DLSU Research Congress 2025
De La Salle University, Manila, Philippines
June 26 to 28, 2025

Evaluation of the Antioxidant Property and Total Phenolic Content of Wooly Joint Prickly Pear (*Nopalea cochenillifera* (Linn.) Salm-Dyck)

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Abstract: This study evaluated the antioxidant activity, total phenolic content, and bioactive components of *Nopalea cochenillifera* cladodes and fruits. Moreover, the effects of conventional cooking techniques on the antioxidant activity and total phenolic content of the cladodes were also assessed. DPPH, or 2,2-diphenyl-1-picrylhydrazyl, assay revealed that the cladodes and fruits exhibited antioxidant activity, with the cladodes being more potent than the fruits. However, the Folin-Ciocalteau assay revealed that the fruits contained more phenolic compounds than cladodes. Blanching, boiling, steaming, and microwaving decreased the DPPH scavenging activity of cladodes, which could be due to the loss of bioactive compounds by leaching, thermal degradation, and oxidation. Similar results were observed for the Folin-Ciocalteu assay, wherein the cooking techniques decreased the total phenolic content (TPC) of the dichloromethane extract of the raw cladodes. In contrast, no significant difference in total phenolic content between the raw and processed cladodes was found in the methanol extract, except for the boiling method. It was also observed that methanol extracts obtained a higher DPPH scavenging activity and total phenolic content than dichloromethane extracts. The results clearly showed that *N. cochenillifera* cladodes and fruits could be exploited for their antioxidant activity and bioactive compounds. This suggests that *N. cochenillifera* is a potential medicinal food. At the same time, methanol was found to be a more efficient solvent in extracting phenolic compounds and possibly other antioxidant compounds. Lastly, the results showed that the conventional cooking techniques negatively affected the DPPH scavenging activity and total phenolic content of cladodes.

Key Words: *N. cochenillifera*, antioxidant, total phenolic content, cooking

1. INTRODUCTION

Oxidative stress can induce the development and progression of certain diseases, such as diabetes (Folli et al., 2011) and cancer (Waris & Ahsan, 2006). Although synthetic antioxidants have already been developed, their low solubility, moderate antioxidant activity, and adverse effects on human health have restricted their application (Rani et al., 2018). Considering this, antioxidants have been mainly sought from plants. *Nopalea cochenillifera*, has been used in folk medicine to treat certain diseases. However, the species has limited scientific information regarding its biological properties that can be used for pharmaceutical applications and drug development (Fabela-Illescas et al., 2022).

Few studies reported the cladodes' antioxidant activity, while the fruit had no existing publications. A study reported the *in vitro* antioxidant activity of *N. cochenillifera* cladodes against numerous radicals (Fabela-Illescas et al., 2022). Similarly, Magaña-Cerino et al. (2020) reported the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of the methanolic extract of the cladodes. At the same time, the effects of seasonal variability on the antioxidant and total phenolic content (TPC) of the cladodes have already been reported, with the cladodes harvested during the rainy season having a higher antioxidant activity and TPC than the ones harvested during the dry season (Alves et al., 2017). A study by Lamnoi et al., (2018) showed that drying the cladodes increases antioxidant activity. However, because the cladodes can either be eaten raw or cooked, no studies have been conducted on the effects of conventional cooking techniques such as blanching, boiling, steaming, and microwaving on antioxidant activity and TPC. This study aims to determine the effects of conventional cooking techniques on the antioxidant properties and TPC of *N. cochenillifera*.

2. METHODOLOGY

2.1 Materials and Equipment

A Telstar Freeze dryer was used to lyophilize the samples. Methanol, dichloromethane, and glass containers were used for maceration of the sample with frequent agitation using a vortex mixer and DLAB centrifuge was used. Micropipettes and tips, 96-well plates. Solvents such as methanol, dichloromethane, and acetonitrile were used to extract and reconstitute the samples for the assays. The assays were performed using FLUOstar® Omega microplate reader.

