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"Research on the antioxidant properties of Radish Leaf Extract from Split Leaf and Plate Leaf species"

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1. Introduction

This study explores the antioxidant properties of radish leaves (from Split Leaf and Plate Leaf varieties) to provide scientific data supporting the future use of radish leaf extract in cosmetic formulations. It also aims to identify the variety with stronger antioxidant activity, which could help farmers improve radish yield and added value. Plant-derived phenolic compounds in radish leaves have gained attention in the pharmaceutical, health food, and cosmetic industries, and this study lays the foundation for developing natural skincare ingredients.

2. Materials and Methods

2-1 Equipment

Bio tek elisa reader、Ultrasonic Cleaner（aslmtg DC300）、pH Meter 、VORTEX-2 GENIE、Centrifuge MINI-6K、Di gital water bath SB-100**,** EYELA、Mettler toledo Balance、Hot air circulation oven（DO30）。

2-2 . Chemical

	Reagent	Supplier
1	Gallic acid monohydrate, 98%	Uni-Onward Corp.
2	Sodium carbonate, ≥99.5%, MW = 105.99 g/mol	Uni-Onward Corp.
3	Trichloroacetic acid, ≥99%, MW = 163.38 g/mol	Uni-Onward Corp.
4	Potassium dihydrogen phosphate, 98%, MW = 136.09 g/mol	Echo Chemical Co., Ltd.
5	Potassium hydrogen phosphate, 98%, MW = 174.2 g/mol	Echo Chemical Co., Ltd.
6	Potassium hexacyanoferrate(III), 99.98%, MW = 329.24 g/mol	Uni-Onward Corp.

7	Folin–Ciocalteu's phenol reagent, 2N, MW = 94.111 g/mol	Uni-Onward Corp.
8	2,2-Diphenyl-1-picrylhydrazyl (DPPH), ≥95%, MW = 394.32 g/mol	Uni-Onward Corp.
9	Butylated hydroxytoluene (BHT), MW = 220.35 g/mol	Uni-Onward Corp.
10	Potassium peroxodisulfate, MW = 270.32 g/mol	Uni-Onward Corp.
11	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), MW = 548.68 g/mol	Uni-Onward Corp.
12	Tris(hydroxymethyl)aminomethane, ≥99.8%, MW = 121.14 g/mol	Echo Chemical Co., Ltd.
13	Hydrochloric acid, 12N, MW = 36.46 g/mol	Echo Chemical Co., Ltd.
14	Ethanol, 95%, MW = 46.07 g/mol	Echo Chemical Co., Ltd.
15	Iron(III) chloride, 97%, MW = 270.29 g/mol	Uni-Onward Corp.
16	Distilled and deionized water	

2-3 . Extraction of Radish Leaf Samples:

Radish leaves were divided into three groups: A1 (Split Leaf type), A2 (Common Plate Leaf type), and A3 (Organic Plate Leaf type). For each group, 5 g of leaves were mixed with 25 g of distilled water. The mixtures were incubated in a water bath at 60°C for 180 minutes. After incubation, the samples were filtered using a vacuum filtration system. The remaining residues were dried and weighed. The sample concentration was calculated, then diluted appropriately for absorbance measurements.

2-4 . DPPH Radical Scavenging Assay:

The DPPH radical scavenging assay was conducted following the method of Gamba et al. (2021) with slight modifications. Briefly, 100 µL of sample, 400 µL of Tris-HCl buffer, and 500 µL of DPPH solution in ethanol were mixed and shaken. The mixture was kept in the dark at room temperature for 20 minutes. After incubation, 200 µL of the mixture was transferred to a 96-well plate, with three replicates per sample. Absorbance was measured at 517 nm using an ELISA reader. The scavenging activity was calculated. A standard curve was generated using different concentrations of BHT in ethanol.

2-5 . $ABTS^{+}$ Radical Scavenging Assay

The $ABTS^{+}$ radical scavenging assay was performed based on Gamba et al. (2021) with modifications. ABTS stock solution was prepared and incubated at 4°C in the dark for 16 hours. The solution was then diluted with distilled water to an absorbance of 0.7 ± 0.02 at 734 nm. In the assay, 70 µL of sample and 630 µL of diluted ABTS solution were mixed and shaken. The

mixture was incubated in the dark for 10 minutes. Afterward, 200 µL was transferred to a 96-well plate, with three replicates per sample. Absorbance was measured at 734 nm. The scavenging percentage was calculated, and a standard curve was prepared using BHT in ethanol.

