academicJournals

Vol. 10(40), pp. 732-747, 25 October, 2016
DOI: 10.5897/JMPR2016.6207
Article Number: E010AA961417
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Genetic diversity and seasonal chemical profile by ^{1}H NMR and cytotoxic activity in *Opuntia* and *Nopalea* genres

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Received 22 July, 2016; Accepted 12 October, 2016

The cactus pear (*Opuntia* spp.) is known to have bioactive compounds which work in the prevention of various diseases, especially cancer. The objectives of the study were to characterize the chemical profile and genetic diversity, through chromatic tests and ^{1}H NMR, using multivariate analysis, and assess the cytotoxic potential of cactus pear varieties of *Opuntia* and *Nopalea* genera grown in the semi-arid region of Brazil in dry and wet seasons. In the study of chemical prospecting and cytotoxic activity, crude ethanol extracts from cladodes of varieties (IPA-100003, IPA-100004, IPA-200021, IPA-200205, IPA-200008, IPA-200149 and IPA-200016) were used. The cytotoxic activity was evaluated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl bromide tetrazolin] against HCT-116 (human colorectal), SF-295 (human glioblastoma) and OVCAR-8 (human ovarian cancer). The group of chemicals which stand out are the carbohydrates and glycosylated substances, lipids (fatty acids and steroids) and phenolic compounds (flavonoids), which vary both between the botanical varieties studied and among the collection periods. The analysis of the NMR spectra of ^{1}H cactus pear varieties by methods of multivariate analysis finds genetic diversity among the materials in the dry and rainy seasons. The ethanol extract (50 µg ml^{-1}) shows limited growth inhibitory effect on the cancer cell lines examined. It is concluded that the cytotoxic activity of cactus pear cladodes is attributed to phenolic compounds especially the flavonoids. It is recommended that population at risk utilize these materials as preventative natural dietary supplements against cancer.

Key words: Anticancer, cactus pear, flavonoids, food analysis, forage, phenolics, semiarid.

INTRODUCTION

The cactus pear of the genera *Opuntia* and *Nopalea* are widely (about 550.000 ha) cultivated in the semi-arid region of northeastern Brazil, and represent an important forage option in the dry season. This region is characterized by having an average temperature greater than 30°C, high rate of annual evaporation, greater than 2000 mm, and average of less than 750 mm rainfall, concentrated in a single period of 3 to 5 months, in addition, in some years the lack of rain is prolonged, resulting in periods of drought (Araújo et al., 2005; Medeiros et al., 2005).

Due to the cactus pear having morphophysiological mechanisms to absorb small amounts of rain water and reduce water loss through transpiration, it is well-adapted to arid and semi-arid regions, where water is a limiting factor in agricultural production. For this reason, cultivation of the cactus pear is a viable alternative source of income for inhabitants of these regions in the dry season, who depend in agriculture for their livelihood (Oliveira et al., 2010). Due to the increase in arid and semi-arid areas and the reduction of water resources in the world, cactuses are gaining importance in human and animal food, medicine, cosmetics and the pharmaceutical industry (Shedbalkar et al., 2010).

The World Health Organization (WHO) has expressed its position on the need to value the use of medicinal plants in the health field, taking into account that 80% of the population uses plants or their preparations as regards the primary health care. Beside this, there are several auxiliaries, including the social and economic spheres, which work in advancing health through the use of medicinal plants. Since there are social classes with low financial power what have no access to allopathic medicine (Nascimento et al., 2016). In this context, only in 2011, phytotherapy in Brazil generated revenues R$ 1.1 billion. Therefore, the popular and institutional interest has grown to strengthen phytotherapy in the Unified Health System (SUS) as the use of medicinal plants and their rituals provides an economical way healing for most of the population, contributes significantly to the attention primary health (Nascimento et al., 2016).

Since 2007, the SUS provides herbal plant derived, such that currently offers the use of 12 herbal medicines of the National List of Essential Medicines (RENAME) available SUS (Holy Bramble, Guaco, Artichoke, Aroeira, Cascara, Devil's claw, Isoflavone-of-soy, Catnail, Mint, Aloe, Willow, Plantago) all these plants adapted the conditions of the Brazilian semi-arid (Nascimento et al., 2016).

The Ministry of Health issued in February, 2009, the National Relationship Medicinal Plants of Interest to the SUS (RENISUS). This list includes 71 plants species that have the potential to generate products of interest to SUS. The list view was and is directing studies and research that could subsidize the maintenance of herbal ratio available for use by the population, with safety and efficacy for the treatment of some diseases (Nascimento et al., 2016). Due to increased public interest in the use of these plants and their therapeutic potential, the scientific community has been searching for ways to obtain new herbal (Peixoto et al., 2014).

The species *Opuntia ficus-indica* is known to be an important source of bioactive compounds, such as betalains, polyphenols, carotenoids, vitamin C and minerals. The plant also prevents disease via its antioxidant, anticancer, neuroprotective and anti-proliferative properties, in addition to being used in the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes, inflammation and pain management (Morales et al., 2012). Nuclear magnetic resonance spectroscopy (NMR) is a powerful analytical tool for the characterization of heterogeneous materials from natural sources. Among the main features of the natural sources, isotopes ^{1}H, ^{13}C and ^{31}P are the most commonly used in the studies, as they are the most abundant elements in nature and due to the sensitivity of their nuclei. So the high-resolution NMR technique, such as ^{1}H, is a viable alternative in the study of organic compounds due to the simplicity and the importance of information generated (Iulianelli and Tavares, 2011).

Due to a wide genetic diversity in *Opuntia* and *Nopalea* genera, with about 300 species (Mondragón-Jacobo and Pérez-González, 2001), there is a need to characterize the varieties grown in Brazil in order to get nutritional information and bioactive properties. The objectives of the study were to characterize the chemical profile and genetic diversity, through chromatic tests and ^{1}H NMR, using multivariate analysis, and assess the cytotoxic potential of cactus pear varieties of *Opuntia* and *Nopalea* genera grown in a semi-arid region of Brazil during the dry and rainy seasons.

MATERIALS AND METHODS

Plant

In the study, cladodes of different varieties of three years old cactus pear were used, collected in the experimental station of the Agronomic Institute of Pernambuco (IPA), located in the city of Arcoverde, State of Pernambuco, Brazil. The materials used

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are listed in Table 1. Secondary and tertiary cladodes of each variety were collected from six plants, at 8:00 am on February 19, 2013 (dry season) and on May 10, 2013 (rainy season). After collection the material was cleaned with brush, cut into small pieces (2 to 3 cm in length) and dried in a forced air circulation oven at 55°C, where it remained for 72 h until constant weight. The dried material was crushed in a Willey® type mill and packed in sealed plastic pots.

Ethanol extraction

The extraction of the crushed material (10 g) was performed with 95% ethanol at room temperature, covering the entire sample with the solvent. The samples were in contact with the solvent for eight days, and stirred daily, renewing the extractor solvent every two days. After this period, the extracts were filtered and concentrated using a rotary evaporator under reduced pressure at 40 °C, yielding the crude ethanol extract, and kept in glass vials sealed at room temperature (22°C) until use (Vizcaino et al., 2007).

Chemical prospection

One milligram of each sample (crude ethanolic extract) was used for the identification of the major classes of chemicals, using the protocols described in Matos (2009). The intensity of the color and/or appearance of a precipitate in the performance of chemical reactions were interpreted as responses to the tests. The alkaloids were detected by precipitation method using a reactive *Bouchardat* (A), *Mayer* (B), *Dragendorf* (C) and *Bertrand* or silico-tungstic acid (D); Steroids were detected by the *Liebermann-Burchard* reaction; tannins by precipitation methods with iron salts and gelatin; flavonoids detected by the reactions of *Shinoda* e *Taubouk*; saponins by the agitation of the aqueous extract with persistent foaming (Desoti et al., 2011).