2.2 Sample Collection and Preparation

The young cladodes and the ripe fruits were collected from a single plant source. The cladodes and fruits were washed, and the thorns were removed using a sharp knife. The cladodes were chopped into cubes and divided into five (5) 150-gram portions. Blanching was done by adding the cladodes into 300 mL boiling distilled water (100 °C) for two (2) minutes, and immediately placed in an ice bath. Boiling was carried out by adding the cladodes to 300 mL of boiling distilled water (100 °C) for fifteen (15) minutes. Steaming was carried out by exposing the cladodes to steam (100 °C) for fifteen (15) minutes in an enclosed container. Microwaving was carried out for five (5) minutes at 800 W. The samples were placed in a zip-lock bag and were lyophilized using the Telstar Freeze Dryer. The freeze-dried samples were pulverized and stored in the refrigerator before extraction.

2.3 Sample Extraction

Two (2) grams of the samples were soaked in 20 mL of methanol or dichloromethane in a glass container for three days at room temperature with frequent agitation using a vortex mixer (Bandiola, 2018). The sample labels are shown in Table 1.

Table 1. Abbreviations of the Different Samples

METHANOL EXTRACTS		DICHLOROMETHANE EXTRACTS	
MCR	Raw Cladodes	**DCR**	Raw Cladodes
MFR	Raw Fruits	**DFR**	Raw Fruits
MCBL	Blanched Cladodes	**DCBL**	Blanched Cladodes
MCBO	Boiled Cladodes	**DCBO**	Boiled Cladodes
MCS	Steamed Cladodes	**DCS**	Steamed Cladodes
MCM	Microwaved Cladodes	**DCM**	Microwaved Cladodes

The samples were then transferred to 15 ml centrifuge tubes and centrifuged for 20 minutes at 3000 rpm (Arive et al., 2017). The supernatants were collected and transferred to pre-weighed containers. After which, methanol and dichloromethane were boiled off in a water bath. The recovered crude extracts were freeze-dried overnight.

2.4 DPPH Assay

The protocol used was from Widowati et al. (2021) with modifications. A stock solution of DPPH (80 µg/mL or 202.9 µM) was prepared in methanol. Various concentrations of the extracts (250 – 4000 µg/mL for methanol and 300-5000 µg/mL for dichloromethane) were prepared. In the 96-well plate, 100 µL of the sample extract and 100 µL of DPPH were added. Methanol was used as the negative control. Quercetin and ascorbic acid served as positive controls. The plate was shaken at 100 rpm for 30 seconds and incubated in the dark for 30 minutes. The absorbance was read at 517 nm. The scavenging activities were then calculated using the equation below, and the IC_{50} values were determined.

$$\%SA = [A_C - A_S/A_C] \times 100$$

Where %SA is the percentage scavenging activity. A_C is the absorbance of the negative control, and A_S is the absorbance of the sample at 517 nm.

2.5 Folin-Ciocalteu Assay

The protocol used was from Monter-Arciniega et al. (2018) with modifications. Stock solutions prepared during the DPPH assay were also used in this assay. Briefly, 20 µL of the sample solution was mixed with 80 µL of Folin-Ciocalteu reagent. This was followed by adding 100 µL of sodium carbonate (Na_2CO_3). The plate was shaken at 100 rpm for 30 seconds before and after adding sodium carbonate and incubated in the dark for 30 minutes. The samples were read at 746 nm, and each sample was analyzed in triplicate. A calibration curve was generated using gallic acid (0.488 to 31.25 µg/mL) and was used to determine the total phenolic content (TPC). The total phenolic content of the sample extracts was initially expressed as µg GAE (gallic acid equivalent)/mL, and was converted to mg GAE/g DW (dried weight).