2-4 . Determination of Total Phenolic Content (TPC)

The TPC was determined following the method of Parsaei et al. (2013) with slight modifications. Solutions of 7.5% sodium carbonate and 10% Folin reagent were prepared. In the assay, 100 µL of sample was mixed with 500 µL of 10% Folin reagent and shaken. After standing in the dark at room temperature for 5 minutes, 400 µL of 7.5% sodium carbonate was added and mixed. The mixture was incubated in the dark for 30 minutes. Then, 200 µL was transferred to a 96-well plate, with three replicates per sample. Absorbance was measured at 765 nm. Gallic acid was used to prepare a standard curve.

2-5 . Determination of Reducing Power

The reducing power was determined according to the method of Chiou et al. (2009) with modifications. Solutions of 0.2 M potassium phosphate buffer, 10% TCA, 1% potassium ferricyanide, and 1% ferric chloride were prepared. For the assay, 200 µL of sample, 200 µL of phosphate buffer, and 200 µL of potassium ferricyanide were mixed and shaken. The mixture was heated in a water bath at 50°C for 20 minutes, then cooled to room temperature. Next, 200 µL of 10% TCA was added, followed by centrifugation at 6000 rpm for 5 minutes.After centrifugation, 600 µL of the supernatant was mixed with 600 µL of distilled water and 120 µL of 1% ferric chloride (or 720 µL of water if ferric chloride was not added). The mixture was shaken and kept in the dark at room temperature for 10 minutes. Finally, 200 µL was transferred to a 96-well plate, with three replicates per sample. Absorbance was measured at 700 nm. A standard curve was prepared using ascorbic acid.

3. Results

3-1. DPPH Radical Scavenging Assay

Using BHT as the standard, the calibration curve was obtained as shown in Figure 1, with the equation $y = 0.4666x + 9.233$ ($R^2 = 0.9963$). As shown in Figure 2, the IC50 values for DPPH free radical scavenging were:Split Leaf type (normal farming) : 74.32,Common Plate Leaf type (normal farming) : 40.16,Organic Plate Leaf type (natural farming) : 66.74,BHT (standard) : 87.37.Among them, the antioxidant ability ranked as : Common Plate Leaf type > Organic Plate Leaf type > Split Leaf type > BHT(standard).

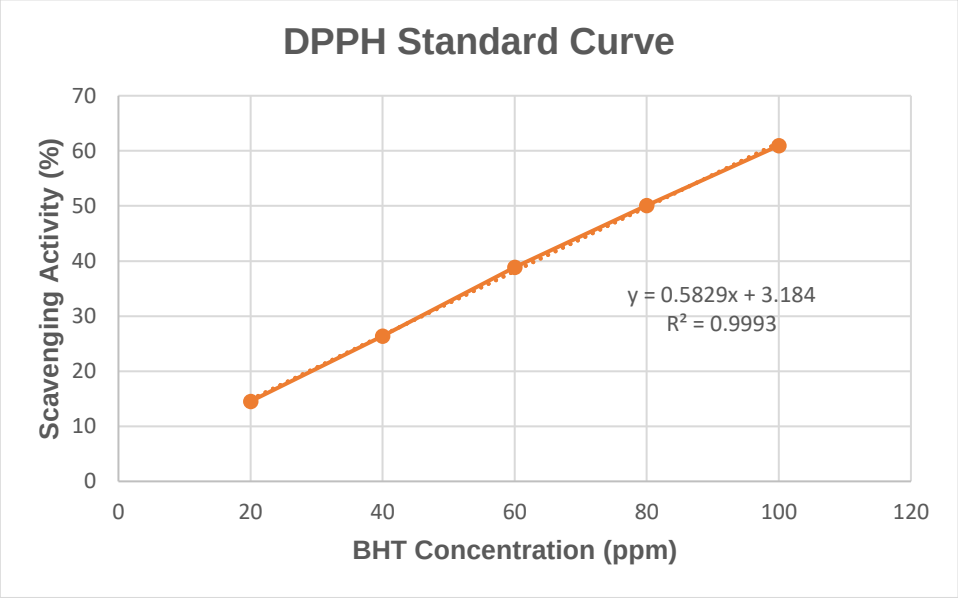

Figure 1. IC50 values of DPPH scavenging for different radish leaf types.