^{1}H NMR spectra

^{1}H NMR spectra of crude ethanolic extracts were obtained from s 200 MHz NMR Oxford® spectrometer. For analysis of the ^{1}H core sample solutions were prepared using approximately 20 mg of extract and 0.6 ml of deuterated methanol (99.95%) as solvent. Samples were placed into NMR tubes five mm in diameter and subsequently in a five mm probe. Typical acquisition parameters included frequency of observation of 200.0 MHz, acquisition time 4.0 s, spectral 3200.0 Hz window, pulse width 0.0 µs, number of accumulations (410), interval between pulses 1.0 s. The spectra were processed by MestReNova® program version 6.1.0 and the chemical shifts are expressed in ppm (Iulianelli and Tavares, 2011; Prestes et al., 2012).

Determination of cytotoxic activity

The evaluation of the cytotoxic effect of crude ethanolic extracts on human tumor cells was performed by MTT test [3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl bromide tetrazolin] in triplicate. The tumor cell lines OVCAR-8 (human ovarian), HCT-116 (human colorectal) and SF-295 (human glioblastoma), used in this study were provided by the National Cancer Institute (USA), which were cultured in RPMI® 1640, supplemented with fetal bovine serum (10%) and antibiotics (1%), kept at a constant temperature of (37°C) and CO_2 (5%). The cells were distributed into 96 well plates at following densities: 0.1×10^6 cells ml^{-1} (OVCAR-8 and HCT-116) and 0.7×10^5 cells ml^{-1} (SF-295). The crude ethanolic extract from cladodes of cactus pear varieties were diluted in pure sterile dimethyl sulfoxide (DMSO) at a concentration 50 µg ml^{-1}, and added to the wells, which were maintained for 72 h under controlled conditions of temperature (37°C) and CO_2 (5%). After this period, the plates were centrifuged (1500 rpm / 15 min) and the supernatant discarded. Each well received 150 µL of MTT solution (0.5 mg/ml), and the plate was kept for 72 h in a climatised oven. At the end of the period, the plates were centrifuged again (3000 rpm / 10 min), the supernatant discarded and the pellet suspended in 150 µl of DMSO. Quantification of low salt in living cells was performed in a spectrophotometer plates at 595 nm (Mosmann, 1983). An intensity scale was used to assess the cytotoxic potential of the samples tested: Sample with no activity (NA), with little activity (LA, inhibiting cell growth varying from 1 to 50%) with moderate activity (MO, inhibition of cell growth ranging from 50 to 75%) and much activity (MA, growth inhibition ranging 75 to 100%).

Statistical analysis

The experimental design used was randomized blocks, with seven treatments, represented by the varieties with three repetitions. The experimental plots consisted of two plants. Analyses were performed in triplicate, and the results were expressed as mean ± standard deviation using the Excel program of Microsoft Office® 2010. Genetic diversity among the varieties was estimated using the measure of similarity expressed by the Jaccard index, according to Cruz et al. (2012). Hierarchical clustering method UPGMA (Unweighted Pair Group Method with Arithmetic Mean), Optimization method of Tocher (Rao, 1952) and method of Principal Component Analysis (Cruz et al., 2012) were carried out. The analyses of these data were carried out with the help of the statistical program, GENES® - Computer Application in Genetics and Statistics (Cruz, 2001).

RESULTS AND DISCUSSION

Chemical prospection

The results of the phytochemical screening of crude ethanol extract of varieties of cactus pear, *Opuntia* and *Nopalea,* in the dry and rainy seasons are shown in Table 2. Of the tests for the identification of the main classes (alkaloids, steroids, tannins, flavonoids and saponins) present in the cactus pear of cladodes the presence of flavonoids and steroids in all varieties were detected in both periods studied (dry and wet). The presence of tannins was detected in the IPA-200149 variety in the dry season, and the IPA-100003, IPA-200016, IPA-200205 varieties in the rainy season. The presence of flavonoids and steroids on cladodes of cactus pear (*Opuntia ficus indica*) was cited by Brás (2011) and Soares (2012), the presence of tannins in the cactus pear cladodes was reported by Mendez et al. (2012) studying *Opuntia ficus indica*, and Bari et al. (2012) researching *Opuntia monacantha*, corroborating the results obtained in this work. This means that the species of the genera *Opuntia* and *Nopalea* have secondary metabolites with bioactive properties and can act to prevent disease.

The results of the phytochemical profile of cactus pear varieties for the flavonoids and tannins indicate a difference between the varieties and collection periods, since the intensity of the response of the samples were

Table 1. Varieties of cactus pear, genres *Opuntia* e *Nopalea*, used in the study and grown in the state of Pernambuco, Brazil.

N°	Varieties	Espécie	Common name
1	IPA-100003	*Opuntia fícus indica*	IPA-20
2	IPA-200016	*Opuntia stricta*	Elephant Ear Mexican
3	IPA-200008	*Opuntia atropes*	F-08
4	IPA-100004	*Nopalea cochenillifera*	Small palm
5	IPA-200021	*Nopalea cochenillifera*	F-21
6	IPA-200205	*Nopalea cochenillifera*	IPA-Sertânia
7	IPA-200149	*Opuntia larreri*	-

Table 2. Phytochemical profile of crude ethanol extract of varieties of cactus pear, *Opuntia* and *Nopalea* genres during the dry and rainy seasons.

Varieties	Alkaloids				Steroids	Tanins		Flavonoids		Saponins
	A	B	C	D	(*Liebermann-Burchard*)	Gelatin 0.5%	$FeCl_3$ 2%	Magnesium tape (*Shinoda*)	Fluorescence (*Taubouk*)	Foam
Dry season										
IPA-100003	-	-	-	-	+	-	-	+	++	-
IPA-200016	-	-	-	-	+	-	-	+	++	-
IPA-200008	-	-	-	-	+	-	-	++	++	-
IPA-100004	-	-	-	-	+	-	-	+	+	-
IPA-200021	-	-	-	-	+	-	-	+	++	-
IPA-200205	-	-	-	-	+	-	-	+	++	-
IPA-200149	-	-	-	-	+	+	+	++	+++	-
Rainy season										
IPA-100003	-	-	-	-	+	-	+	+++	+++	-
IPA-200016	-	-	-	-	+	+	+	+++	+++	-
IPA-200008	-	-	-	-	+	-	-	+++	+++	-
IPA-100004	-	-	-	-	+	-	-	+++	+++	-
IPA-200021	-	-	-	-	+	-	-	+++	+++	-
IPA-200205	-	-	-	-	+	-	+	+++	+++	-
IPA-200149	-	-	-	-	+	-	-	++	++	-

Key: (A) *Bouchardat*, (B) *Mayer*, (C) *Dragendorf*, (D) *Bertrand* ou Silico-tungstic acid; "+++" (High intensity), "++" (medium intensity), "+" (low intensity), "-" (negative reaction).

different, comparing them to their listed white (reagents absence of extracts) furthermore, depending on the collection period phytochemical class has been detected or not (Table 2). The flavonoid results for both methods of investigation Shinoda (tape-magnesium) and Taubouk (fluorescence), was more intense in the rainy season for all varieties except the IPA-200149, whose variety stood out in the dry season when the second method (Taubouk) was used. These results confirm the difference in composition and content of secondary metabolites between species, botanical varieties and among the seasons of collection of the material. The production and/or accumulation of secondary metabolites in the rainy season is probably an adaptation of the genera *Opuntia* and *Nopalea* to withstand the dry season. Where these substances accumulated in the rainy season would act as antioxidants natural against oxidative stress suffered by the plant in the dry season.

The secondary metabolites (phenolic compounds, terpenes and nitrogen compounds) are related to osmotic adjustment and protection against reactive oxygen species (ROS), as well as to stabilize proteins and cell membranes in plants exposed to abiotic and biotic stresses (Ramakrishna and Ravishankar, 2011; Rodziewicz et al., 2014).

