

# The Effect of Post-harvest Treatment on Antioxidant Properties of the White and Red Dragon Fruit

Nahashon Mwaura<sup>1</sup>, Justus Wambua Mukavi<sup>1,2</sup>, Beth Ndunda<sup>1</sup>, Joyce Gatiiria Njagi Kithure<sup>1</sup>, Leonidah Kerubo Omosa<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry, University of Nairobi, Nairobi, KENYA.

<sup>2</sup>Institute of Pharmaceutical Biology and Phytochemistry, University of Münster, Corrensstrasse, Münster, GERMANY.

## ABSTRACT

**Introduction:** Dragon fruits are widely used for their preventive and therapeutic effects against many chronic conditions due to the wide range of biological qualities associated with betalains and phenolic compounds. However, detailed reports on the antioxidant properties of various parts of the white and red dragon fruits and preferable post-harvest treatment modes are lacking and hence the current study. **Objectives:** To evaluate the antioxidant properties of air and oven-dried red and white dragon fruits grown in Meru, Kenya. **Materials and Methods:** Pre-developed thin layer chromatographic plates were sprayed with 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) to evaluate the extracts of *Hylocereus undatus* and *Hylocereus polyrhizus* for their preliminary free radical scavenging activity. The extracts exhibiting the most promising antioxidant activity were subjected to additional investigation to determine their radical scavenging activity using spectroscopy. **Results:** 6 air-dried samples displayed possible antioxidant activity in the initial TLC technique, compared to four oven-dried samples. The ability of moderately polar extracts to scavenge free radicals was significantly lower ( $IC_{50}=1.290-2.152$ ) than that of polar extracts ( $IC_{50}=0.497-0.768$ ). The white-fleshed dragon fruit pulp and the red-fleshed dragon fruit skin were found to have higher radical scavenging activity ( $92.65\pm 0.001$  and  $54.62\pm 0.002$ , respectively). **Conclusion:** The antioxidant activity of the dragon fruit pulp does not appear to be impacted by oven drying at 40°C; however, the activity of the fruit skin and bracts is significantly reduced. The dragon fruit part, extraction solvent system and the drying conditions all impact the antioxidant activity.

**Keywords:** Antioxidant activity, Dragon fruit, *Hylocereus*, 1,1-Diphenyl-2-picrylhydrazyl (DPPH).

## Correspondence:

**Prof. Leonidah Kerubo Omosa**

Department of Chemistry, University of Nairobi, P. O. Box 30197-00100, Nairobi, KENYA.

Email: leoomosa@gmail.com,  
lkerubo@uonbi.ac.ke

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## INTRODUCTION

Oxidative stress is characterized broadly as a shift in the pro-oxidant-antioxidant balance in favor of the former that results in potential harm due to the numerous and different impacts that oxygen toxicity can have on a cell. Oxidative stress is now understood to be a key player in the pathophysiology of a wide range of illnesses, including cerebrovascular diseases, cancer, Parkinson's disease, arteriosclerosis and pregnancy problems.<sup>1,2</sup> When the body's natural antioxidant defenses are overwhelmed by the generation of free radicals such as reactive oxygen species, oxidative stress results.<sup>2</sup> Free radicals can be defined as any chemical species that contains unpaired electrons. Unpaired electrons make an atom or molecule more chemically reactive. The Hydroxyl radical (HO<sup>•</sup>), Superoxide anion (O<sub>2</sub><sup>•-</sup>), Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), transition metals like iron and copper, Nitric

Oxide (NO) and peroxynitrite (ONOO) are examples of free radicals that are frequently encountered in nature.<sup>3</sup>

Antioxidants, on the other hand, are protective mechanisms that counteract the harm brought on by free radicals. Antioxidants are found in a variety of dietary supplements, nutraceuticals and functional food additives. They support health promotion and food preservation by preventing oxidation processes.<sup>4,5</sup> The potential harmful effects of synthetic antioxidants in foods, however, have raised significant concerns. Consequently, the demand for new, safe and cheap natural antioxidants has increased.<sup>6</sup> Antioxidants derived from plants can prevent the production of free radicals and/or stop the spread of oxidative stress. The phenolic acids, phenolic diterpenes, flavonoids and volatile oils are typically responsible for this antioxidant activity.<sup>7</sup>

