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ANTIOXIDANT PECTIN FROM CACTUS OPUNTIA DILLENII GROWING IN NINH THUAN COAST, VIETNAM: EXTRACTION, PHYSIC-CHEMISTRY CHARACTERIZATION AND ANTIOXIDANT ACTIVITY

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Keywords:

Antioxidants, Cactus, Extraction, Infrared-freeze drying, *Opuntia dillenii*, Pectin, Vietnam

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ABSTRACT: The extraction condition, physic-chemistry characterization, and antioxidant activity of pectin from cactus *Opuntia dillenii* found commonly growing in the Ninh Thuan coast, Vietnam, were studied. The extraction conditions of antioxidant pectin content, such as temperature, time, cactus-to-solvent ratio, solvent pH, and extraction numbers, were surveyed. A correlation between pectin content and antioxidant activities, physic-chemistry characterization such as FTIR spectra, sugar composition, viscosity, solubility ability, and esterification degree of antioxidant pectin were also analyzed. The results showed that the extraction condition strongly affected ($p < 0.05$) and close correlation ($R^2 > 0.9$) on pectin content and antioxidant activities. The pectin content and the antioxidant activities got the highest value at the condition (pH 4, the solvent/material of 6/1 (v/w), the extracting time of 90 min, and extracting temperature of 90 ºC). Glucuronic and galacturonic acid of antioxidant pectin occurred from 20.920 min to 21.765 min and 20.915 min to 21.779 min, respectively. Pectin possessed an esterification degree of 62.38 ± 0.94 %, viscosity of 0.893 ± 0.051 Pa.s, and fully solubility in 90 ºC. They fully dispersed into hot water and formed gelation in cold water. Antioxidant pectin of cactus *Opuntia dillenii* grown in the Ninh Thuan coast is useful for application into the foods industry.

INTRODUCTION: Pectin is an active poly-saccharide existing in different plants, for example, cactus, seaweed, and seagrass.

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Inside, pectin content in cactus is the highest evaluated, compared to different plants. Pectin structure includes uronic acids (D-glucuronic acid, D-galacturonic acid, L-iduronic acid, and D-mannuronic acid), groups of natural methyl esters, groups of carboxyl amide, and carboxyl groups with the linkages of 1-4-glycoside [1], are divided into two groups (high methoxy pectin (DM > 50%) and low methoxy pectin (DM < 50%)). Their average molecular weight is from 50.000 to 150.000 Dalton. Pectin in cactus possesses value

bioactivities, for example, antioxidant [2-4], antibacterial [5-7], reducing blood sugar, cholesterol reduction, against UV radiation, wound treatment, improving the excretion of heavy metal [8, 9]. Therefore, pectin is useful for the product of nutrition, health, and disease [10]. In different products such as jams, jellies, probiotic beverage, salad sauces, ice cream, and yogurt, pectin absence is also found because of their ability of thickener, stabilizer, suspending agent, emulsifier, flavor booster, and food preservation [11]. The content, the biological activity, structural characteristics, and the applicating effect of pectin depend on a growth area and species of cactus, harvest time and the method of treatment and extraction [12, 13]. Physical-chemistry, structure characterization, bioactive of pectin from cactus fruit peel *Opuntia dillenii* was noticed [14] and from the cactus trunk was not found.

In Vietnam, cactus commonly grows in nature, used as a medicinal plant for support treatment of spinal pain, and food for sheep [15, 16]. The notices on physical-chemistry, structure characterization, bioactive of pectin from cactus grown on the Vietnam coast did not found. Therefore, antioxidant pectin and a suitable extraction condition will be useful more for an effective increase of production and application of pectin into foods, beverages, and pharmacy. Changing laws, a correlation between pectin content and antioxidant activities, and an impact of extraction condition on antioxidant pectin content shown in the current study. The things can open a new trend for cactus development grown in the coast into foods, functional food, and pharmacy, contributing an effective control of the extraction condition for biological activity pectin.

MATERIALS AND METHODS: Material preparation: Brush cactus *Opuntia dillenii* growth in Ninh Thuan province, Vietnam was collected in the Ninh Thuan coast, Vietnam and the storage and transportation of the samples to the laboratory were in the cold condition under 10 ºC. In the laboratory, the cactus was cleaned and dried under a radiation-heat with an air rate of 1.5 m/s by an infrared-freeze drying machine. After drying until 15% humidity, the cactus was crushed to the powder and stored at 4 ºC. The results in the study expressed on the dry weight of powder. All chemicals using in the analysis originated from Sigma-Aldrich, except for double-distiller water. The voucher specimens (NT-XR-17072018) of brush cactus *Opuntia dillenii* was authenticated and deposited by Dr. Luong Anh Dung in Da Lat University.

FIG. 1: BRUSH CACTUS OPUNTIA DILLENII

Extraction of Antioxidant Pectin: Extracting antioxidant pectin from the cactus powder was by using the dynamic maceration technique. Some factors surveying in extract process conclude pH (1, 2, 3, 4, 5, 6, 7 and 8), the ratio of solvent-to-material (S/M) (1/1, 2/1, 3/1, 4/1, 5/1, 6/1, 7/1, 8/1 (v/w)), time (30, 60, 90, 120, 150 and 180 min), temperature (70, 80, 90, 100 ^{o}C), times of repeated extraction (1, 2 and 3 times). After extraction, filtering the crude extract was by using the technique of vacuum filter. The supernatant precipitation was vortexed to 96% cold-acidic ethanol according to the ratio of 2/1 (v/w) for 30 min and kept the mixture for 2 h. After filtering the mixture for precipitation collection and the precipitate cleaning was continuously vortexes with 70% acidic ethanol according to the ratio of 2/1 (v/w) for 30 min and kept for 2 h. Then, the cleaned precipitate solubilization was in double-distilled water at 90 ^{o}C for 30 min and centrifuged at 6.000 rpm for 5 min removing insoluble fraction and the supernatant storage at 4 ^{o}C for further, study.

