation therapy, as opposed to lemon aromatherapy, can lower blood sugar levels in diabetics more steadily. The goals of both interventions are to suppress stress feedback and promote physical relaxation through relaxation exercises. In a relaxed condition, the parasympathetic nervous system will take over, which can slow down the heart's rate of contraction and increase the body's production of the hormone insulin, which the body needs for blood glucose homeostasis.

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REFERENCES

Abubacker, M.N. and Deepalakshmi, T. (2013) 'In vitro Antifungal Potentials of Bioactive Compound Methyl Ester of Hexadecanoic Acid Isolated from Annona muricata Linn. (Annonaceae) Leaves', *Biosciences Biotechnology Research Asia*, 10(2), pp. 879–884. Available at: https://doi.org/10.13005/bbra/1211.

B. Prabawati, N.B. *et al.* (2021) 'Edible Flowers: Antioxidant Compounds and Their Functional Properties', *Horticulturae*, 7(4), p. 66. Available at:https://doi.org/10.3390/horticulturae7040066.

Carreira-Casais, A. *et al.* (2021) 'Benefits and Drawbacks of Ultrasound-Assisted Extraction for the Recovery of Bioactive Compounds from Marine Algae', *International Journal of Environmental Research and Public Health*, 18(17), p. 9153. Available at: https://doi.org/10.3390/ijerph18179153.

Chairunnisa, S., Wartini, N.M. and Suhendra, L. (2019) 'Pengaruh Suhu dan Waktu Maserasi terhadap Karakteristik Ekstrak Daun Bidara (Ziziphus mauritiana L.) sebagai Sumber Saponin', *Jurnal Rekayasa dan Manajemen Agroindustri*, 7(4), pp. 551–560.

Dayu Ardani, P., Suminarti, N.E. and Nugroho, A. (2017) 'Respon Tanaman Kentang Hitam (Solenostemon Rotundifolius) pada Berbagai Jumlah dan Frekuensi Pemberian Air', *Biotropika - Journal of Tropical Biology*, 5(3).

Du, H. *et al.* (2015) 'Methylation mediated by an anthocyanin, O - methyltransferase, is involved in purple flower coloration in Paeonia', *Journal of Experimental Botany*, 66(21), pp. 6563–6577. Available at: https://doi.org/10.1093/jxb/erv365.

Francenia Santos-Sánchez, N. *et al.* (2019) 'Antioxidant Compounds and Their Antioxidant Mechanism', in *Antioxidants*. IntechOpen. Available at:https://doi.org/10.5772/intechopen.8527.

Hafizah, N. and Mukarramah, R. (2017) 'Aplikasi Pupuk Kandang Kotoran Sapi pada Pertumbuhan dan Hasil Tanaman Cabai Rawit (Capsicum frustescens L.) di Lahan Rawa Lebak', *ZMIP Ziraa'ah Majalah Ilmiah Pertanian*, 42(1).

Handayani, H., Sriherfyna, F.H. and Yunianta, Y. (2015) 'Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan: Pelarut dan Lama Ekstraksi)', *Jurnal Pangan Dan Agroindustri*, 4(1).

Hawkesford, M. *et al.* (2012) 'Functions of Macronutrients', in *Marschner's Mineral Nutrition of Higher Plants*. Elsevier, pp. 135–189. Available at: https://doi.org/10.1016/B978-0-12-384905-2.00006-6.

Khanam, U.K.S. and Oba, S. (2013) 'Bioactive substances in leaves of two amaranth species, Amaranthus tricolor and A. hypochondriacus', *Canadian Journal of Plant Science*, 93(1), pp. 47–58. Available at: https://doi.org/10.4141/cjps2012-117.

Kruk, J. *et al.* (2022) 'Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise', *The Journal of Physiological Sciences*, 72(19).

Lin, Y. *et al.* (2019) 'Effect of Plant Growth-Promoting Rhizobacteria at Various Nitrogen Rates on Corn Growth', *Agricultural Sciences*, 10(12), pp. 1542–1565. Available at: https://doi.org/10.4236/as.2019.1012114.

Mujipradhana, V.N. (2018) 'Aktivitas Antimikroba dari Ekstrak Ascidian Herdmania Momus pada Mikroba Patogen Manusia', *Jurnal Ilmiah Pharmacon*, 7(3). Available at: https://doi.org/https://doi.org/10.35799/pha.7.2018.20601.

Noor, N.Q.I.M. *et al.* (2021) 'Application of Green Technology in Gelatin Extraction: A Review', *Processes*, 9(12), p. 2227. Available at: https://doi.org/10.3390/pr9122227.

Nugraheni, M., Hamidah, S. and Auliana, R. (2016) 'The Effect of Coleus Tuberosus Containing Resistant Starch Type 3 Crackers on Hypercholesterolemia Suffering Animals', *Jurnal Penelitian Saintek*, 21(1), pp. 21–31.