^{1}H NMR spectra

The chemical shift observed in the ^{1}H NMR spectrum of

crude ethanol extract from varieties of cactus pear *Opuntia* and *Nopalea* in the dry and rainy season are shown in Table 3. The ^{1}H signal located between 0.8 to 3.0 ppm refers to lipids (diterpenes, fatty acids and steroids) in the sample; signals between 3.2 and 5.0 ppm are related to heteroatoms (OH, NH, SH, PH, BH) glycosylated substances; signals between 5.0 and 5.8 refer to the anomeric carbon ^{1}H, referring to carbohydrates; and the range between 6.0 and 8.0 ppm refers to ^{1}H attached to the aromatic ring, phenolic compounds in general are included and the range 9 to 10 ppm refers to aldehyde ^{1}H (Iulianelli and Tavares 2011; Ribeiro and Souza, 2007). The profile of the ^{1}H NMR spectra of cactus pear varieties signals towards divergence of substances among chemical compounds present groups in the *Opuntia* and *Nopalea* genres, both within species and between species and time of collection of material (Table 3, Figures 1 and 2).

In the dry season, in the *Opuntia* (V1, V2, V3, V7), there is the presence of fatty acids, steroids, carbohydrates and phenolics. However, range V2 does not detect the presence of phenolic compounds (Figure 1). *Nopalea* (V4, V5 and V6) show the existence of carbohydrates, fatty acids, steroids, but the presence of phenolic compounds was not detected (Figure 2). In the rainy season, in the *Opuntia* (V1, V3, V7), there is the presence of fatty acids, steroids, carbohydrates and phenolics. In the variety V2 fatty acids and phenolic compounds were not detected, only the presence of carbohydrate (Figure 1). *Nopalea* (V4, V5 and V6) shows the existence of carbohydrates, fatty acids, steroids and phenolic compounds. However, for the V4 the existence of fatty acids was not noticed (Figure 2). The failure to detect phenolic compounds, steroids and fatty acids in the extract of some cactus pear varieties is probably due to the limited power of the equipment in addition to possible interference between the substances present in the sample, since the analyses were carried out in ethanol extract. In these same samples the presence of phenolic compounds, steroids and fatty acids were detected in all varieties in both periods studied (dry and wet) in the phytochemical screening (Table 2).

The cactus pear varieties studied show more expressive levels of carbohydrates, glucoside substances and phenolic compounds in the rainy season than in the dry season. However, fatty acids and steroids at that time are less significant compared to the dry period (Figures 1 and 2). Ribeiro et al. (2010), studying the carbohydrates (galactose, xylose, arabinose, glucose, fructose, rhamnose, sucrose and uronic acid) present in the cactus pear cladodes (*Opuntia* spp.), varieties (giant, round, copena F1 and clone 20), grown in Northeastern Brazil, reported variations in the amount of total sugars and composition between genotypes, cladodes order, and collection season of the material. The researchers reported that the total carbohydrates in cactus pear cladodes (giant and clone 20) were higher in the rainy season, corroborating the results obtained in this work. However, in varieties (round and copena F1) the total carbohydrate content was higher in the dry season.

Sánchez-Rodríguez et al. (2011 and 2012), studying the influence of water stress in the profile of phenolic compounds reported differences between content and composition of phenolic compounds, flavonoids and their glycosides between tomato varieties, depending on the availability of water to which they were submitted, some genotypes present higher content and composition of phenolic compounds, flavonoids and their glycosides under irrigation, other contents were higher in conditions of water stress. Overall, the authors report that total phenolic compounds in varieties are higher under irrigation, supporting the results presented in this paper where the secondary metabolites in particular the phenolic compounds accumulated in the rainy season contributes in the osmotic adjustment and protection against reactive oxygen species, as well as to stabilize proteins and cell membranes in plants exposed to water stresses in dry season (Ramakrishna and Ravishankar, 2011; Rodziewicz et al., 2014).

El-Kaoua et al. (2006), investigating the influence of drought on the content of fatty acids in wheat (*Triticum aestivum* L.) varieties (Nasma and Tigre), reported that water stress changes the amount and composition of fatty acids. Water stress reduced the total fatty acid content in both varieties studied. This reduction was more dramatic for unsaturated octadecatrienoic acid (18:3) in parallel there was an increase in the percentage of saturated fatty acids (16:0 and 18:0). Furthermore, the lipid composition (glycolipids and phospholipids) was reduced by drought stress in both varieties. However, there was an increase in neutral lipids (diacylglycerol and triacylglycerol) under water stress. The authors considered the synthesis of neutral lipids as a mechanism of action against water stress. In addition, free fatty acids, released during the water deficit by action of lipases on polar lipids, can be stored in triacylglycerols to prevent oxidation by free radicals and active forms of oxygen. The authors also reported that the reduction octadecatrienoic acid (18:3) in wheat plant is related to the formation of methyl jasmonate (Me-JA) through 12-oxophytodienoic acid (12-OPDA) in chloroplasts. The (Me-JA) is considered a growth and development regulator in plants, and together with other cyclopentanone molecules, particularly jasmonic acid (JA) and its conjugates of amino acids influence various metabolic processes relating to tolerance to water stress. Bourgou et al. (2010), investigating the content and composition of fatty acids and essential oils in Black Cumin (*Nigella sativa*) under salt stress reported that both the content, as well as the composition of fatty acids and essential oils were modified by salt stress. The content of total fatty acids was reduced, however the percentage of linoleic acid (18:2) was increased. Furthermore, the content of essential oils increased

Table 3. Chemical shift observed in the ^{1}H NMR spectrum of crude ethanol extract of cactus pear varieties of *Opuntia* and *Nopalea* genres in the dry season and rainy.

Hydrogen	Chemical shifts (ppm) of cactus pear samples													
	Dry season							Rainy season						
	V1	V2	V3	V4	V5	V6	V7	V1	V2	V3	V4	V5	V6	V7
^{1}H of lipids	0.86	1.13	0.86	0.89	0.86	0.89	0.86	1.27	1.26	0.86	1.28	1.26	0.68	0.83
	0.89	1.17	0.89	1.17	0.89	1.28	0.89	1.59		0.90	2.69	1.57	0.88	0.86
	1.02	1.20	1.13	1.28	1.01	2.15	1.13	2.04		0.97		2.03	0.99	0.94
	1.13	1.28	1.17	2.15	1.28		1.17			1.01		2.06	1.12	0.98
	1.17	2.15	1.20		1.59		1.20			1.28		2.27	1.27	1.24
	1.20		1.28		2.04		1.28			1.59		2.31	1.58	1.56
	1.23		1.59		2.07		1.59			1.77		2.34	2.03	2.01
	1.28		2.04		2.28		2.15			2.08			2.27	2.04
	1.59		2.32		2.30		2.32			2.29			2.31	2.21
	1.98				2.32					2.32			2.34	2.25
	2.04				2.77					2.58			2.76	2.29
	2.15									2.80				2.73
	2.27													2.76
	2.31													
^{1}H linked heteroatoms	3.20	3.54	3.35	3.39	3.47	3.37	3.35	3.18	3.13	3.32	3.34	3.49	3.62	3.30
	3.40	3.58	3.39	3.44	3.62	3.39	3.39	3.23	3.18	3.34	3.52	3.63	3.63	3.49
	3.43	3.62	3.43	3.61	3.66	3.44	3.58	3.53	3.21	3.38	3.65	3.65	3.64	3.62
	3.46	3.65	3.44	3.65	3.68	3.61	3.61	3.66	3.34	3.52	3.67	3.67	3.66	3.64
	3.61	3.71	3.54	3.70	3.71	3.65	3.65	3.68	3.35	3.78	3.70	3.69	3.68	3.67
	3.65	3.75	3.58	3.74	3.74	3.70	3.68	3.70	3.49	3.82	3.79	3.70	3.69	3.72
	3.68	3.78	3.61	3.78	3.78	3.74	3.70	3.79	3.62	3.88	3.82	3.73	3.72	3.75
	3.70	3.84	3.65	3.83	3.82	3.77	3.71	3.82	3.64	4.00	3.89	3.76	3.76	3.78
	3.74	3.86	3.70	4.05	4.08	3.98	3.74	3.90	3.67	4.06	4.00	3.79	3.78	4.47
	3.78	4.08	3.71	4.07	4.45	4.01	3.78	4.06	3.68	4.51	4.06	3.85	4.47	4.51
	3.83	4.35	3.74	4.11		4.03	3.83	4.51	3.69	4.55	4.51	4.04	4.51	
	3.98	4.39	3.78	4.44		4.05	4.07	4.55	3.73		4.55	4.47	4.62	
	4.01	4.45	3.83	4.48		4.07	4.44		3.76			4.51		
	4.05	4.49	4.07			4.44	4.48		3.79					
	4.07	4.52	4.44			4.48			3.82					
	4.12	4.59	4.48						3.86					
									4.04					
									4.09					
									4.37					
									4.47					
									4.51					
									4.52					
									4.59					
^{1}H linked anomeric carbon	5.37	5.09	5.37	5.37	5.09	5.08	5.09	5.42	5.11	5.14	5.14	5.11	5.11	5.11
	5.39	5.11	5.39	5.39	5.11	5.10	5.10		5.13	5.16	5.16	5.13	5.13	5.13
	5.88	5.37			5.37	5.37	5.37		5.38	5.40	5.62	5.38	5.37	5.30
		5.39			5.39	5.39	5.39		5.39	5.42		5.40	5.39	5.37
		5.57			5.48				5.59	5.62				5.38
		5.59												
^{1}H linked aromatic ring	6.18	6.40	6.09	6.18			6.19	6.69	6.40	6.22	6.23	6.00	6.72	6.58
	6.19		6.17	6.19			6.20	6.70	6.41	6.42	6.33	6.30	7.39	6.59