Dragon fruit, commonly known as pitahaya or pitaya is a plant that is native to the tropics. It is a member of the genus *Hylocereus* (Cactaceae), which has approximately 16 species.<sup>8</sup> The fruit is typically spherical, oval, or pear-shaped, with many tiny, delectable black seeds scattered throughout and pulp that is sweet or sour in flavor.<sup>9</sup> In Kenya, red dragon fruit (*H. polyrhizus*)



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and white dragon fruit (*H. undatus*) are the two species that are most commonly encountered with both having red skin. The red and white varieties of dragon fruit differ only in that the red dragon fruit also has red pulp and pink bracts, whereas the white dragon fruit only has white pulp and greenish bracts.<sup>10</sup> The red dragon fruit is typically utilized as a food additive due to its color, while the native Americans utilize the white dragon fruit's pulp as a form of homeopathic medicine to treat cuts and bruises.<sup>11</sup> Both species have a wealth of different nutrients, vitamins and minerals as well as excellent therapeutic values. They can decrease cholesterol, improve digestion, strengthen the immune system and help with weight loss. The fruits have also been used to manage inflammation, cancer, heart disease, diabetes, brain dysfunction and other degenerative illnesses.<sup>12-17</sup> Betalains,

phenolics and dietary fibers have been identified as the primary bioactive constituents in dragon fruits.<sup>13,18-20</sup>

The utilization of dragon fruits as natural antioxidants has been the subject of numerous earlier studies to ascertain their total phenolic content and antioxidant activity.<sup>12,14,21-24</sup>

However, the antioxidant properties of the bracts, peels and flesh of both the white and red dragon fruits, either oven-dried and air-dried, have not been comprehensively compared. Additionally, the antioxidant properties of various solvent extracts of the aforementioned fruit parts have not yet been documented. In view of the growing interest in finding promising new sources of natural antioxidants, the current work set out to examine the antioxidant effects of various solvent extracts of oven-dried and air-dried bracts, peels (skin) and pulps of *H. polyrhizus* and *H. undatus*.

**Table 1: Percentage yield of the extracts of white Dragon fruit.**

Solvent system for extraction	Fruit part	White Dragon fruit					
		Air dried powder (g)	Extract (g)	% Yield	Oven dried powder	Extract (g)	% Yield
50% CH <sub>2</sub> Cl <sub>2</sub> / <i>n</i> -hexane	Pulp	10.09 (A-WP 1)	0.06	0.60	10.09 (O-WP 1)	0.42	4.14
	Skin	10.09 (A-WS 1)	0.13	1.33	10.09 (O-WS 1)	0.41	4.11
	Bracts	10.09 (A-WB 1)	0.12	1.16	10.09 (O-WB 1)	0.38	3.75
100% CH <sub>2</sub> Cl <sub>2</sub>	Pulp	10.09 (A-WP 2)	0.03	0.29	10.09 (O-WP 2)	0.01	0.06
	Skin	10.09 (A-WS 2)	0.03	0.27	10.09 (O-WS 2)	0.02	0.22
	Bracts	10.09 (A-WB 2)	0.12	1.16	10.09 (O-WB 2)	0.13	1.25
50% CH <sub>2</sub> Cl <sub>2</sub> /MeOH	Pulp	10.09 (A-WP 3)	1.00	9.91	10.09 (O-WP 3)	2.47	24.45
	Skin	10.09 (A-WS 3)	0.08	0.80	10.09 (O-WS 3)	0.15	1.52
	Bracts	10.09 (A-WB 3)	0.12	1.16	10.09 (O-WB 3)	0.25	2.50
100% MeOH	Pulp	10.09 (A-WP 4)	3.33	33.00	10.09 (O-WP 4)	0.53	5.28
	Skin	10.09 (A-WS 4)	0.16	1.60	10.09 (O-WS 4)	0.04	0.43
	Bracts	10.09 (A-WB 4)	0.12	1.19	10.09 (O-WB 4)	0.50	5.00