Pamungkas, E.M.P., Dewi, L. and Tapilouw, M.C. (2022) 'Penambahan angkak (monascus purpureus) pada tempe dalam peningkatan antioksidan', *Teknologi Pangan : Media Informasi dan Komunikasi Ilmiah Teknologi Pertanian*, 13(2), pp. 144–155. Available at: https://doi.org/10.35891/tp.v13i2.292.

Pietta, P.-G. (2000) 'Flavonoids as Antioxidants', *Journal of Natural Products*, 63(7), pp. 1035–1042. Available at: https://doi.org/10.1021/np9904509.

Purwanto, D., Bahri, S. and Ridhay, A. (2017) 'Uji Aktivitas Antioksidan Ekstrak Buah Purnajiwa (Kopsia Arborea Blume.) dengan Berbagai Pelarut', *Kovalen Jurnal Riset Kimia*, 3(1).

Ratnayani, R. *et al.* (2021) 'Karakteristik Tepung Kulit Kentang (Solanum Tuberosum) sebagai Bahan Pangan Sumber Kalium', *J-KESMAS: Jurnal Kesehatan Masyarakat*, 7(2), p. 137. Available at: https://doi.org/10.35329/jkesmas.v7i2.1993.

Riedl, K.M. *et al.* (2002) 'Antioxidant Activity of Tannins and Tannin-

Protein Complexes: Assessment In Vitro and In Vivo', in, pp. 188–200. Available at: https://doi.org/10.1021/bk-2002-0807.ch014.

Rioba, N.B. *et al.* (2015) 'Effects of nitrogen, phosphorus and irrigation frequency on essential oil content and composition of sage (Salvia officinalis L.)', *Journal of Applied Research on Medicinal and Aromatic Plants*, 2(1), pp. 21–29. Available at: https://doi.org/10.1016/j.jarmap.2015.01.003.

Rondang Tambun *et al.* (2017) 'Pengaruh Ukuran Partikel, Waktu dan Suhu pada Ekstraksi Fenol dari Lengkuas Merah', *Jurnal Teknik Kimia USU*, 5(4), pp. 53–56. Available at: https://doi.org/10.32734/jtk.v5i4.1555.

S. Teixeira, T. *et al.* (2017) 'Antioxidant Potential and its Correlation with the Contents of Phenolic Compounds and Flavonoids of Methanolic Extracts from Different Medicinal Plants', *Revista Virtual de Química*, 9(4), pp. 1546–1559. Available at: https://doi.org/10.21577/1984-6835.20170090.

Saloner, A. and Bernstein, N. (2022) 'Nitrogen Source Matters: High NH4/NO3 Ratio Reduces Cannabinoids, Terpenoids, and Yield in Medical Cannabis', *Frontiers in Plant Science*, 13. Available at: https://doi.org/10.3389/fpls.2022.830224.

Scher, C.F., Brandelli, A. and Noreña, C.Z. (2015) 'Yacon Inulin Leaching during Hot Water Blanching', *Ciência e Agrotecnologia*, 39(5), pp. 523–529. Available at: https://doi.org/10.1590/S1413-70542015000500011.

Sukma, I.W.A., Harsojuwono, B.A. and Arnata, I.W. (2017) 'Pengaruh Suhu dan Lama Pemanasan Ekstraksi terhadap Rendemen dan Mutu Alginat dari Rumput Laut Hijau Sargassum sp', *Jurnal Rekayasa dan Manajemen Agroindustri*, 5(1).

Supriwanti, S., Warsidah, W. and Prayitno, D.I. (2023) 'Characterization of Seaweed Caragenan Eucheuma cottonii and Its Aplication as Edible coating', *BERKALA SAINSTEK*, 11(2), p. 114. Available at: https://doi.org/10.19184/bst.v11i2.33865.

Thirugnanasambandham, K., Sivakumar, V. and Prakash Maran, J. (2014) 'Process optimization and analysis of microwave assisted extraction of pectin from dragon fruit peel', *Carbohydrate Polymers*, 112, pp. 622–626. Available at: https://doi.org/10.1016/j.carbpol.2014.06.044.

Utami, Y.P. *et al.* (2017) 'Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem (Clerodendrum minahassae Teisjm. & Binn.)', *Journal of Pharmaceutical and Medicinal Sciences*, 2(1).

Zhang, Q.-W., Lin, L.-G. and Ye, W.-C. (2018) 'Techniques for extraction and isolation of natural products: a comprehensive review', *Chinese Medicine*, 13(20).

Zou, T.-B. *et al.* (2014) 'Ultrasound-Assisted Extraction of Mangiferin from Mango (Mangifera indica L.) Leaves Using Response Surface Methodology', *Molecules*, 19(2), pp. 1411–1421. Available at: https://doi.org/10.3390/molecules19021411.