Table 3. Cont'd.

	6.71	6.19	6.38	7.05	6.70	6.61	6.44	6.59	7.41	7.16
	6.78	6.38		7.06	6.71	6.63	6.73	6.60		7.39
	7.11	6.39		7.09	6.72	6.73	6.97	7.40		7.40
	7.13	6.41		7.13	6.73	6.91	7.34	7.41		7.56
	7.15	6.67			7.04	6.94	7.46			
	7.16	6.68			7.06	7.43	7.57			
		6.68			7.07	7.44	7.62			
		6.86			7.08	7.56	7.94			
		6.88			7.08	7.95	8.04			
		6.92			7.11	8.06				
		6.93			7.12					
		7.31			7.14					
		7.94			7.43					
		7.95			7.44					
		8.02								
		8.02								
		8.03								
		8.06								
		8.07								
^{1}H linked aldehyde					9.53	9.52	9.53	9.53	9.52	9.52	9.50

Table 4. Grouping of the seven varieties of cactus pear of *Opuntia* and *Nopalea* genres grown in the semiarid region of Pernambuco, based on the ^{1}H NMR spectrum in the dry season, the similarity measure expressed by the Jaccard index and the Tocher optimization method.

Group	Varieties
I	4, 5, 2, 3, 6 and 1
II	7

Table 5. Grouping of the seven varieties of cactus pear of *Opuntia* and *Nopalea* genres grown in the semiarid region of Pernambuco, based on the ^{1}H NMR spectrum in the rainy season, the similarity measure expressed by the Jaccard index and the Tocher optimization method.

Group	Varieties
I	1 and 7
II	3 and 5
III	4
IV	2
V	6

under conditions of salt stress. The increase detected in the lipid substances in cactus pear in the dry season is probably due to increased waxes, cutin, suberin, triterpenoids, neutral lipids, essential oils, carotenoids, tocopherols, ABA, jasmonate, as well as the accumulation of fatty acids, palmitic, linoleic and linolenic acids, as these molecules are increased under drought (Buchanan et al., 2009).

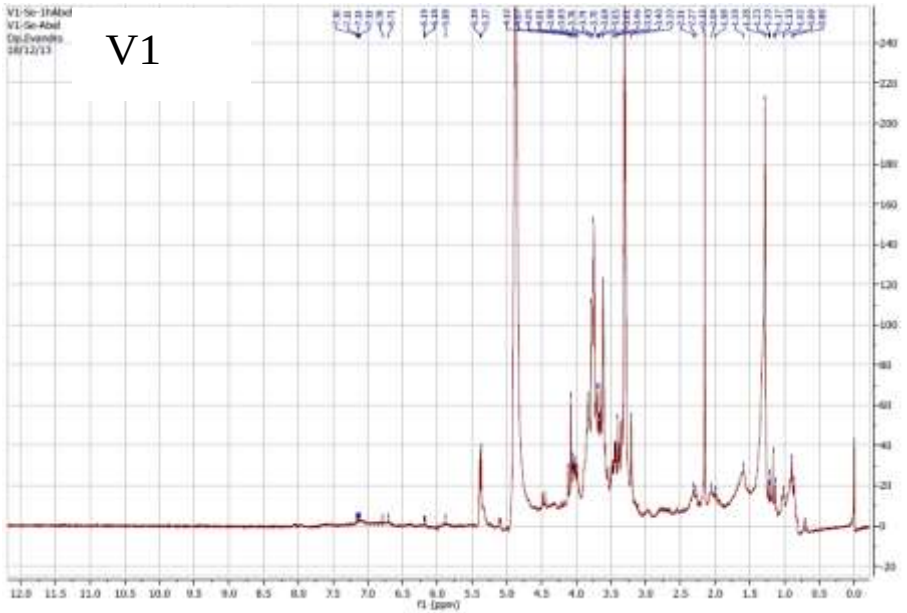
Thus, the storage of carbohydrates, glucosides substances and phenolic compounds in the rainy season will serve as molecules, that will act against water stress during the dry season (Tao et al., 2015; Zhong et al., 2010). In addition, the accumulation of some fatty acids and steroids in the dry season could also contribute to tolerance to water stress (Bourgou et al., 2010; El-Kaoua et al., 2006; Yeilaghi et al., 2012).