Quantification of Pectin Content: Quantification of pectin content based on uronic acid content according to the description of Beda *et al.*, 2014 [17].

Pectin content (% Anhydrous uronic acid) = 176 × 100 × Total uronic acid / 1000

Inside, the evaluation of uronic acid was according to the developed method by Siew [18]. The dissolve of the pectin sample was in 0.4 mL of distilled water according to the sample-to-water ratio of 0.1 %. Adjusting the solution pH was to 1.6 by using 4 M sulfamic–potassium sulphamate acid and vortex. Adding 2.5 mL solvent B (H_2SO_4 and 0.0125 M

sodium tetraborate) was then into the compound. The mixture assimilation was in cold condition again. After the mixture boiling was for 20 min and cooled in ice. Adding 80 µL meta-hydroxydiphenol was continuously into the cold mixture and vortex. Keeping the mixture was for 20 min. Finally, the measurement of absorbance was at a wavelength of 520 nm with a D-galacturonic acid standard.

Determination of Antioxidant Activity:

Total Antioxidant Activity: Total antioxidant activity was determined according to describe by Vu *et al.*, 2017 [19]. 900 µl of distilled water and 3 ml of solution A (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added into 100 µl of the sample, in turn, and vortexed. The mixture incubation was for 90 min at 95 °C. The absorbance measurement of the mixture was at a wavelength of 695 nm with the standard of ascorbic acid.

Reducing Power Activity: Reducing power activity was determined by the described by Honggao *et al.*, [20]. Firstly, adding 0.5 ml phosphate buffer (pH 7.2) and 0.2 ml of 1% $K_3[Fe(CN)_6]$ into 500 µl sample, in turn, and kept in 20 min at 50 °C. 500 µl of 10 % CCl_3COOH, 300 µl distilled water, and 80 µl of 0.1% $FeCl_3$ was then added into the mixture, respectively. Finally, the absorbance measurement was at the wavelength of 655 nm, and the standard of $FeSO_4$.

Characterization of Antioxidant Pectin:

Solubility of Pectin: Dissolving 0.3 g pectin powder into 20 ml distilled water at 90 °C for 30 min and then filtered through membrane Whatman no. 01. After evaluating pectin content for the filtrate. The insoluble fraction was calculated by using the difference of 0.3 g pectin powder and dissolved pectin content.

Sugar Composition of Antioxidant Pectin: The analyzation of sugar composition was on the HPLC system - Jasco Inc., Tokyo, Japan. Pump PU980 connect detector RI (RI830). Column Aminex HPX-87-Ca (300-7.8 mm) (Bio-Rad Laboratories, Hercules, California, USA).

Elution Condition: flow rate of 0.5 ml/min, injection volume of 20 µl, column temperature of 80 °C. The mobile phase using in the analysis was 0.05 mM H_2SO_4 solution.

Viscosity Measurement of Pectin: determination of pectin viscosity was on the machine Brookfield.

Fourier Transform Infrared Spectroscopy (FTIR) of Antioxidant Pectin: FTIR spectra record of pectin was on IR Equinox 50, Bruker. Compressing pectin powder was into tablets with potassium bromide (KBr). The transmission (%) was in the 4,000-500 cm^{-1} region.

Determination of Esterification Degree of Pectin: The determination of the esterification degree of pectin was according to the methodology of Mohamed *et al.*, (2016) [21]. Pectin powder (0.05 g) was dissolved in 50 mL of distilled water and kept for 12-15 h at 50 °C. Adjusting the solution was to a pH of 8.5 by using 0.05 mol/L NaOH and ticked V_1. Realizing the saponification process was by adding the same volume of 0.5 mol/L NaOH, and kept for 30 min at 30 °C. Neutralizing the compound was then by 0.5 mol/L HCl. The excess of HCl was treated by adding 0.05 mol/L NaOH and named V_2. Calculation of esterification degree of pectin was as follow:

$$DE\ (\%) = (V_2 / V_1 + V_2) \times 100 \ldots\ldots\ldots (Eq.\ 1)$$

Data analysis: Each experiment was triplicated (n=3). The values are expressed under a mean ± standard deviation. The analysis of ANOVA and regression were by using soft Excel 2007. Remove the unnormal values by using the method of Dulcan.

RESULTS AND DISCUSSIONS:

Extraction of Antioxidant Pectin:

Effect of Various pH Solvent on Pectin Content and Antioxidant Activities: Antioxidant pectin content extracting from cactus changed according to the change of solvent pH and was affected by various pH solvent ($p < 0.05$). The highest value of pectin content, total antioxidant activity, and reducing power (RP) got at pH 4, corresponded to 231.99 ± 6.45 mg uronic acid equivalent/g DW, 10.02 ± 0.28 mg ascorbic acid equivalent/g DW and 1.02 ± 0.03 mg $FeSO_4$ equivalent/g DW **Fig. 2**, respectively, compared to other pH solvent. Pectin content and antioxidant activities got the minimum value at pH 8. The closing correlation between pectin content and antioxidant activities was found ($R^2 > 0.8$) in the study.