A: Air-dried; O: Oven-dried; WP: White pulp; WS: White skin; WB: White bracts. 1: 50% CH<sub>2</sub>Cl<sub>2</sub>/*n*-hexane; 2: 100% CH<sub>2</sub>Cl<sub>2</sub>; 3: 50% CH<sub>2</sub>Cl<sub>2</sub>/MeOH; 4: 100% MeOH.

## MATERIALS AND METHODS

### Sample collection

The twigs and fruits of *H. undatus* and *H. polyrhizus* were collected from a farmer based in Imenti Central, Meru County-Kenya, in February 2022. The samples were identified by a Taxonomist at the University of Nairobi Herbarium, where voucher specimens, HP/001/2022 and HU/001/2022 were deposited.

### Sample preparation and extraction

The bracts, skin and pulp of both red and white dragon fruits were separated using a clean scalpel and each part was divided into two equal portions. One portion was oven-dried at 40 °C for 24 h, while the other portion was dried under shade in free air circulation for 21 days. The separately dried samples were milled in a blender and the powder obtained was sieved using 1 mm sieve sizes to obtain particles of even grade. Their weights were recorded in Tables 1 and 2. Each plant species had 12 samples

that were labelled and separately extracted sequentially using different solvent systems starting with 50% dichloromethane ( $\text{CH}_2\text{Cl}_2$ )/*n*-hexane (1); 100%  $\text{CH}_2\text{Cl}_2$  (2); 50%  $\text{CH}_2\text{Cl}_2$ /methanol (MeOH) (3) and 100% MeOH (4). The extracts were then filtered using filter paper and the filtrates were concentrated *in vacuo* using a rotary evaporator. Their weights were recorded (Tables 1 and 2) and the percentage yield (% yield) was calculated as follows:

$$\% \text{Yield} = [(\text{weight of extract}) / (\text{weight of dry matter})] \times 100\%$$

### Test for antioxidant activity

The ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity against *H. undatus* and *H. polyrhizus* extracts was evaluated using the procedure described by Hsu and colleagues, with a few minor adjustments.<sup>25</sup> A fresh piece of aluminum foil was used to measure and transfer 10 mg of DPPH into a beaker. To make a 0.25  $\mu\text{M}$  solution, MeOH (50 mL) was

**Table 2: Percentage yield of the extracts of red Dragon fruit.**

Solvent system for extraction	Fruit part	Red Dragon fruit					
		Air dried powder (g)	Extract (g)	% Yield	Oven dried powder	Extract (g)	% Yield
50% $\text{CH}_2\text{Cl}_2$ / <i>n</i> -hexane	Pulp	12.33 (A-RP 1)	0.01	0.08	12.33 (O-RP 1)	0.29	2.34
	Skin	12.33 (A-RS 1)	0.15	1.18	12.33 (O-RS 1)	0.21	1.70
	Bracts	12.33 (A-RB 1)	0.07	0.58	12.33 (O-RB 1)	0.46	3.75
100% $\text{CH}_2\text{Cl}_2$	Pulp	12.33 (A-RP 2)	0.02	0.16	12.33 (O-RP 2)	0.02	0.13
	Skin	12.33 (A-RS 2)	0.07	0.59	12.33 (O-RS 2)	0.03	0.21
	Bracts	12.33 (A-RB 2)	0.07	0.58	12.33 (O-RB 2)	0.15	1.25
50% $\text{CH}_2\text{Cl}_2$ /MeOH	Pulp	12.33 (A-RP 3)	0.18	1.46	12.33 (O-RP 3)	2.67	21.67
	Skin	12.33 (A-RS 3)	0.05	1.18	12.33 (O-RS 3)	0.42	3.40
	Bracts	12.33 (A-RB 3)	0.29	2.33	12.33 (O-RB 3)	0.62	5.00
100% MeOH	Pulp	12.33 (A-RP 4)	8.06	65.37	12.33 (O-RP 4)	2.21	17.89
	Skin	12.33 (A-RS 4)	0.15	1.18	12.33 (O-RS 4)	0.21	1.70
	Bracts	12.33 (A-RB 4)	0.57	4.65	12.33 (O-RB 4)	0.31	2.50