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity in Raw Samples

Extracts with antioxidant activity are found to have increasing antioxidant capacity as the extract concentration is increased (Rahman et al., 2015). This trend was observed for all of the methanol and dichloromethane extracts of the raw cladodes and fruits. The increase in scavenging activity as the concentration of the extracts increased was found to be significant, indicating the presence of antioxidant activity for MCR, MFR, DCR, and DFR.

The IC_{50}, or the half maximal inhibitory concentration, is useful in determining the potency of the extracts as antioxidants. It is the concentration of the extract required to inhibit 50% of the DPPH radicals. As such, a low IC_{50} value corresponds to a high scavenging activity or high antioxidant activity (Rezig et al., 2020). Table 2 summarizes the average IC_{50} values of each extract.

Table 2. IC_{50} values of raw cladodes and fruits against quercetin and ascorbic acid

SAMPLE	IC_{50} (µg/mL)
MCR	406.85 ± 2.07
MFR	660.94 ± 22.96
DCR	611.31 ± 33.94
DFR	1008.88 ± 66.88
quercetin	1.08 ± 0.06
ascorbic acid	6.20 ± 0.45

The DPPH result for the methanol extract of *N. cochenillifera* cladodes was consistent with existing studies. For instance, the methanol extract of *N. cochenillifera* cladodes showed antioxidant activity by DPPH assay in the publications of Fabela-Illescas et al. (2018) and Magaña-Cerino et al. (2020).

The IC_{50} value for the methanol extract of *N. cochenillifera* cladodes (MCR) was found to be 406.85 ± 2.07 µg/mL, which was significantly lower than the IC_{50} of the methanol extract of *N. cochenillifera* fruits (MFR), with IC_{50} value of 660.94 ± 22.96 µg/mL. MCR was significantly more potent in the DPPH assay compared to the MFR. The difference in potency could be attributed to their differences in the amount and presence of bioactive compounds Andreu et al., 2017). Furthermore, qualitative phytochemical screening revealed that the cladodes and fruits of *N.*

cochenillifera differed in terms of phytochemicals present (Pooja and Vidyasagar, 2016).

The dichloromethane extracts of the raw cladodes and fruits also contained antioxidant activity, but their IC_{50} values were significantly higher than the methanol extract counterparts. For instance, DCR has an IC_{50} of 611.31 ± 33.94 µg/mL, which was significantly higher than MCR. Similarly, DFR had a significantly higher IC_{50} value than MFR, with an (IC_{50} = 1008.88 ± 66.88 µg/mL). Despite the differences in IC_{50} values, the trend of cladodes having a higher antioxidant capacity than the fruits was consistent for both methanol and dichloromethane extracts. Furthermore, it could also be said that the antioxidant activity was higher in the methanol extracts than in the dichloromethane extracts, suggesting that the solvent used can be considered a great factor in influencing the antioxidant activity of extracts.

3.2 Effects of Conventional Cooking Techniques on the Antioxidant Activity

The effect of conventional cooking techniques on the antioxidant activity of *N. cochenillifera* cladodes was also assessed. It was found that the methanol and dichloromethane extracts of the blanched, boiled, steamed, and microwaved cladodes still exhibited antioxidant activity. It was found that the IC_{50} values of the processed cladodes increased significantly compared to the raw samples in both the methanol and dichloromethane extracts (see Table 3), indicating that conventional cooking methods negatively affected the DPPH scavenging activity of the cladodes. At the same time, the antioxidant activity of the methanol extracts of the processed samples was stronger than that of the dichloromethane extracts, further suggesting that methanol was better in extracting antioxidant compounds than dichloromethane.