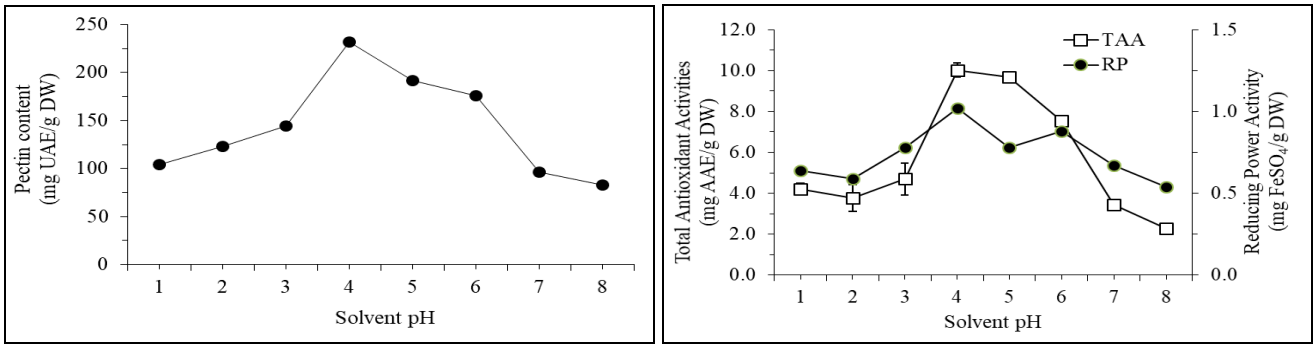

FIG. 2: EFFECT OF SOLVENT PH ON PECTIN CONTENT AND ANTIOXIDANT ACTIVITY

Pectin content, total antioxidant activity, and reducing power changed according to the model of levels 5, 5, and 6, corresponding to the equations 2, 3, and 4, under the impact of solvent pH from 1 to 8. The regression equations have shown complex fluctuations of the target functions under the influence of factors in the extraction process.

$Y = 0.1255\ x^5 - 1.5784\ x^4 + 0.7254\ x^3 + 42.495\ x^2 - 102.55\ x + 166.1\ R^2 = 0.9209$ … (2)

$Y = 0.0122\ x^5 - 0.1872x^4 + 0.6277\ x^3 + 1.5396\ x^2 - 7.5767x + 9.874\ R^2 = 0.9559$ …(3)

$Y = 0.0006\ x^6 - 0.0168\ x^5 + 0.1975\ x^4 - 1.1715x^3 + 3.6034\ x^2 - 5.1603\ x + 3.19\ R^2 = 0.842$…..(4)

A correlation between pectin content and anti-oxidant activity (TAA, RP) under the effect of various extracting conditions such as pH solvent, solvent-to-material ratio, extracting time, extracting temperature, and times of repeated extraction was good ($R^2 > 0.9$), except for total antioxidant activity ($R^2 = 0.48$) and reducing power activity ($R^2 = 0.59$) under the impact of solvent-to-material ratio and extracting temperature, respectively **Table 1**.

TABLE 1: A PEARSON CORRELATION BETWEEN PECTIN CONTENT AND ANTIOXIDANT ACTIVITY (TAA, RP) UNDER THE EFFECT OF VARIOUS EXTRACTING CONDITIONS

Various Extracting Conditions	Total Antioxidant Activity (TAA)	Reducing Power Activity (RP)
Various pH solvent	0.96	0.92
Solvent-to-material ratio	0.48	0.90
Extracting time	0.91	0.94
Extracting temperature	0.99	0.59
Times of repeated extraction	0.99	0.99

It means that pectin possessed and played a important role in antioxidant activity. Function groups of pectin possessed both total antioxidant activity and reducing power activity, and it was different when function groups were affected by extracting temperature ($R^2 = 0.65$) **Table 2**. It said that the extracting temperature impact on pectin content and antioxidant activity were higher than other input factors.

TABLE 2: A PEARSON CORRELATION BETWEEN TOTAL ANTIOXIDANT ACTIVITY AND REDUCING POWER ACTIVITY UNDER THE EFFECT OF VARIOUS EXTRACTING CONDITIONS

Various Extracting Conditions	Reducing Power Activity (RP)
Various pH solvent	0.86
Solvent to-material ratio	0.72
Extracting time	0.98
Extracting temperature	0.65
Times of repeated extraction	0.99

The pectin content and antioxidant activity were higher in comparison to that of Gopi *et al.,* on cactus *Opuntia ficus* Indica [22] and similar to that of citrus [23]. The regression analysis between the pectin content and the antioxidant activity according to the change of solvent pH showed the model of level 5.

Cactus cells structure and pectin structure are less affected by solvent pH 1, compared to solvent pH 8. Different pH solvents impacted different the linkage between the pectin molecule and hemicellulose caused a significant difference in pectin molecules liberation. Effect of solvent to the material ratio on pectin content and antioxidant activities. Antioxidant pectin content extracting from cactus *Opuntia dillenii* was strongly affected by the S/M ($p < 0.05$). The maximum point of the model had happened as the S/M of 6/1 (v/w). At the maximum peak, pectin content corresponded to 259.973 ± 8.241 mg uronic acid equivalent/g DW, TA corresponded to 3.558 ± 0.125 mg ascorbic

acid equivalent/g DW, and RP corresponded to 1.256 ± 0.037 $FeSO_4$ equivalent/g DW **Fig. 3.**

The model of levels 5, 5, and 6 exhibited the change of pectin content, total antioxidant activity and reducing power under the impact of the solvent-to-material ratio from 1/1 to 8/1 (v/w), corresponding to the equation 5, 6 and 7.