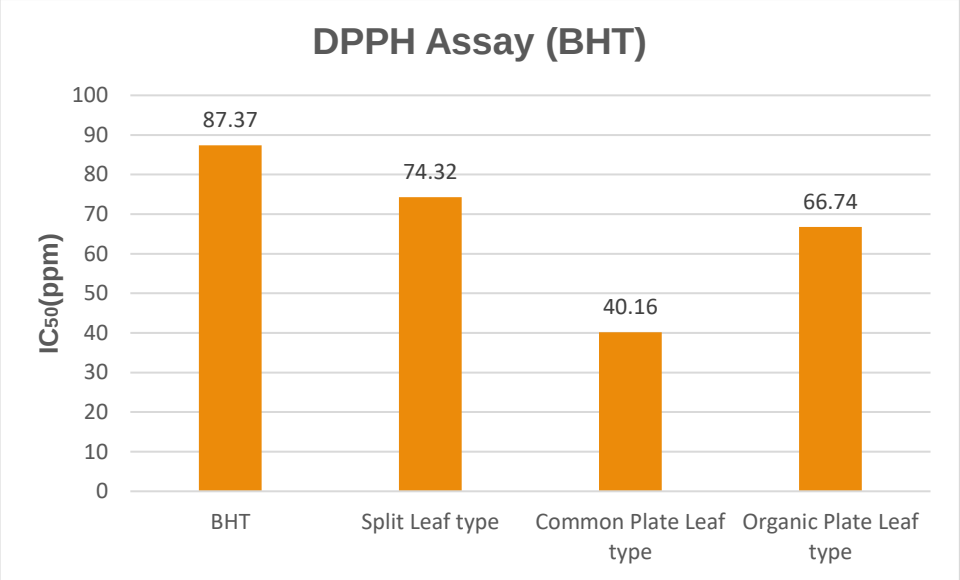

Figure 2. DPPH free radical scavenging capacity IC50 of different varieties and planting methods

3-2. ABTS⁺ Radical Scavenging Assay

Using BHT as the standard, the calibration curve was obtained as shown in Figure 3, with the equation y = 0.6562x + 5.943 (R^2 = 0.999).

As shown in Figure 4, the IC50 values for ABTS⁺ free radical scavenging were : Split Leaf type : 16.41, Common Plate Leaf type : 11.58 , Organic Plate Leaf type : 14.21, BHT (standard) : 67.14 . The antioxidant ability ranked as :Common Plate Leaf type > Organic Plate Leaf type > Split Leaf type > BHT (standard).