A: Air-dried; O: Oven-dried; RP: Red pulp; RS: Red skin; RB: Red bracts. 1: 50%  $\text{CH}_2\text{Cl}_2$ /*n*-hexane; 2: 100%  $\text{CH}_2\text{Cl}_2$ ; 3: 50%  $\text{CH}_2\text{Cl}_2$ /MeOH; 4: 100% MeOH.

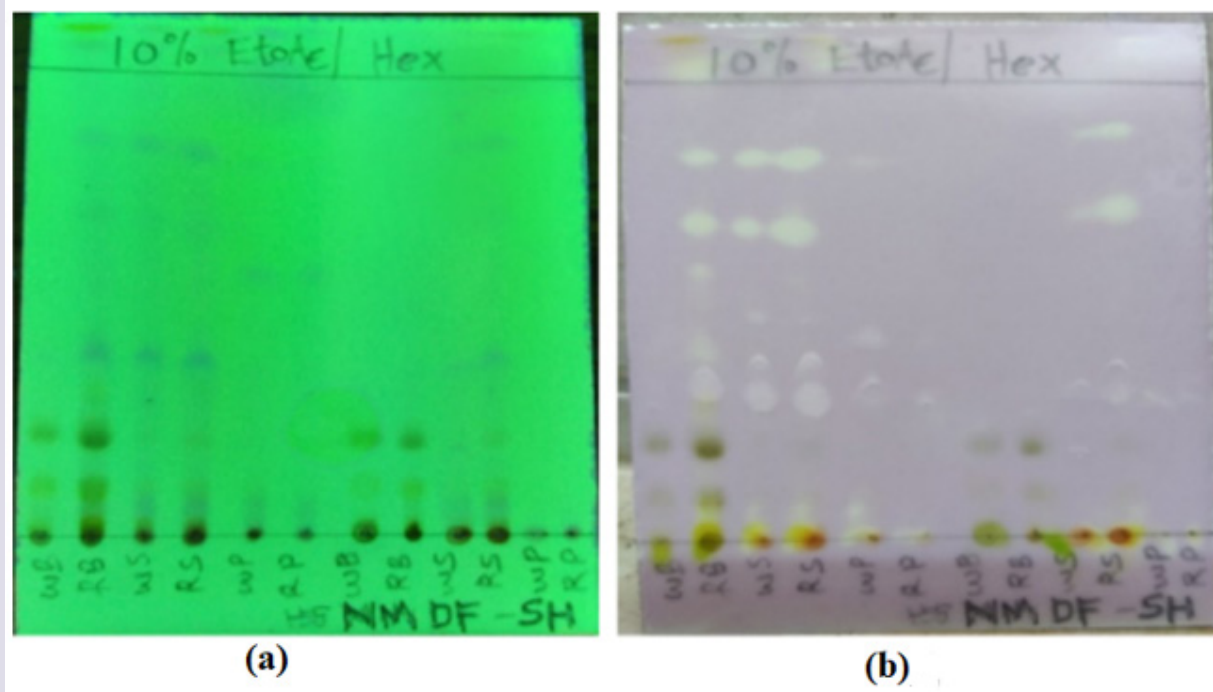
added, mixed and then transferred to a 100 mL volumetric flask and filled to the mark using methanol. First, preliminary screening was carried out using TLC, an inexpensive and quick approach that allows the direct visual comparison of profiles of metabolites found in plant material, in order to identify the most promising extracts. 5 mg of each extract was dissolved in 2 mL of appropriate solvent and shaken for complete dissolution. Using a micropipette, an aliquot of each extract was then applied manually on preparative TLC plates with inorganic fluorescent indicator binder. After air drying, the plates were developed using different mobile phases in a pre-saturated TLC chamber at room temperature. For example, the moderately polar extracts were developed using 10 or 15% Ethyl Acetate (EtOAc)/*n*-hexane, whereas the polar fractions were developed using CH<sub>2</sub>Cl<sub>2</sub>:EtOAc:MeOH (6:2:2). After development, the plates