Table 3. IC_{50} values of processed cladodes

SAMPLE	IC_{50} (µg/mL)	SAMPLE	IC_{50} (µg/mL)
MCR	406.85 ± 2.07	DCR	611.31 ± 33.94
MCBL	496.32 ± 44.09	DCBL	1001.75 ± 71.87
MCBO	534.21 ± 46.44	DCBO	1083.28 ± 98.10
MCS	615.78 ± 20.72	DCS	1043.81 ± 22.77
MCM	918.39 ± 32.19	DCM	1192.75 ± 22.92

The effects of cooking methods on the antioxidant activity of plants can vary Miglio et al. (2007) and Mehmood and Zeb (2020). In general, cooking can modify the physical and chemical properties of vegetables (Mehmood and Zeb, 2020). These techniques could lead to leaching, thermal destruction, and oxidation of bioactive compounds present in plants, leading to a decrease in the antioxidant activity of the cladodes. In addition, cutting the cladodes could have decreased its antioxidant activity. For instance, Dovene et al. (2019) stated that there could be a loss in antioxidant activity due to cutting because of the disruption of the cell membrane, forcing existing phenolic compounds to combine with oxidative enzyme systems like polyphenol oxidase. As a result, phenolic compounds will be oxidized, decreasing the antioxidant activity.

3.3 Total Phenolic Content of Raw Samples

The total phenolic content (TPC) of the methanol and dichloromethane extracts of the raw cladodes and fruits is summarized in Table 4.

Table 4. Total phenolic content of raw cladodes and fruits expressed in mg of gallic acid equivalents (mg GAE) per g of dry weight (DW)

SAMPLE	mg GAE/g DW
MCR	1.34 ± 0.18
MFR	2.43 ± 0.16
DCR	0.16 ± 0.02
DFR	0.22 ± 0.04

The methanol fruit extracts (MFR) contained the highest TPC, giving a value of 2.43 ± 0.16 mg of gallic acid equivalents per g of dry weight (mg GAE/g DW). This was followed by the methanol cladode extracts (MCR), which were significantly lower than MFR, giving a value of 1.34 ± 0.18 mg GAE/g DW. On the other hand, the dichloromethane extracts of fruits and cladodes showed a total phenolic content of 0.22 ± 0.04 mg GAE/g DW and 0.16 ± 0.02 mg GAE/g DW, respectively.

Fabela-Illescas et al. (2022) reported that the TPC of MCR (2.0792 ± 0.0074 mg GAE/ g DW) was higher than the observed value. This could be attributed to the solvent used and the extraction. In addition to this, factors such as the maturity stage, geographic conditions, and other environmental conditions could also play an important role in the TPC (Moussa-Ayoub et al., 2014). However,

comparing the TPC to the results obtained in the DPPH assay showed contradicting observations, as both methanolic (MCR) and dichloromethane extracts of cladodes (DCR), which were found to have a higher antioxidant capacity than their fruit counterparts (MFR and DFR). It is important to note that antioxidant activity does not equate to individual bioactive compounds but rather, it functions in a synergistic way, where the cumulative effects of bioactive compounds, trace elements, metals, and other food constituents are also taken into account (Saura-Calixto et al., 2009).

The methanol extracts of the raw samples obtained a significantly higher TPC than the dichloromethane extracts. Haminiuk et al. (2014) mentioned that polyphenols are of high polarity. Thus, using a polar solvent such as methanol would prove to be more efficient in extracting phenolic compounds. A similar result was observed in the publication of Ammar et al. (2015). This is similar to the DPPH assay, wherein the methanol extracts have a significantly more potent antioxidant activity than the dichloromethane extracts.

3.4 Effects of Conventional Cooking Techniques on the Total Phenolic Content

All of the cooking techniques significantly decreased the TPC of cladodes in the dichloromethane extracts (see Table 5). However, contradicting results were observed for the blanched, steamed, and microwaved cladodes in the methanol and dichloromethane extracts. This could be due to the cooking technique having different effects on the various types of compounds found within the extracts (Irondi et al., 2017). The phenolic compounds in the methanol extracts may have been more stable after being subjected to the cooking techniques than those in the dichloromethane extracts.