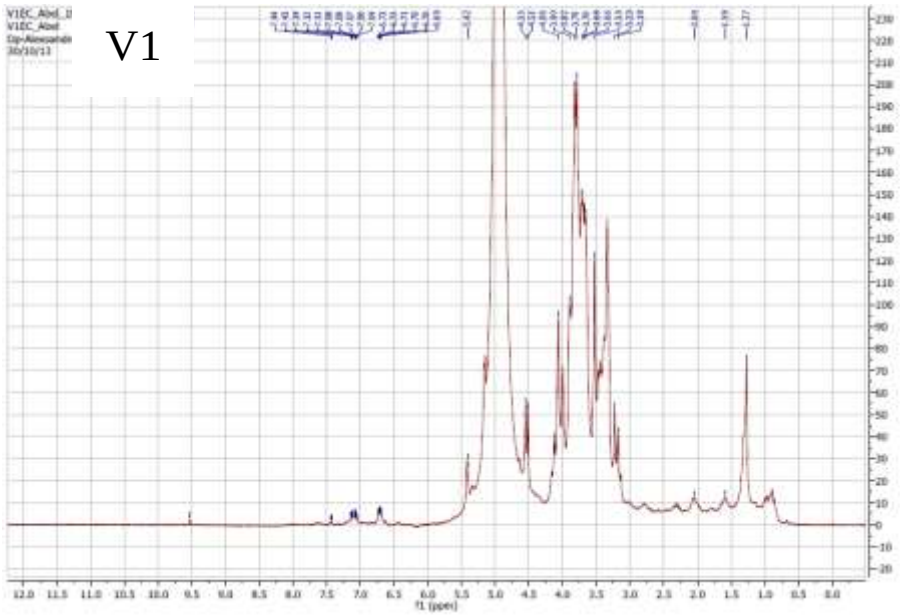
Genetic diversity through ^{1}H NMR

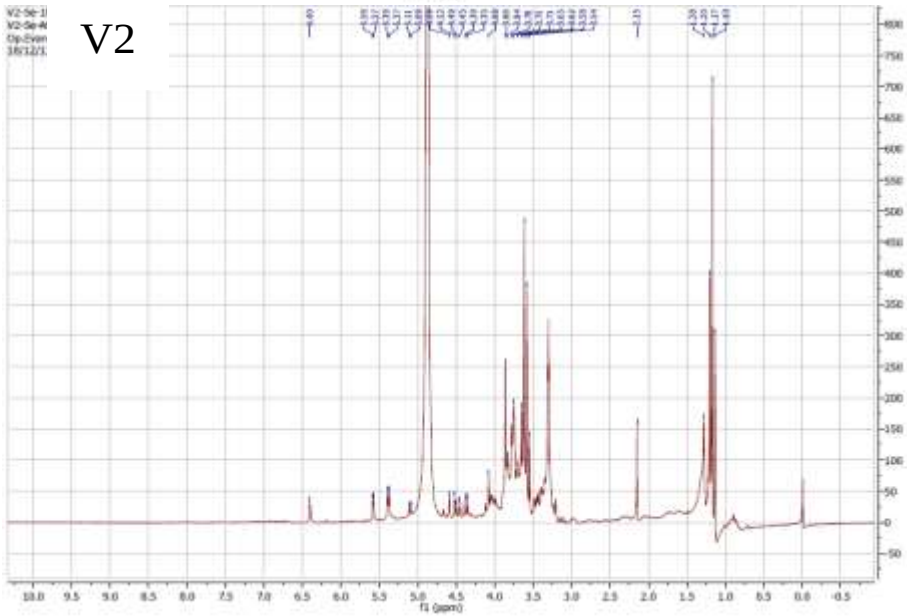
The analysis of the ^{1}H NMR spectra of cactus pear varieties by methods of multivariate analysis found genetic diversity among the materials in the dry and rainy seasons. Furthermore, there was a difference in identifying groups of similar subjects in the two seasons (Tables 4 and 5, Figures 3 to 6). In the dry season, using the Tocher grouping method, the varieties were grouped into two distinct groups. Group I was represented by genotypes 1, 2, 3, 4, 5 and 6, and group II was represented by genotype 7 (Table 4). By hierarchical clustering method UPGMA, genotypes were grouped into

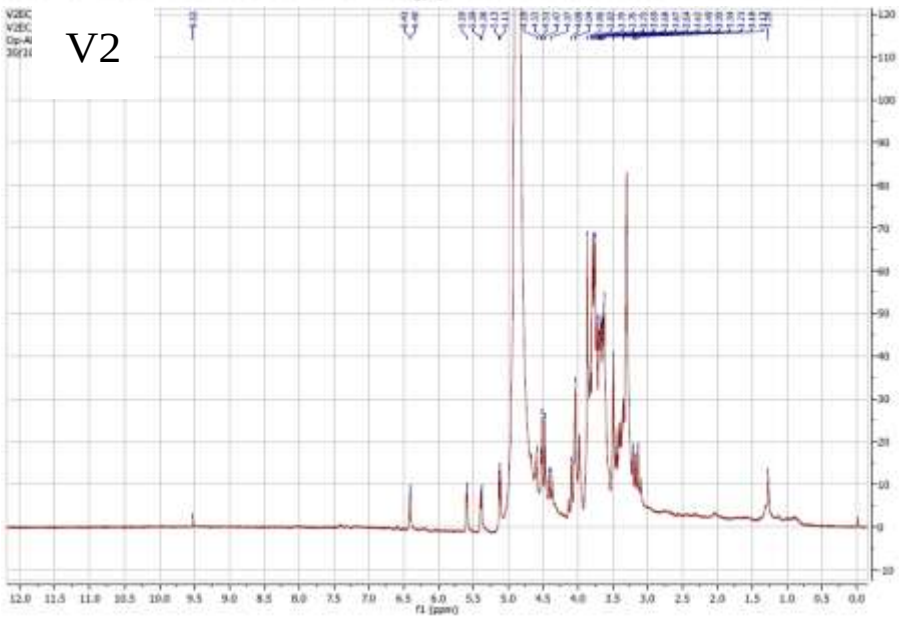
Dry season

Rainy season









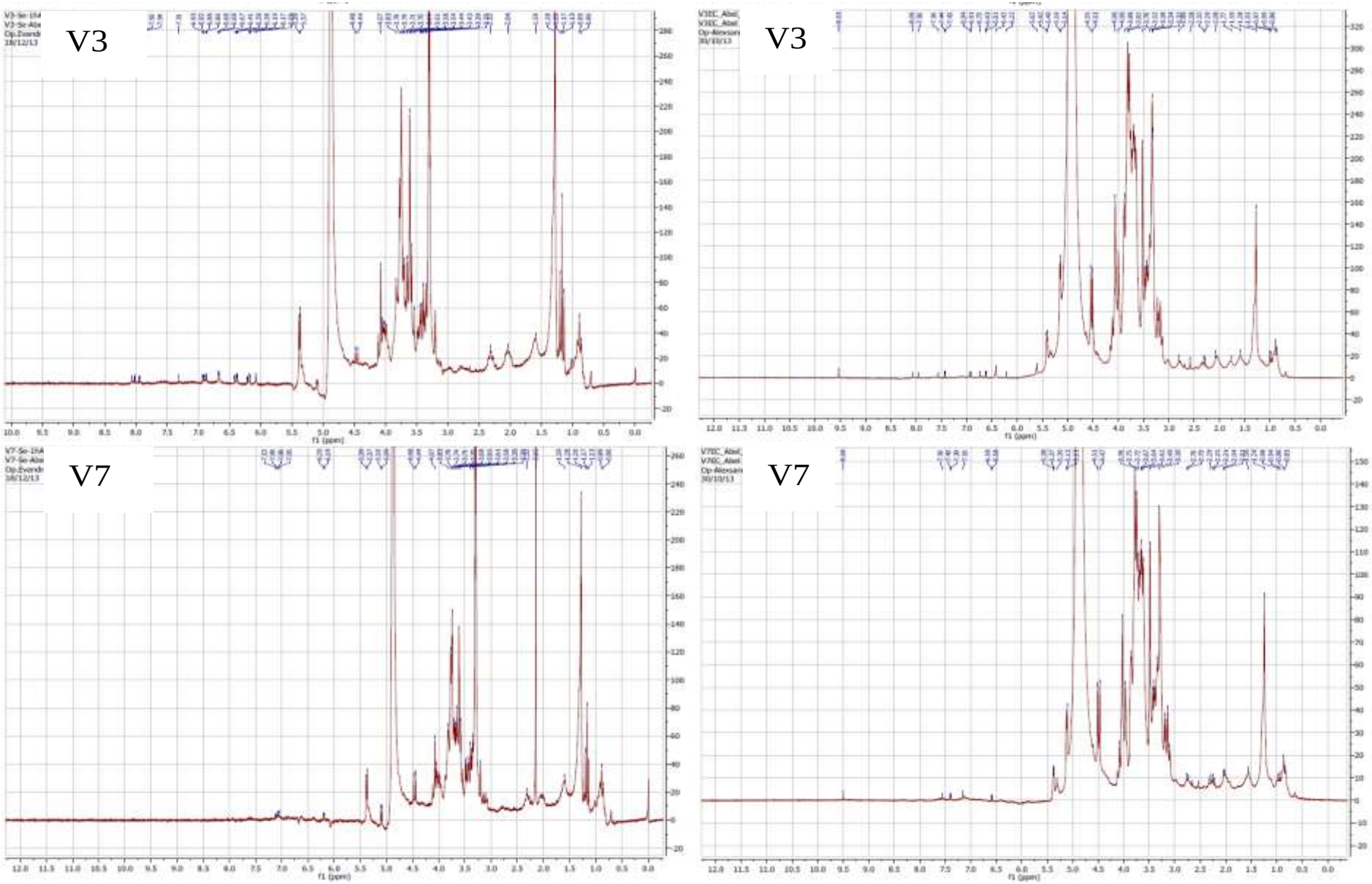
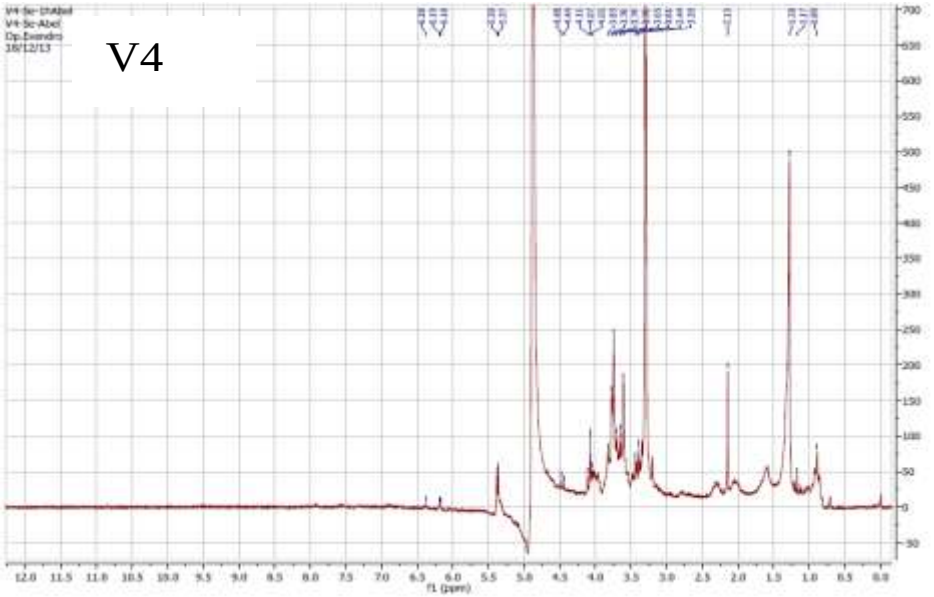
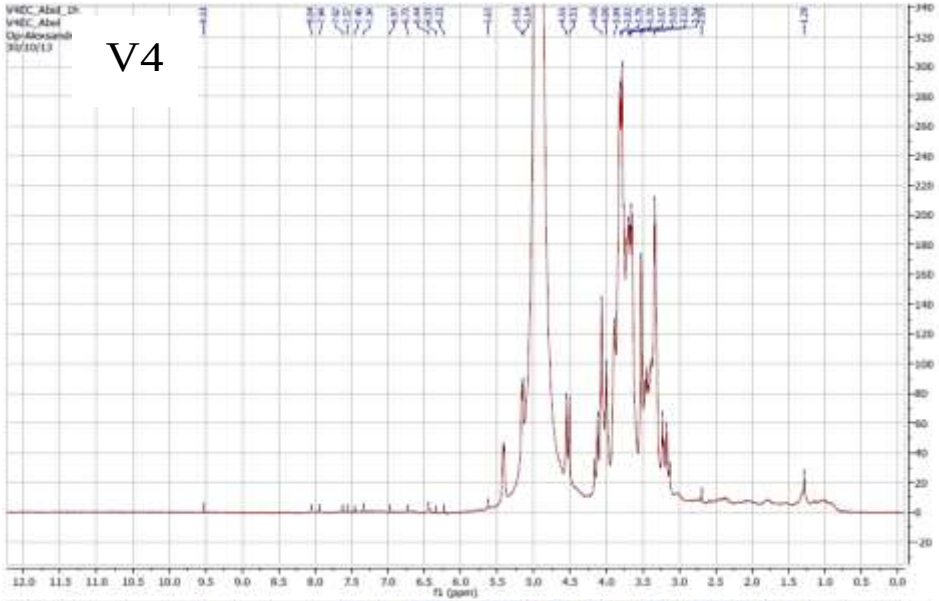