were visualized under UV light at 254 nm and further by spraying with 0.25 μM DPPH solution using TLC sprayer. Separation zones' antioxidant activities were noticed almost instantly upon spraying as white spots on a purple background. Further study of the radical scavenging activity using a spectroscopic technique was performed on the extracts showing the most promising antioxidant activity (Table 3).

Following a set of five serial dilutions (factor of 2; ranging from 10 mg/mL to 0.625 mg/mL) of each extract, 1 mL of each dilution was pipetted and thoroughly mixed with 2 mL of 0.25 μM DPPH solution and incubated in the dark for 30 min. The absorbance was measured at 517 nm against a blank (DPPH+Solvent) using a UV-vis spectrophotometer. Since the standard positive controls (ascorbic acid and quercetin) have a higher antioxidant

**Table 3: Selected extracts for further spectroscopic study of the radical scavenging activity.**

Solvent system of extraction	White flesh Dragon fruit (Code)		Red flesh Dragon fruit (Code)	
	Air dried	Oven dried	Air dried	Oven dried
50% CH <sub>2</sub> Cl <sub>2</sub> / <i>n</i> -hexane	-	-	-	-
	1 (A-WS 1)	1 (O-WS 1)	1 (A-RS 1)	0
100% CH <sub>2</sub> Cl <sub>2</sub>	0	0	1 (A-RS 2)	1 (O-RP 2)
50% CH <sub>2</sub> Cl <sub>2</sub> /CH <sub>3</sub> OH	1 (A-WP 3)	1 (O-WP 3)	0	0
100% CH <sub>3</sub> OH	0	1 (O-WP 4)	1 (A-RP 4)	0
Total	2	3	4	1



**Figure 1:** TLC chromatograms obtained for solvent extracts of *H. undatus* and *H. polyrhizus*: (a) TLC chromatogram visualized at 254 nm; (b) antioxidant profiles (visualized using 0.25 μM DPPH solution). Mobile phase: hexane (Hex); Ethyl acetate (EtOAc) (90:10 vol/vol).

activity, the working concentrations ranged from 6.250  $\mu\text{g/mL}$  to 0.391  $\text{mg/mL}$ . Each sample analysis was done in triplicate and expressed as a mean average. The percentage Radical Scavenging Activity (% RSA) was calculated as  $\% \text{ RSA} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance of control}] \times 100\%$ . The  $\text{IC}_{50}$  values of the sample extracts and the standards were determined by performing a nonlinear regression analysis using the GraphPad Prism 9.3.1 software.

## RESULTS AND DISCUSSION

### Percentage yield of the Dragon fruit extracts

There was no discernible pattern in the percentage yield of the pulps and skins of the white-fleshed dragon fruit for the samples that were air dried and oven dried (Table 1). However, the oven-dried samples had a clearly higher percentage yield than the air-dried ones when it came to the bracts. Following oven and

air drying, the percent yield of the pulp samples of red dragon fruit extract did not reveal any distinctive patterns (Table 2). Oven-dried sample skin extracts were, however, found to have higher yield than air-dried ones, with the exception of the 100%  $\text{CH}_2\text{Cl}_2$  extract. These results demonstrate that the plant part and extraction solvent system had an impact on the drying and extraction yields and is consistent with the published literature.<sup>26</sup> The bracts extract also followed the same pattern as the skin extracts, yielding higher percentages in oven dried samples in comparison to air dried ones, except the 100% methanol extract.