$Y = 0.4591\ x^5 - 9.8177\ x^4 + 75.308\ x^3 - 257.47\ x^2 + 413.95x - 88.939$ $R^2 = 0.9303$… (Eq. 5)

$Y = 0.0036\ x^5 - 0.0802\ x^4 + 0.651\ x^3 - 2.365\ x^2 + 3.8624x - 1.3916$ $R^2 = 0.966$…. (Eq. 6)

$Y = -0.005\ x^6 + 0.1575\ x^5 - 1.9113\ x^4 + 11.337\ x^3 - 33.905\ x^2 + 47.045\ x - 20.338$ $R^2 = 0.952$…(Eq. 7)

When the S/M increased, antioxidant pectin content increased. It was reverse when the S/M decreased. When the S/M increased over 6/1 (v/w), the difference in pectin content and antioxidant activities in various extracting conditions was not significant ($p > 0.05$). Pectin content and antioxidant activities change according to the non-linear model of level 5 were different to the previous results. A strong correlation between pectin content and antioxidant activities was found ($R^2 > 0.7$). A difference for antioxidant pectin content at the S/M ratio from 6/1 to 8/1 (v/w) was not significant. The heating time, the used solvent volume, the heat loss, and the dissolve of unexpected substances into the extracting solvent increased as the ratio increased S/M [24-26].

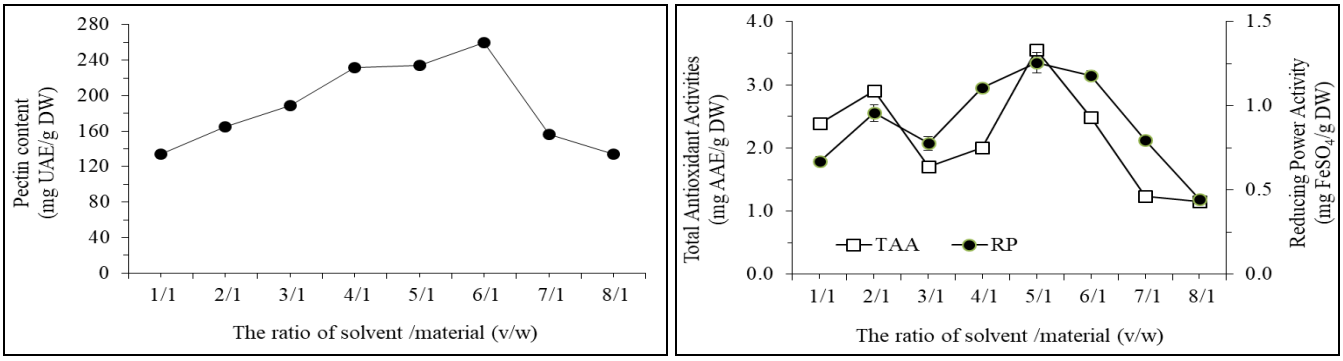

FIG. 3: EFFECT OF SOLVENT/ MATERIAL RATIO ON PECTIN CONTENT AND ANTIOXIDANT ACTIVITY

Effect of Extracting Time on Pectin Content and Antioxidant Activities: Antioxidant pectin content was also affected by the extracting time ($p < 0.05$). The maximum pectin content corresponded to 259.30 ± 5.86 mg uronic acid equivalent/g DW. Highest TA value got 2.48 ± 0.077 mg ascorbic acid equivalent/g DW and highest RP value was 1.22 ± 0.037 mg $FeSO_4$ equivalent/g DW. TA and RP got the lowest value as the extracting time of 180 min, 1.313 ± 0.04 mg ascorbic acid equivalent/g DW and 0.203 ± 0.008 mg $FeSO_4$ equivalent/g DW, respectively **Fig. 4**.

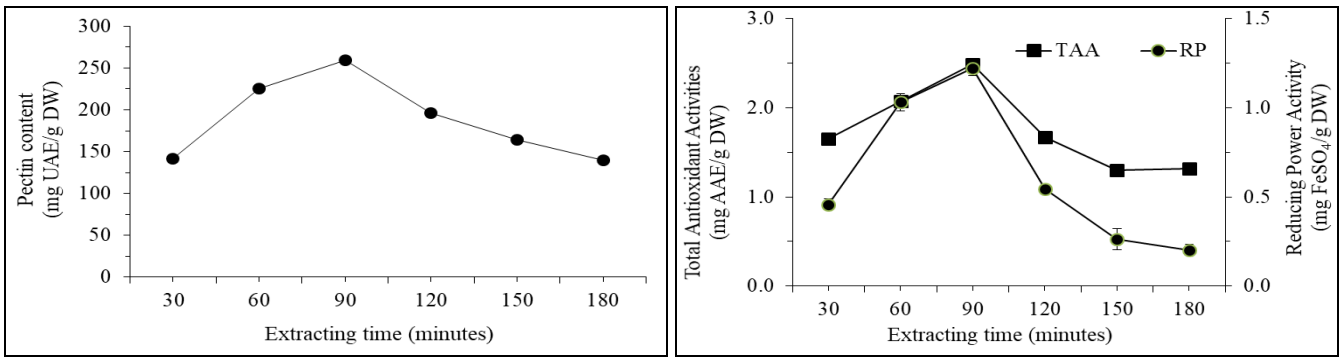

FIG. 4: EFFECT OF EXTRACTING TIME ON PECTIN CONTENT AND ANTIOXIDANT ACTIVITY

The change of pectin content, total antioxidant activity, and reducing power under the impact of the extracting time from 30 to 180 min was according to the model of levels 3, 4, and 3, corresponding to the equation 8, 9, and 10.