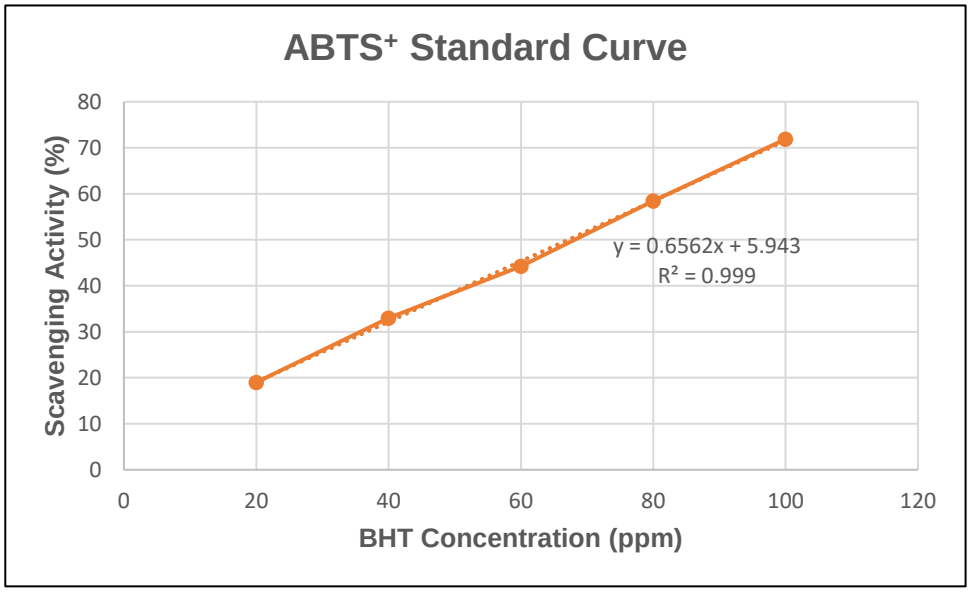

Figure 3. Standard curve of ABTS⁺ scavenging using BHT as the standard.

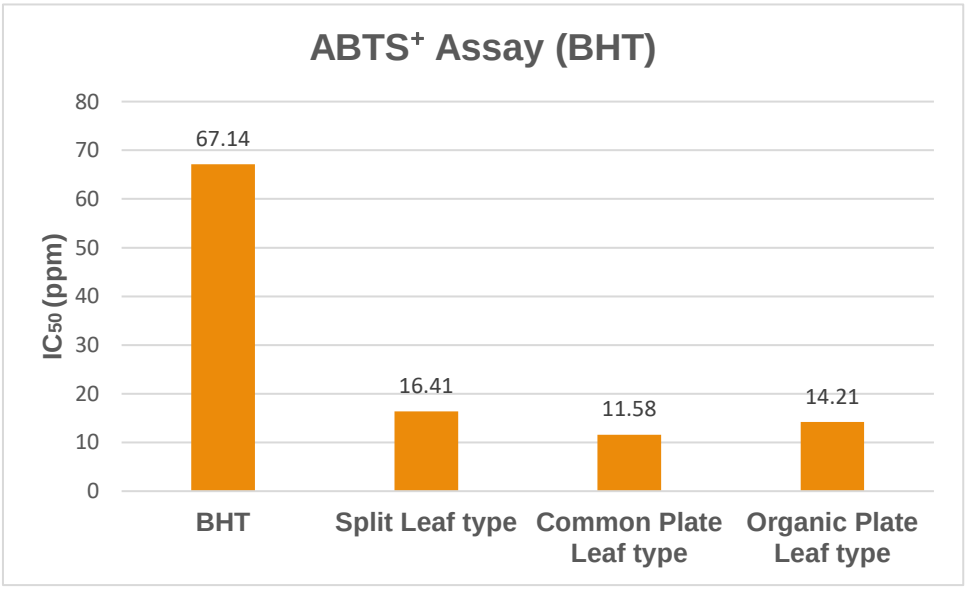

Figure 4. IC50 values of ABTS⁺ scavenging for different radish leaf types.

3-3. Determination of Total Phenolic Content (TPC)

Using gallic acid as the standard, the calibration curve was obtained as shown in Figure 5, with the equation y = 0.0025x + 0.0552 (R^2 = 0.9989). As shown in Figure 6, the relative gallic acid concentrations for total phenolic content were: Split Leaf type : 130.99 ppm, Common Plate Leaf type : 262.12 ppm, Organic Plate Leaf type : 218.79 ppm. The antioxidant capacity ranked as : Common Plate Leaf type > Organic Plate Leaf type > Split Leaf type.