Table 5. Total phenolic content of processed cladodes expressed in mg of gallic acid equivalents (mg GAE) per g of dry weight (DW)

SAMPLE	mg GAE/g DW	SAMPLE	mg GAE/g DW
MCR	1.34 ± 0.18	DCR	0.16 ± 0.02
MCBL	1.14 ± 0.10	DCBL	0.08 ± 0.02
MCBO	0.89 ± 0.09	DCBO	0.06 ± 0.01
MCS	1.53 ± 0.29	DCS	0.10 ± 0.01
MCM	1.31 ± 0.15	DCM	0.06 ± 0.02

Each cooking technique was found to have varying effects on the sample. Blanching decreased the TPC of cladodes, but this was only significant for the dichloromethane extract. High temperatures can quickly disrupt cell wall barriers (Kim et al., 2019), causing the leaching of more phenolic compounds during the blanching process (Irondi et al. 2017). Prolonged heat during boiling could drastically alter the chemical composition of the cladodes. This was observed in the study, as boiling the cladodes for fifteen minutes had significantly decreased their total phenolic content in both the methanol and dichloromethane extracts Ramírez-Moreno et al. (2013). For steaming, varying results were observed. The decrease in the TPC in the dichloromethane extract could be due to the thermal degradation of phenolic compounds present in the cladodes. Similar results were obtained in the study of Lafarga et al. (2018), wherein steaming Brassica vegetables for 15 minutes at 100 °C caused a significant decrease in the TPC. Lastly, it was observed that the TPC of the microwaved cladodes decreased in the methanol and dichloromethane extracts. However, this was only significant for the dichloromethane extracts. The results in microwaved samples could not be attributed to leaching of compounds as observed in boiling and blanching. Microwaving vegetables causes dehydration and destroys phenolic compounds when subjected to microwave heating leading to a decrease in the TPC (Pokkanta et al., 2022) In addition, cutting could also contribute to the decrease in the TPC. Mechanical stress and cutting could lead to changes in its chemical composition. This could expose the plant to external conditions, more susceptible to oxidation. Dovene et al. (2019) suggested that the wounding stress induced by cutting forces phenolic compounds to interact with polyphenol oxidase, which is an oxidative enzyme. As such, since phenolic compounds become oxidized, the antioxidant activity of the natural product is negatively affected as well.

4. CONCLUSIONS

The assay results showed that *N. cochenillifera* cladodes and fruits could be exploited for their antioxidant activity and bioactive compounds and could be used as support in using them as a medicinal food and a source for bioactive compounds that can be screened for future pharmaceutical

applications and drug developments. Furthermore, results showed that methanol was a more efficient solvent in extracting phenolic compounds and possibly other antioxidant compounds than dichloromethane. The results also implied that the various cooking techniques utilized in this research affected the DPPH antioxidant activity and TPC of the cladodes.

It is recommended to vary the cooking parameters for blanching, boiling, steaming, and microwaving to minimize the loss of bioactive compounds and antioxidant activity. Furthermore, the researchers recommend performing other in vitro antioxidant assays to further support the antioxidant capacity of *N. cochenillifera* cladodes and fruits, as well as the effects of conventional cooking techniques on the antioxidant activity of cladodes. Lastly, the antioxidant compounds could be characterized using GC-MS (gas chromatography-mass spectrometry), HPLC (high-performance liquid chromatography), and LC-MS (liquid chromatography-mass spectrometry) of crude extracts, and NMR (nuclear magnetic resonance) spectroscopy of purified fractions.

5. ACKNOWLEDGMENTS

The authors extend their deepest gratitude to Mr. Michael Dominic Ajero, Dr. Maria Carmen Tan, and Dr. Joan Candice Ondevilla for their time, patience, and knowledge in helping us with instrumentation and for constant encouragement. They would also like to thank Dr. Marissa Noel and Dr. Mariafe Calingacion for providing insights and counsel for their research. Lastly, to the Department of Chemistry for providing the materials and chemicals during the experimental process.

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