$Y = 6.2581\ x^3 - 80.124\ x^2 + 291.99\ x - 79.034$ $R^2 = 0.9629$….(Eq. 8)

$Y = 0.0243\ x^4 - 0.2756x3 + 0.8498\ x^2 - 0.4556\ x + 1.4873$ $R^2 = 0.9185$…(Eq. 9)

$Y = 0.0628\ x^3 - 0.7497\ x^2 + 2.501\ x - 1.3807\ R^2 = 0.9492$…..(Eq. 10)

Pectin content and antioxidant activities change according to the non-linear model of level 3, and the maximum peak of the model was at the extracting time of 90 min. Antioxidant pectin content increased as the extracting time increased from 30 to 90 min and decreased as the extracting time increased from 90 to 180 min. At the extracting time of 180 min, extracted pectin content was the lowest. The correlation between pectin content and antioxidant activities was close (R^2 > 0.9). Those demonstrated that the longer time is, the bigger the degradation of pectin structure, and antioxidant activities decrease. The durability of the pectin in the current study was lower than in previous publications (the linkage decomposition of glycosidic only happens was at least 7 h [27]. The lower the molecular weight of pectin is, the higher the solubility in water and dispersion in alcohol are. The long extracting time would cause some disadvantages for the precipitation step; for example, other compounds increase (not pectin) in the extract, the difficulty in the pectin precipitation. The pectin extraction yield decreased as the extracting time increased because of the extracting time increase leading to the decomposition increase of pectin and the depolymerization process of the galacturonan sequence of pectin under the effect of the temperature [28]. It means that the recovery yield and the activity of pectin decrease when the extracting time increase.

Effect of Extracting Temperature on Pectin Content and Antioxidant Activities: Pectin content, TA, and RP were the highest value in the temperature of 90 °C, 259.26 ± 7.75 mg uronic acid equivalent/g DW, 2.49 ± 0.075 mg ascorbic acid equivalent/g DW, and 1.21 ± 0.036 mg $FeSO_4$ equivalent/g DW, respectively. The content and the TA of the pectin decreased to 183.85 ± 5.31 mg uronic acid equivalent/g DW and 1.80 ± 0.046 mg ascorbic acid equivalent/g DW, correspondingly **Fig. 5** when the extracting temperature increased to 100 °C. RP also decreased light, compared to the decrease of TA and pectin content. RP was the minimum value of 0.476 ± 0.014 mg $FeSO_4$ equivalent/g DW as the extracting temperature of 70 °C. Pectin content was significantly dropped from 259.26 ± 7.75 to 183.85 ± 5.31 mg uronic acid equivalent/g DW (75.41 mg uronic acid equivalent/g DW) when the temperature increased to 100 °C, compared to the temperature of 90 °C. The pectin content and the antioxidant activity of pectin was a close correlation (R^2 > 0.95) and changed according to the non-linear function of level 2. The structure of the cactus cell is still lastingness and the linkage between pectin and the cell membrane is stable when the extracting temperature is not higher than 70 ºC. The cleavage of components in the structure of pectin molecules happens stronger under the effect of high temperature, and suitable conditions of pectin extraction range from 80 ºC -90 ºC depending on plant species [29].

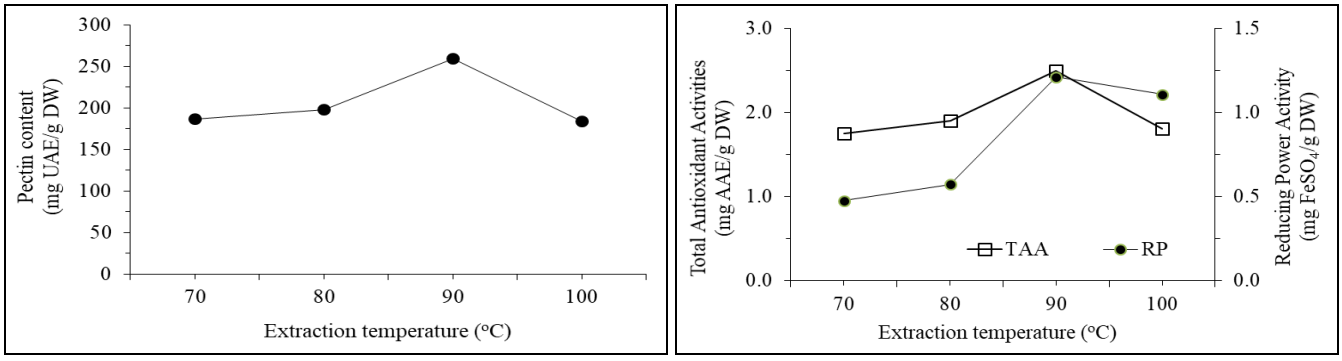

FIG. 5: EFFECT OF EXTRACTION TEMPERATURE ON PECTIN CONTENT AND ANTIOXIDANT ACTIVITY

Effect of Times of Repeated Extraction on Pectin Content and Antioxidant Activity: The efficiency of antioxidant pectin extraction from the cactus was affected by the time of repeating extraction. Pectin content and antioxidant activity was 79.05% at the first time of the extraction, compared to all extraction time. TA and RP corresponded to 2.49 ± 0.068 mg ascorbic acid equivalent / g DW and 1.31 ± 0.037 mg $FeSO_4$ equivalent/g DW, respectively. At the second time of the extraction, pectin content decreased sharply to 45.13 ± 1.46 mg uronic acid equivalent/g DW.

TA and RP corresponded to 0.73 ± 0.026 mg ascorbic acid equivalent/g DW, 0.24 ± 0.007 mg FeSO4 equivalent/g DW, respectively **Fig. 6**. At the third time of extraction, the extraction efficiency (7.19%), TA (0.21 ± 0.008 mg ascorbic acid equivalent/g DW) and RP (0.13 ± 0.005 mg $FeSO_4$ equivalent/g DW) got the lowest value in comparison to all three extractions.