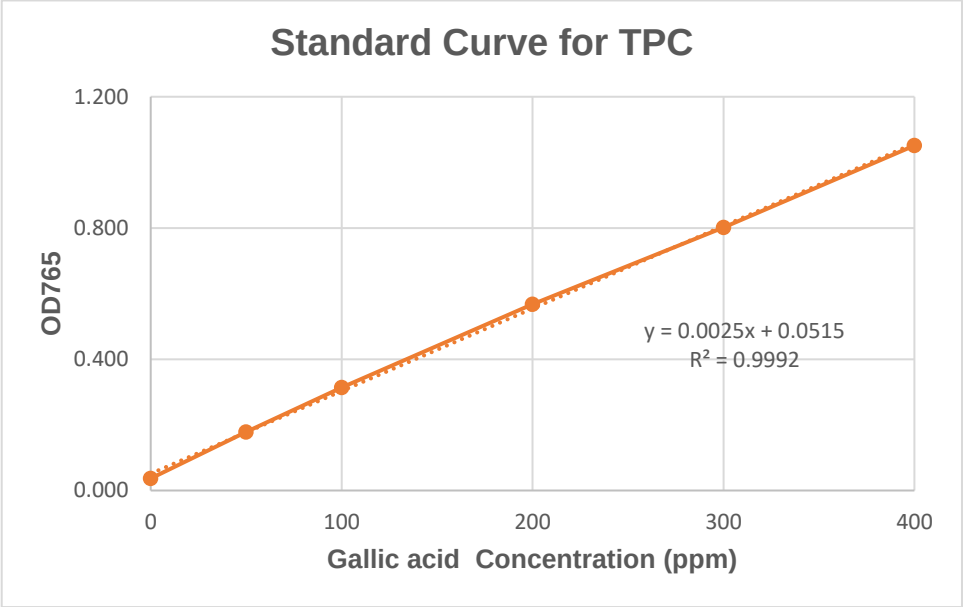

Figure 5.Standard curve of total phenolic content using gallic acid as the standard.

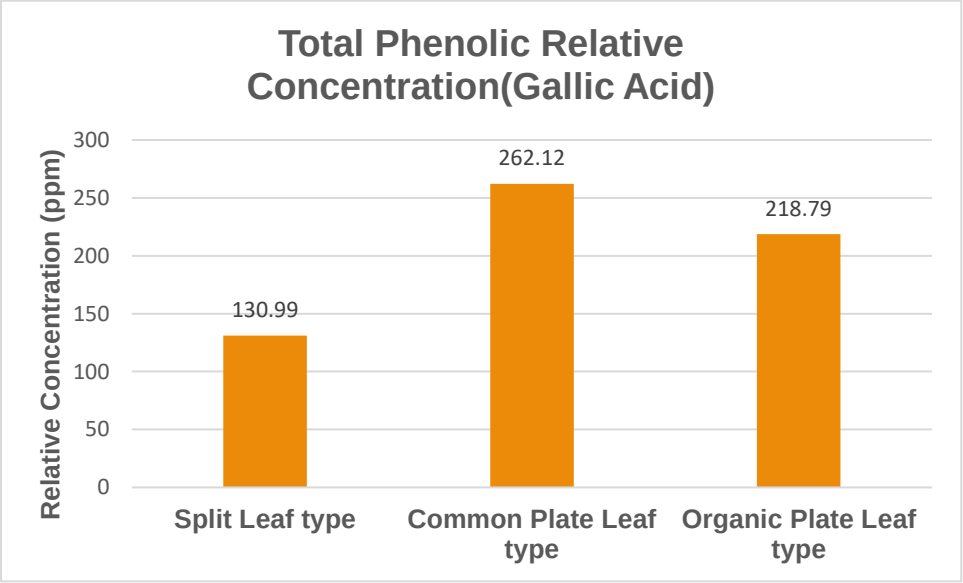

Figure 6. Relative gallic acid concentrations for different radish leaf types.

3-4. Reducing Power Assay

Using ascorbic acid as the standard, the calibration curve was obtained as shown in Figure 7, with the equation y = 0.7389x + 2.8247 (R^2 = 0.9971). As shown in Figure 8, the IC50 values for total reducing power were: Split Leaf type : 50.76, Common Plate Leaf type : 26.50, Organic Plate Leaf type : 27.82. The antioxidant ability ranked as : Common Plate Leaf type > Organic Plate Leaf type > Split Leaf type.