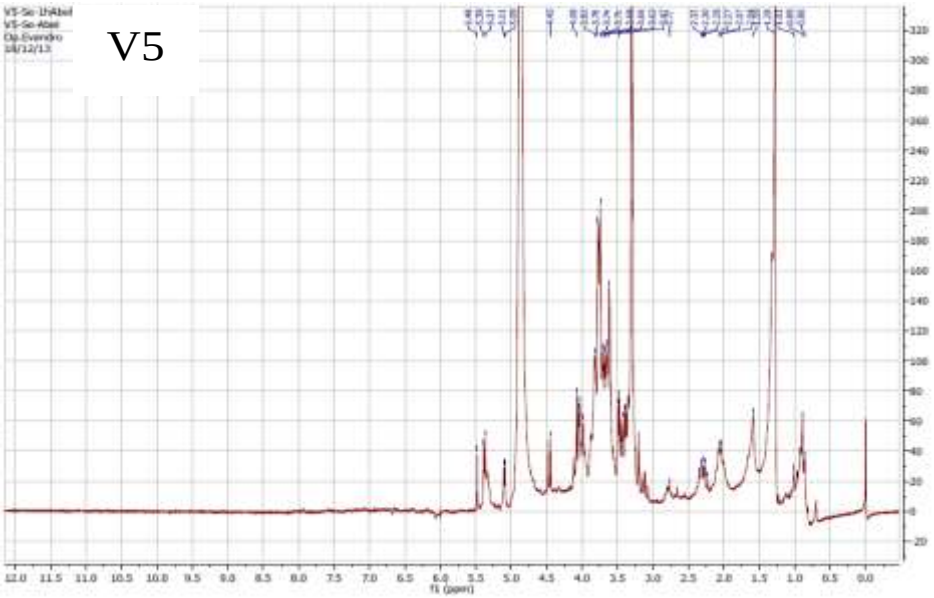
Figure 1. [1]H NMR spectrum of crude ethanol extract of varieties of cactus pear (*Opuntia*), collected in the dry and rainy seasons. (V1) IPA-100003 (*O. ficus indica*), (V2) IPA-200016 (*O. stricta*), (V3) IPA-200008 (*O. atropes*) and (V7) IPA-200149 (*O. larreri*)