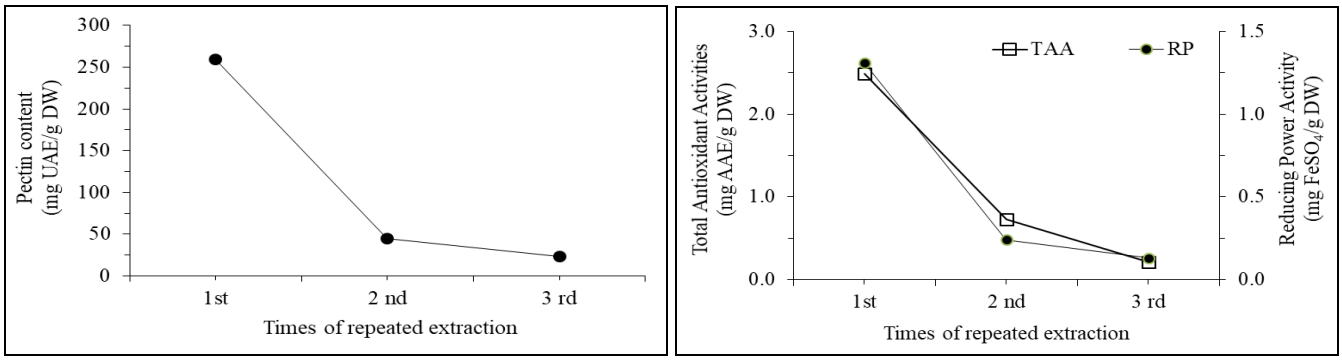

FIG. 6: EFFECT OF TIMES OF REPEATED EXTRACTION ON PECTIN CONTENT AND ANTIOXIDANT ACTIVITY

The content and antioxidant activities of pectin were dropped nearly three times, compared to the first time of extraction. Pectin content and antioxidant activities had a close correlation according to the linear function ($R^2 > 0.9$). The times of repeating extraction impacted on antioxidant pectin content ($p < 0.05$). The pectin color extracting from the cactus was white **Fig. 6**. The color was more beautiful than jackfruit (*Artocarpus heterophyllus*) waste pectin color (yellowish) [30]. The impact of temperature and pH solvent at the second and third extraction times caused the stronger destruction of pectin that led to the efficiency of antioxidant pectin extraction decreased when extraction times increased.

Physic-chemistry Characterization of Antioxidant Pectin:

Viscosity, Solubility Ability and Esterification Degree of Pectin: Pectin was whiter than commercial pectin. The solubility ability of pectin is fully in hot water. Viscosity of pectin got 0.893 ± 0.051 Pa.s. In the current study, the esterification degree of the pectin (62.38 ± 0.94%) was the average level, compared to previous reports.

FIG. 7: PECTIN PREPARED FROM CACTUS

The solubility ability and the viscosity of pectin in hot water were similar to commercial pectin and higher than jackfruit (*Artocarpus heterophyllus*) waste pectin (partly soluble) [30]. The viscosity of the pectin solution was high when the temperature of the solution was the same as room temperature. The phenomenon of water absorption increased when water temperature increased. When pectin powder clumped, the solubility of pectin in the water was less. Thus, it should be the storage of pectin powder in dry conditions and crushed to remove clump before dissolving of pectin into the water. The factors that affect the pectin solubility was determined, for example, time and temperature of dissolving, the rate of stirring in the dissolution process, storage time, and state of pectin powder.

Commercial citrus pectin got the esterification degree of 70.00 ± 0.65 %. The esterification degree of citrus pectin was 63.11 ± 0.25% and 59.92±3.22 % for extracted pectin in citric and nitric solution, respectively [23]. In the current study, the esterification degree of the pectin (62.38 ± 0.94%) was the average level, compared to previous reports. All things suggested that the esterification degree of pectin depended on the condition of extraction and material of pectin extraction. Pectin from cactus *Opuntia dillenii*, commonly growing on the Vietnam coast, especially on Ninh Thuan coast, can be used as a carrier for drug delivery.

Fourier Transform Infrared Spectroscopy and Sugar Composition of Pectin: Antioxidant pectin at the first time extraction and the second time extraction was determined some characterization

by the method of infrared spectroscopy. FTIR spectra exhibited various groups of pectin **Fig. 8**. The study results were analyzed and compared to the notice of Gopi *et al.*, 2015 [22], and other publications, noticed in **Table 3**.