### Preliminary antioxidant results of Dragon fruit extracts

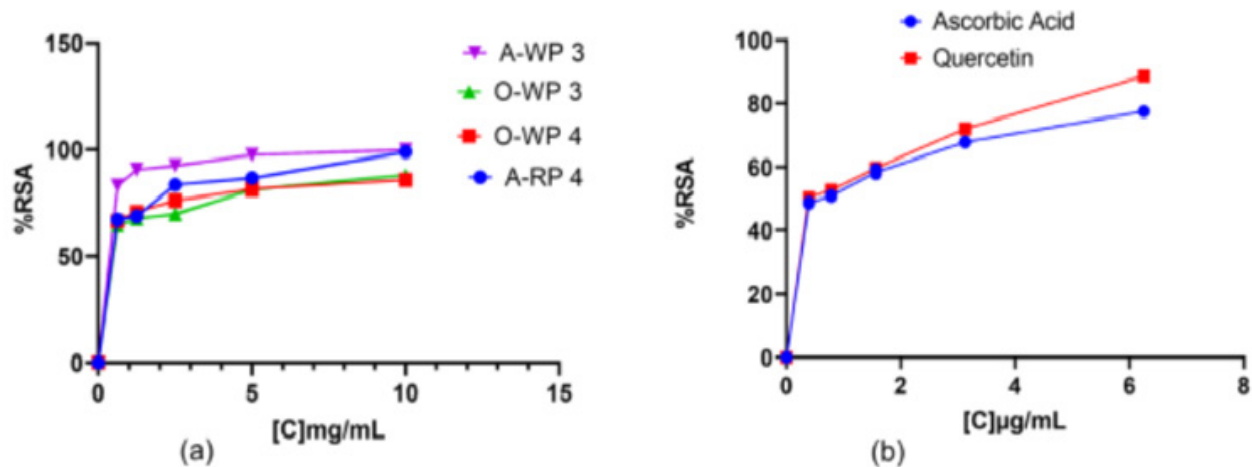
Preliminary DPPH analysis results of the samples using TLC plates are portrayed in Figure 1. From the preliminary TLC-DPPH screening, the white and red fleshed dragon fruits

**Table 4:** Percent free radical scavenging activities and  $\text{IC}_{50}$  values of Dragon fruit extracts and standards using methanol as blank solvent ( $n=3$ ).

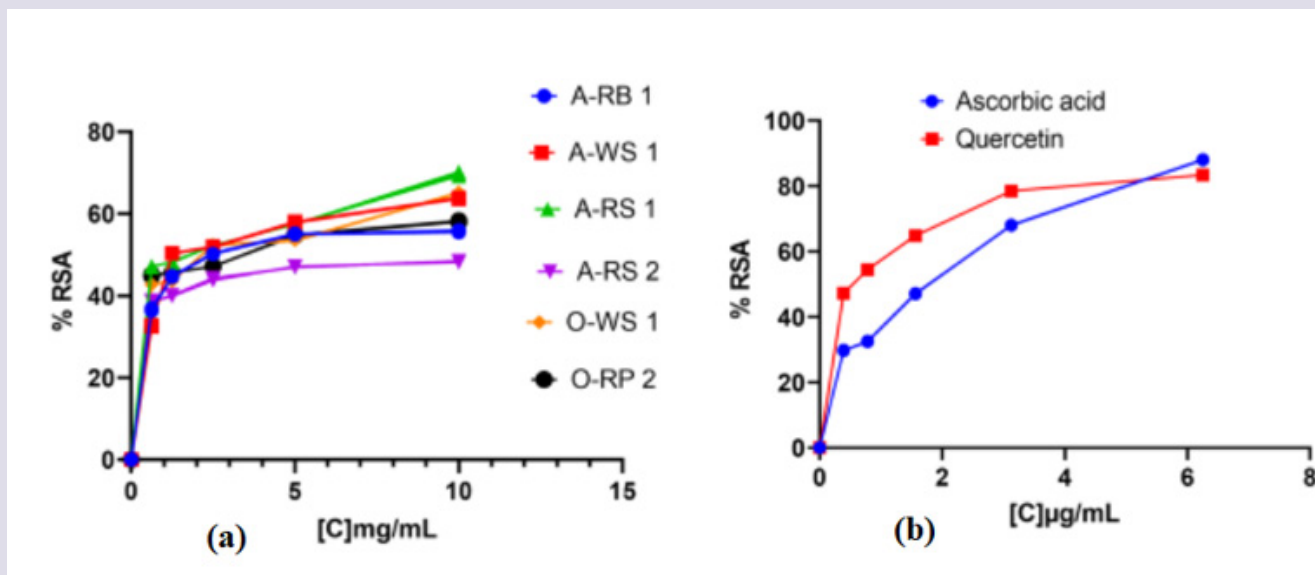
Extract	% Radical Scavenging Activity	$\text{IC}_{50}$ (mg/mL)
A-RP 4	80.95 $\pm$ 0.002	0.689 $\pm$ 0.001
O-WP 3	74.07 $\pm$ 0.001	0.768 $\pm$ 0.001
O-WP 4	76.27 $\pm$ 0.001	0.701 $\pm$ 0.002
A-WP 3	92.65 $\pm$ 0.001	0.497 $\pm$ 0.001
Ascorbic acid	60.69 $\pm$ 0.002	0.727 $\pm$ 0.009
Quercetin	64.65 $\pm$ 0.001	0.692 $\pm$ 0.001