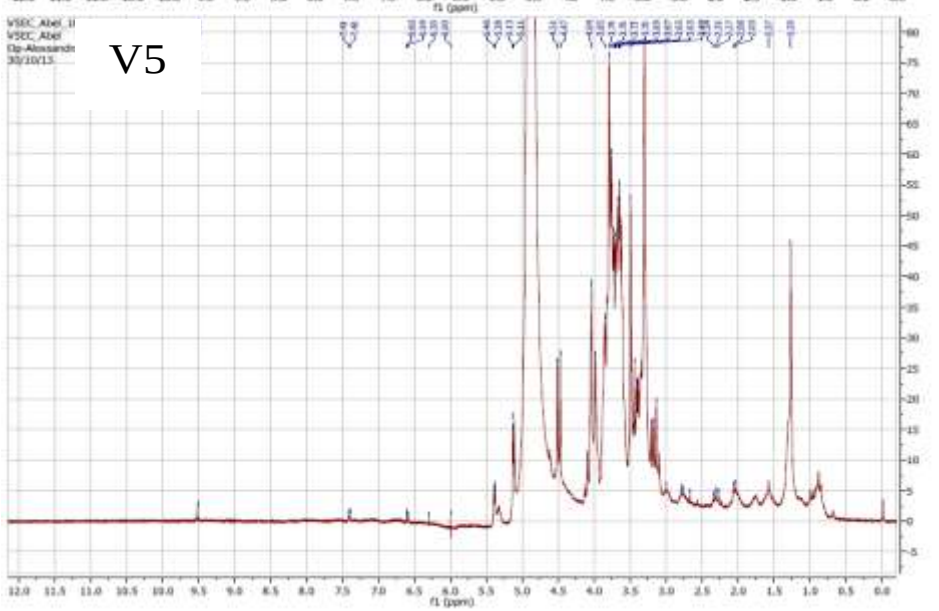
Dry season

Rainy season









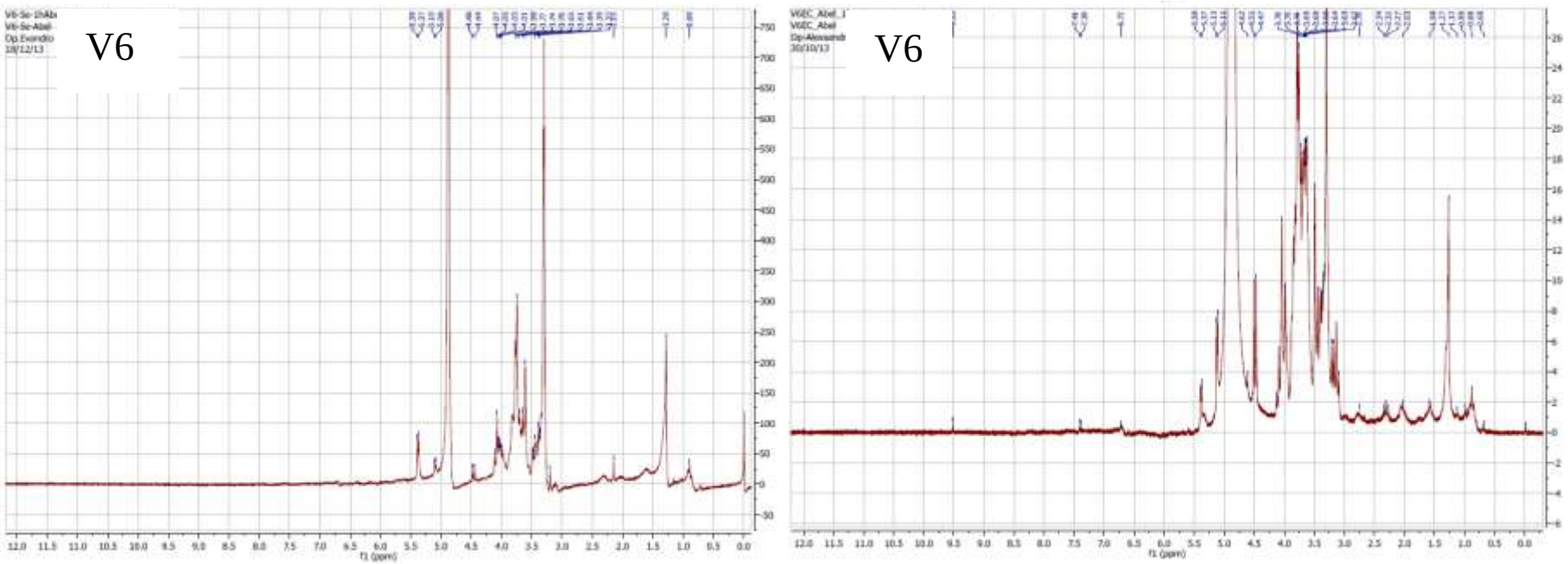


Figure 2. ^{1}H NMR spectrum of crude ethanol extract of varieties of cactus pear (*Nopalea*), collected in the dry and rainy seasons. (V4) IPA-100004 (*N. cochenillifera*), (V5) IPA-200021 (*N. cochenillifera*) and (V6) IPA-200205 (*N. cochenillifera*).

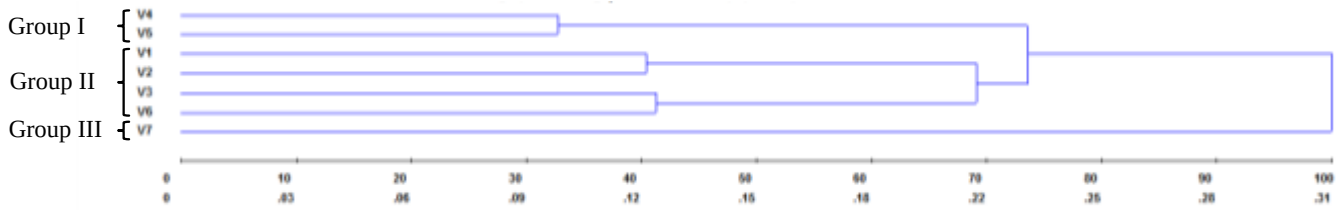


Figure 3. Representative dendrogram grouping by UPGMA seven varieties of cactus pear of *Opuntia* and *Nopalea* genres grown in the semiarid region of Pernambuco, based on the ^{1}H NMR spectrum in the dry season.

four groups considering a cut of 69% of the relative genetic distance, group I represented by genotypes 4 and 5, group II with genotypes 1 and 2, group III at 3 and 6, group IV by 7 (Figure 3). The sorting method of grouping by principal component analysis, the scores dispersions chart, grouped the genotypes into four groups, group I was composed of genotypes 4, 5, 6 and 7, group II by genotype 2, the group III by 1, and the group IV by 3 (Figure 5).

In the rainy season, aging using the Tocher grouping method, the varieties were grouped into

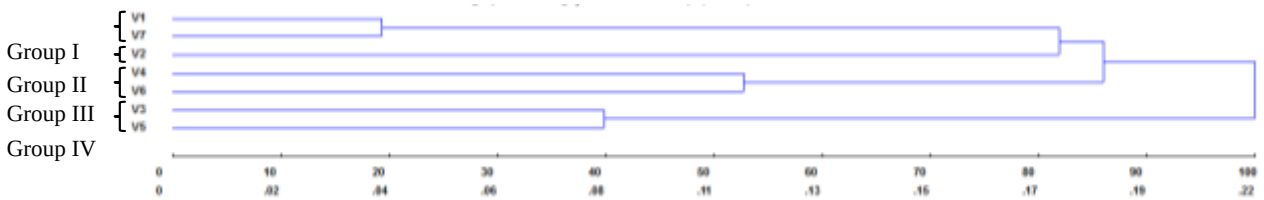


Figure 4. Representative dendrogram grouping by UPGMA seven varieties of cactus pear of *Opuntia* and *Nopalea* genres grown in the semiarid region of Pernambuco, based on the ^{1}H NMR spectrum in the rainy season