TABLE 3: ABSORPTION PEAK ON FTIR OF PECTIN EXTRACTING FROM CACTUS OPUNTIA DILLENII

A Functional Group	Absorption Peak	
	Published	In the Current Study
The C-H stretching	2923 cm^{-1} [31-33]	2926.62 cm^{-1} Fig. 8a; 2929.49 cm^{-1} Fig. 8b
stretching of hydroxyl groups	3432 cm^{-1} [34-36]	3435.96 cm^{-1} Fig. 8a; 3434.80 cm^{-1} Fig. 8b
C=O stretching vibration of the ionic carboxyl group	1624 cm^{-1} [31, 37]	1608.14 cm^{-1} Fig. 8a; 1641.34 cm^{-1} Fig. 8b
Stretching vibration of the COO- group	1400-1450 cm^{-1} [31, 38]	1408.81 cm^{-1} Fig. 8a, 1427.20 cm^{-1} Fig. 8b
C=O stretching vibration of methyl esterified carboxyl groups	1732 cm^{-1} [31, 39]	1743.18 cm^{-1} Fig. 8b
the C-O and C-C vibrational bands of	1000-1200 cm^{-1} [31, 34]	1071.4 - 1249.03 cm^{-1} Fig. 8a, 1027.39 -
The β-D pyranose form of the glucosyl residue	1043 cm^{-1} [31, 40]	1071.04 Fig. 8a, 1027.39 cm^{-1} Fig. 8b
C-O-C stretching, OH bending and CH_3 plane deformation	800-1300 cm^{-1} [22, 41]	777.51 - 1317.95 cm^{-1} Fig. 8a, 765.65 - 1326.49 cm^{-1} Fig. 8b
The O-C-O bending and CH deformation	400-800 cm^{-1} [22, 42]	426.78 - 774.51 cm^{-1} Fig. 8a, 439.29 - 765.65 cm^{-1} Fig. 8b

The stretching peak at 3435.96 cm^{-1} **Fig. 8A** and 3434.80 cm^{-1} **Fig. 8B** corresponded to hydroxyl groups. The absorption peak at 2926.62 cm^{-1} **Fig. 8A** and 2929.49 cm^{-1} **Fig. 8B** exhibited the O-CH_3 group. O-CH_3 group is the methyl ester of galacturonic acid. The absorption peak at 1608.14 cm^{-1} **Fig. 8A** and 1641.34 cm^{-1} **Fig. 8B** attributed C=O stretching vibration of the ionic carboxyl group [31, 37]. C=O stretching vibration of methyl esterified carboxyl groups existed in pectin extracting in 2nd extraction, and an absorption peak at 1743.18 cm^{-1} **Fig. 8B** also attributed to the thing [39] that suggested extracted pectin belong to low methoxyl pectin. The absorption peak at 1071.04 **Fig. 8A** and 1027.39 cm^{-1} **Fig. 8B** showed the β-D pyranose form of the glucosyl residue [40]. The absorption peak 1408.81 cm^{-1} **Fig. 8A** and 1427.20 cm^{-1} **Fig. 8B** corresponded to the asymmetric stretching vibration of the COO- group [38]. The C-O and C-C vibration bands of glycosidic bonds and pyranoid ring were in the region 1071.4 - 1249.03 cm^{-1} **Fig. 8A** and 1027.39 - 1236.32 cm^{-1} **Fig. 8B**. The signal range of 777.51 to 1317.95 cm^{-1} **Fig. 8A** and the signal range of 765.65 to 1326.49 cm^{-1} **Fig. 8B** referenced in the region of CH_3 plane deformation C-O-C stretch and OH bend [41]. The range of 426.78 - 774.51 cm^{-1} **Fig. 8A** and 439.29 - 765.65 cm^{-1} **Fig. 8B** regarded the O-C-O bending and CH deformation [42]. Hence, pectin extracting from the cactus is low methoxyl pectin. Stretch vibration of functional groups on the pectin structure depends on the material and extraction condition. The retention time of glucuronic acid varied from 20.920 min to 21.765 min and that of galacturonic acid from 20.915 min to 21.779 min, respectively **Fig. 9**.

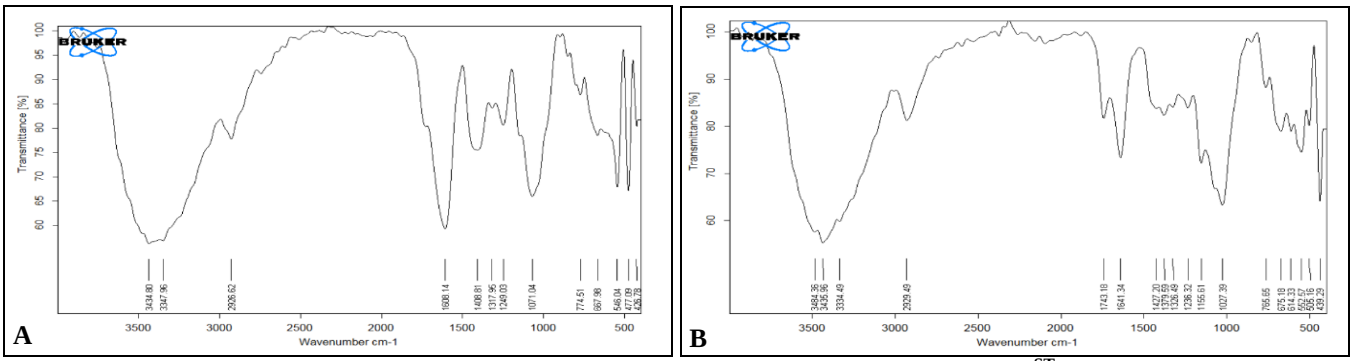

FIG. 8: FOURIER TRANSFORM INFRARED SPECTROSCOPY OF PECTIN: (A) 1ST EXTRACTION PECTIN FTIR; (C) 2ND EXTRACTION PECTIN FTIR