**Table 5:** Percent free radical scavenging activities and  $\text{IC}_{50}$  values of Dragon fruit extracts and standards using acetone as blank solvent ( $n=3$ ).

Extract	% Radical Scavenging Activity	$\text{IC}_{50}$ (mg/mL)
A-RB 1	48.46 $\pm$ 0.001	1.611 $\pm$ 0.008
A-WS 1	51.41 $\pm$ 0.001	1.464 $\pm$ 0.004
A-RS 1	54.62 $\pm$ 0.002	1.290 $\pm$ 0.002
A-RS 2	43.44 $\pm$ 0.002	2.152 $\pm$ 0.039
O-WS 1	52.01 $\pm$ 0.001	1.454 $\pm$ 0.001
O-RP 2	50.62 $\pm$ 0.001	1.521 $\pm$ 0.003
Ascorbic acid	53.26 $\pm$ 0.001	1.501 $\pm$ 0.006
Quercetin	65.64 $\pm$ 0.001	0.677 $\pm$ 0.000



**Figure 2:** (a) Radical scavenging activity of dragon fruit extracts and (b) standards using methanol as blank solvent.



**Figure 3:** (a) Radical scavenging activity of dragon fruit extracts and (b) standards using acetone as blank solvent. A-RB: Air-dried red bracts; A-WS: Air-dried white skin; A-RS: Air-dried red skin; O-WS: Oven-dried white skin; O-RP: Oven-dried red pulp. 1: 50%  $\text{CH}_2\text{Cl}_2/n$ -hexane; 2: 100%  $\text{CH}_2\text{Cl}_2$ .

had five samples, each showing potential antioxidant activity (Table 3). Three samples of the white fleshed fruit pulp and two samples of its skin had potential antioxidant activity, while the bracts lacked any possible antioxidant activity. In comparison to one sample of its bracts, an equal number of samples of the red-fleshed fruit's pulp and skin revealed possible antioxidant activity. Consequently, it can be deduced that the fruit's pulp and skin (peel) consisted of the most active components. Compared to four oven-dried samples, six air dried samples demonstrated potent antioxidant activity. The samples' components might have degraded as a result of oven drying. These results are consistent with findings from research conducted by Bustos and co-authors.<sup>27</sup>