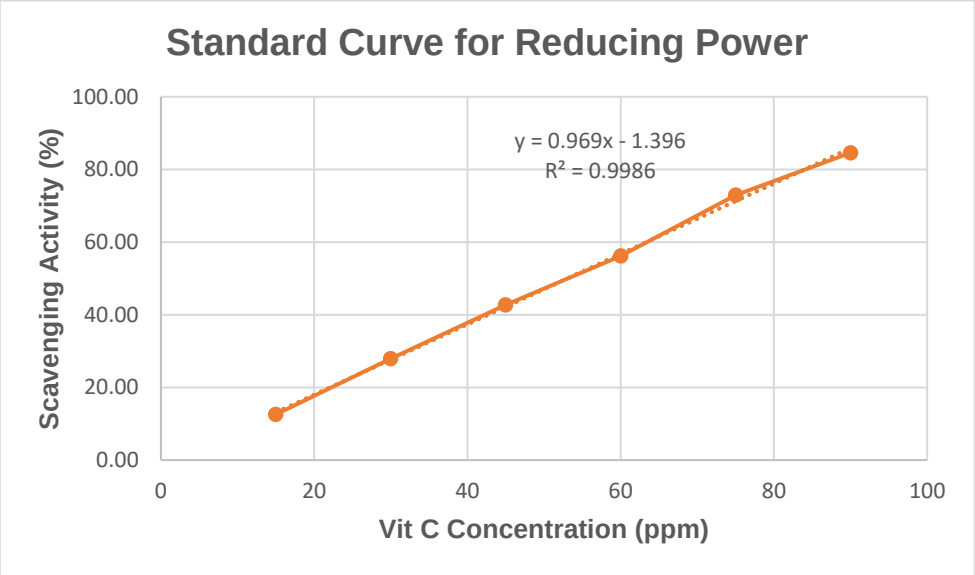

Figure 7.Standard curve of reducing power using ascorbic acid as the standard.

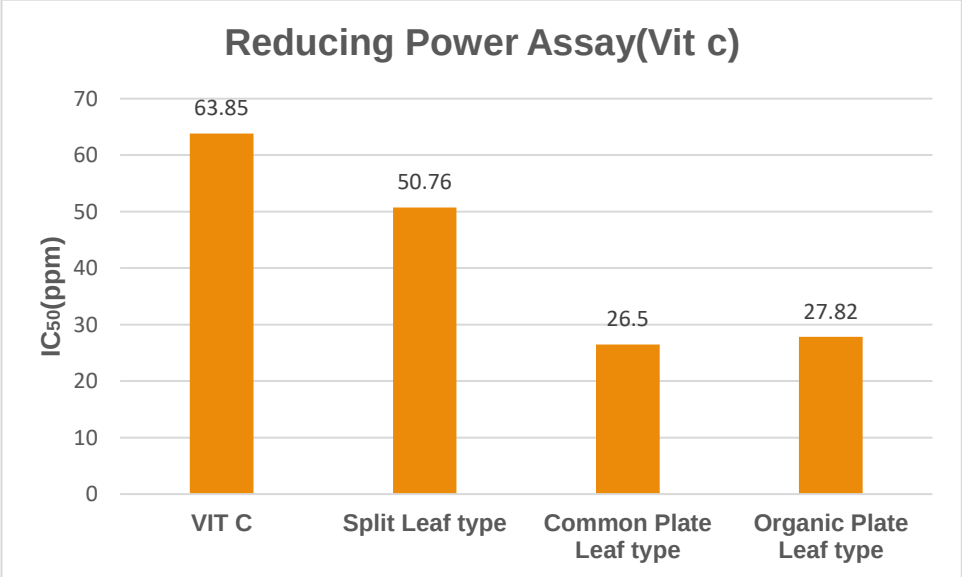

Figure 8. IC50 values of reducing power for different radish leaf types.

4. Conclusion

As shown in the figure above, different types of radish leaves and farming methods showed clear differences in antioxidant activity (Sonam, 2019). Based on the results, the dried leaves of the regular farming Plate Leaf variety had the best performance compared to the other samples and the standards in all four tests: DPPH radical scavenging (IC_{50} = 40.16 µg/mL), $ABTS^{+}$ radical scavenging (IC_{50} = 11.58 µg/mL), total phenolic content (262.12 ppm gallic acid equivalent), and reducing power (IC_{50} = 26.50 µg/mL). This shows that the Plate Leaf radish leaves grown by regular farming have very strong antioxidant activity. These extra radish leaves could be dried and stored to keep their activity and be used as ingredients in cosmetic products.

5. REFERENCES / ACKNOWLEDGEMENT

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