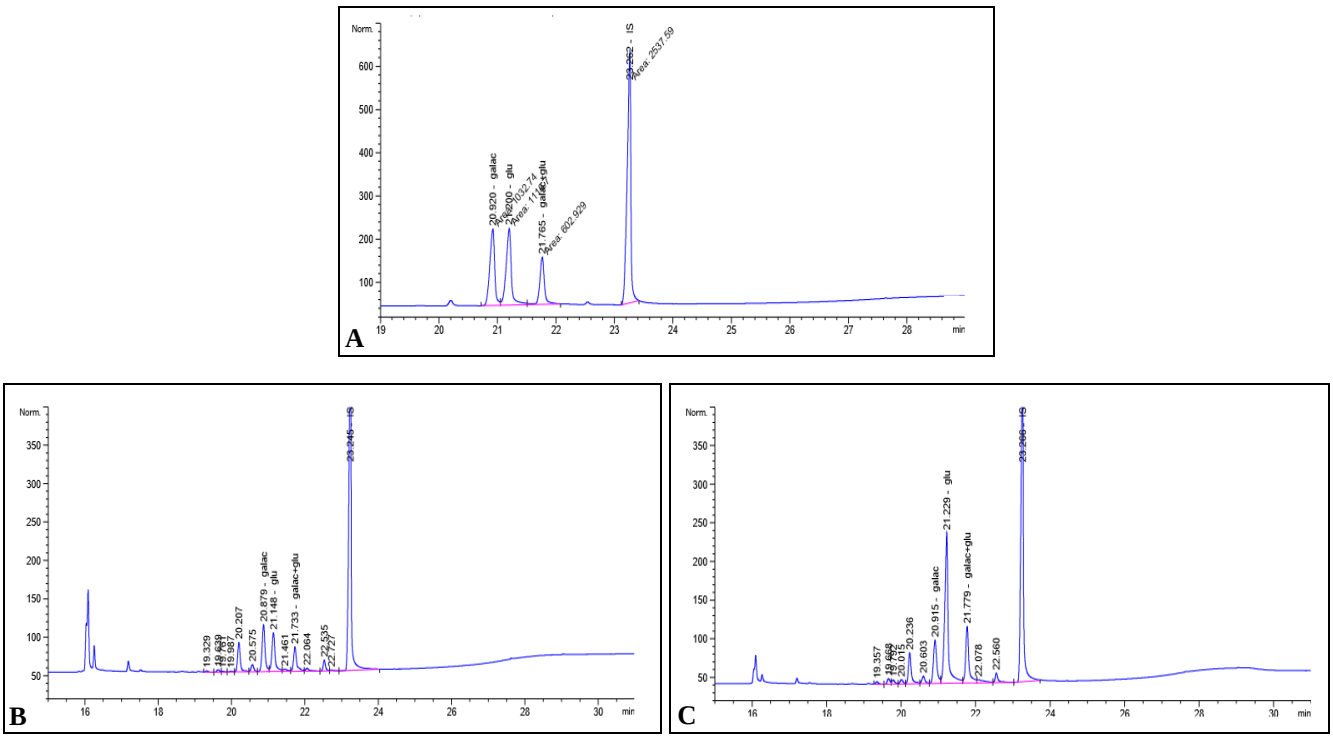

FIG. 9: SUGAR COMPOSITION SPECTROSCOPY OF PECTIN: (A) SUGAR COMPOSITION SPECTROSCOPY OF STANDARD PECTIN; (B) SUGAR COMPOSITION SPECTROSCOPY OF PECTIN EXTRACTED IN 1ST EXTRACTION; (C) FTIR OF PECTIN EXTRACTED IN 2ND EXTRACTION

The peak of glucuronic acid and galacturonic acid appeared in pectin extracting for the first time. The thing showed some linkage degradation between glucuronic acid and galacturonic acid did not happen in the processing of extraction and hydrolysis. Peak areas of glucuronic acid and galacturonic acid were the highest in comparison to all other peaks **Fig. 9**. Compared to extracted solid, collected pectin in the first extraction times was purer than that in the second extraction times because pectin content in the first extraction times was higher than that in the second extraction times. Pectin of cactus *Opuntia dillenii* grown in Vietnam mainly contained glucuronic acid and galacturonic acid, and the direct linkage between glucuronic acid and galacturonic acid existed in pectin. The results showed the pectin structure of cactus trunk *Opuntia dillenii* grown in Vietnam was rhamnogalacturonan backbone [43].

Sugar composition and structure characterization of pectin from cactus fruit *Opuntia dillenii* grown in Indian were different in comparison to pectin from cactus trunk *Opuntia dillenii* grown in Ninh Thuan coast, Vietnam. Components analysis of pectin in various extraction times showed a common point. Glucuronic acid and galacturonic acid exist in collected pectin, and the ratio of glucuronic acid and galacturonic acid was different for collected pectin in various extraction times. The pectin structure would be homogalacturonan or rhamnogalacturonan or both, noticed by Valérie *et al.*, 2018 [44]. Under a condition of high H^+ ion concentration, the hydrolysis of protopectin and the formation of carboxylic acid groups from carboxylate groups were increased [45, 46].

CONCLUSION: Pectin of cactus grown on the Ninh Thuan coast, Vietnam, possessed strongly antioxidant activity ($R^2 > 0.9$). They contained two main compositions (glucuronic acid and galacturonic acid) and dissolved into hot water with an esterification degree of 62.38 ± 0.94%. Pectin exhibited white color, the viscosity of 0.893 ± 0.051 Pa.s, and fully solubility in 90 °C. Antioxidant activities and pectin content were strongly affected by input factors of the extraction condition, for example, temperature, pH, time a ratio of solvent-to-material, and times of repeated extraction ($p < 0.05$). The highest pectin content and highest antioxidant activities (TA and RP) corresponded to 109.781 ± 0.0159 mg uronic acid equivalent / g DW and (1.536 ± 0.009 mg ascorbic acid equivalent/g DW and 0.301 ± 0.019 mg $FeSO_4$ equivalent/g DW), respectively, were at the temperature of 90 °C with the S/M ratio (6/1, v/w)

for 90 min and acidic solvent (pH 4). They can fully deploy on the industrial scale for application into the food industry and pharmacy.

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