### Spectrophotometric analysis results of antioxidant activity of Dragon fruits

The % RSA and the  $\text{IC}_{50}$  values of the 10 extracts that showed remarkable initial antioxidant activity are presented in Figures 2-3 and Tables 4-5, respectively. All of the analyzed extracts demonstrated potent antioxidant activity when compared to the applied standards (ascorbic acid and quercetin). Methanol was utilized as the blank solvent for the polar extracts, which were composed of 50%  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  and 100%  $\text{CH}_3\text{OH}$ , respectively. The air-dried white fleshed dragon fruit pulp (A-WP 3) stood out as being the most active of all of them (92.65% RSA;  $\text{IC}_{50}=0.497$ ). Additionally, compared to the air-dried extracts, the oven dried extracts (O-WP 3 and O-WP 4) showed decreased radical scavenging activity (Table 4). It is once again evident that oven drying decreases the antioxidant activity of dragon

fruit pulp. This is consistent with research showing that some chemicals are heat-sensitive and deteriorate when heated.<sup>28</sup>

In comparison to polar extracts, the radical scavenging activity of the moderately polar extracts was considerably reduced. This is, for instance, demonstrated by the values of O-RP 2 (50.62%;  $\text{IC}_{50}=1.52$  mg/mL; Table 5) in comparison to other pulp samples in Table 4, where O-WP 3 (74.07%;  $\text{IC}_{50}=0.768$  mg/mL) was more active. This can be due to phytochemicals in polar extracts having a higher capacity to scavenge DPPH free radicals. Since the DPPH radical can accept both an electron and a hydrogen atom, the polar extracts' considerably higher free radical scavenging activity suggests that they contain more protic phytochemicals than the moderately polar extracts, which makes it easier for hydrogen atom transfer to occur.<sup>29</sup> This is consistent with observations in the literature that show the antioxidant activity of dragon fruits is typically caused by polar glycosylated betalains, glycosylated flavonoids and other phenolic compounds.<sup>8</sup>

When a mixture of the sample extracts with DPPH was incubated in the dark for 30 min, the DPPH was decolorized by the skin of the red-fleshed fruit and the pulp of the white fleshed one. The standards also yielded comparable results. There was no significant change in the color of DPPH by the bracts and pulp of the red fleshed fruit and skin of the white fleshed fruit. These findings conflict with what was observed in Tables 4 and 5, as some samples (such as A-RP 4) showed a strong radical scavenging activity but no discernible color change. This suggests that in order to fully exploit the antioxidant effects of these fruits, pure compounds from the fruit parts should be isolated as well as formulations developed and their antioxidant potency determined after isolated.

Our study is the first in-depth analysis of the antioxidant activity of distinct solvent extracts of both the white and red dragon fruit parts under various post-harvest treatment conditions. The antioxidant activity of dragon fruits was shown to be influenced by the fruit part and drying conditions in this study and it was also revealed that the extraction solvent is vital.

## CONCLUSION

This study highlights the significance of postharvest treatment in determining the antioxidant potency of different parts of white and red dragon fruits. The air-dried parts of both fruit varieties exhibited the highest antioxidant activities, with the 50% methanol in dichloromethane extract of the air-dried white dragon fruit pulp showing the highest radical scavenging activity ( $92.65 \pm 0.001\%$ ) and an  $IC_{50}$  value of  $0.497 \pm 0.001$ . The choice of solvent systems was crucial, with polar solvents proving most effective in extracting antioxidant compounds. Among the different parts, the pulp and skins of both white and red dragon fruits demonstrated the highest antioxidant activities, while the bracts showed minimal potential.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**DPPH-1**: 1-Diphenyl-2-picrylhydrazyl; **RSA**: Radical scavenging activity; **HO**: Hydroxyl radical; **O<sub>2</sub><sup>•-</sup>**: Superoxide anion; **H<sub>2</sub>O<sub>2</sub>**: Hydrogen peroxide; **NO**: Nitric oxide; **ONOO**: Peroxynitrite; **CH<sub>2</sub>Cl<sub>2</sub>**: Dichloromethane, **MeOH**: Methanol; **EtOAc**: Ethyl acetate; **UV**: Vis; **TLC**: Thin layer chromatography; **UV-Vis**: Ultraviolet-visible spectroscopy; **A**: Air-dried; **O**: Oven-dried; **WP**: White pulp; **WS**: White skin; **WB**: White bracts; **RP**: Red pulp; **RS**: Red skin; **RB**: Red bracts.

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