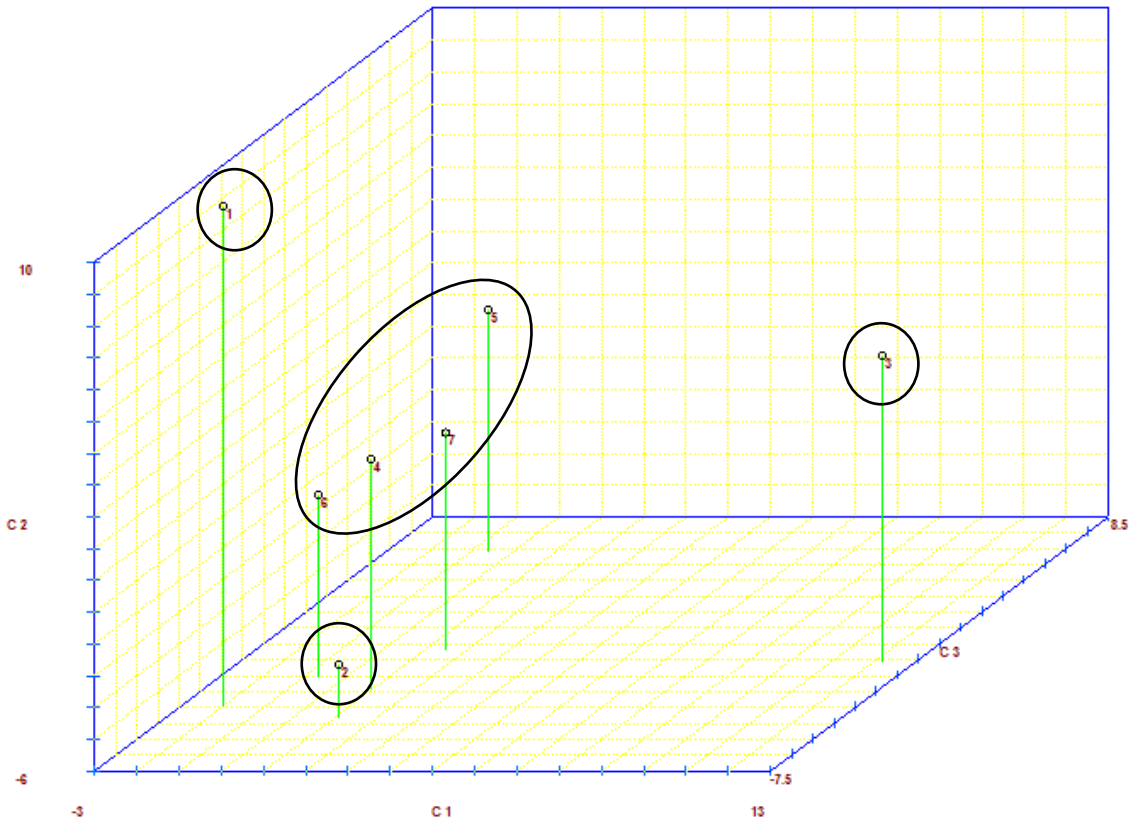


Figure 5. Graphical dispersion of the seven varieties of cactus pear *Opuntia* and *Nopalea* genres, from the first, second and third main component (C1, C2, C3) based on the ^{1}H NMR spectrum of the dry season

five distinct groups, group I represented by genotypes 1 and 7, group II by genotypes 3 and 5, group III by 4, group IV by 2 and group V by 6 (Table 5). The UPGMA method grouped genotypes into four distinct groups, considering a cut of 82% relative genetic distance, group I was represented by varieties 1 and 7, group II by 2, Group III by 4 and 6, the group IV by 3 and 5 (Figure 4). The principal components method grouped the varieties into five distinct groups, group I represented by varieties 2, 5 and 6, group II by 1, Group III by 7, group IV by 3 and group V by 4 (Figure 6).

The principal component analysis shows that the use of the first three variables were sufficient to account for almost 71% and 67% of the total variation obtained between the seven genotypes in the dry and rainy seasons, respectively (data not shown). Thus, a reasonable description of the genetic diversity of genotypes can be made by these components in two-dimensional or three-dimensional plane. According to Silva and Padovani (2006), it is necessary that the first main components accumulate at least 70% of the total variation to explain the variability manifested among individuals, leading to interpretation of the phenomenon with considerable simplification of the features in two-dimensional or three-dimensional plane.

Considering the consensus of most of the groups in the

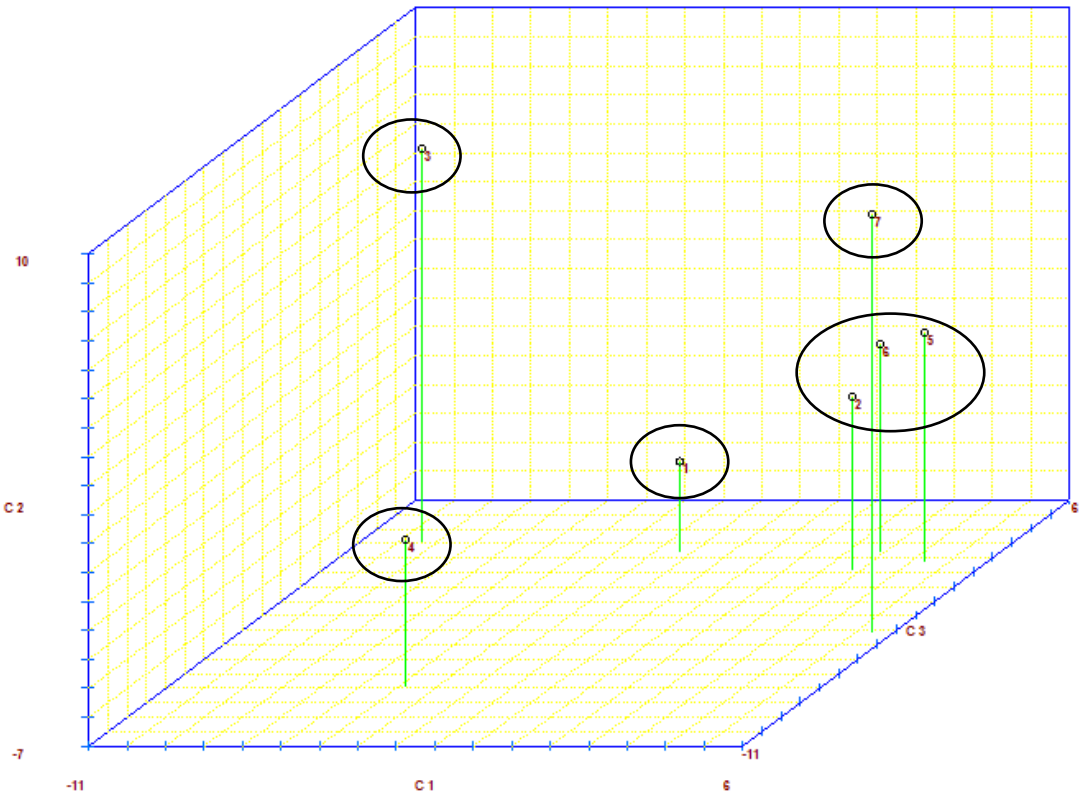


Figure 6. Graphical dispersion of the seven varieties of cactus pear *Opuntia* and *Nopalea* genres, from the first, second and third main component (C1, C2, C3) based on the ^{1}H NMR spectrum of the rainy season

dry season, genotypes 1 and 2; 4, 5 and 6; 3 and 6; were considered similar, and variety 7 considered different from the others. In the rainy season, the multivariate analysis were different to group similar genotypes, accordingly, varieties 1 and 7, as well as 3 and 5 were considered similar. Varieties 4 and 2 were classified different from the others. This means that the varieties grouped in the same group in each season are not different in their chemical composition.

Determination of cytotoxic activity

The results of the cytotoxic activity against human cancer cell ethanol extract of cactus pear varieties (*Opuntia* and *Nopalea*), collected in the dry and rainy seasons are shown in Figure 7. The response of cactus pear extracts in the concentration used has little or no activity (cell growth inhibition lower than 50%) against human cancer cell lines used in the study. Cytotoxic activity of the extracts against cancer cells varied among varieties, sampling stations and between the material and cell types.

The cactus pear extracts that had the best responses against the growth of cancer cells HCT-116 (human colorectal) were: IPA-200008 (V3), IPA-100004 (V4), IPA-200021 (V5) and IPA-200205 (V6) (dry season), and IPA-100003 (V1), IPA-200016 (V2), IPA-100004 (V4) and IPA-200021 (V5) (rainy season), reducing average growth by around 14% and 17%, respectively for the dry and rainy seasons. For cell SF-295 (human glioblastoma) reduction in growth was around 24% for the extracts of varieties IPA-100003 (V1), IPA-200016 (V2), IPA-100004 (V4), IPA-200021 (V5) and IPA-200205 (V6) (dry season), and 33% for the IPA-200016 (V2) (rainy season). For OVCAR-8 cells (human ovary) reduction in growth was around 16% for the two periods between the varieties IPA-200021 (V5) and IPA-200205 (V6) (Figure 7). The growth inhibition of cancer cells was higher in the rainy season, with the exception IPA-200008 (V3) and IPA-200205 (V6) for HCT-116; IPA-200205 (V6) for SF-295; IPA-100003 (V1) and IPA-200149 (V7) for SF-295 (Figure 7). The sensitivity of cancer cells to the extracts of cactus pear varieties differ among cell types. The SF-295 cells (human glioblastoma) were the most sensitive, followed by HCT-116 (human colorectal) and OVCAR-8 (human ovarian) (Figure 7).

The results presented in this study are consistent with those reported by Chavez-Santoscoy et al. (2009), studying the anticancer activity of fruit extracts of nine species of cactus pear (*Opuntia* spp.) on the proliferation of breast cancer cells (MCF-7), prostate (PC3), colorectal

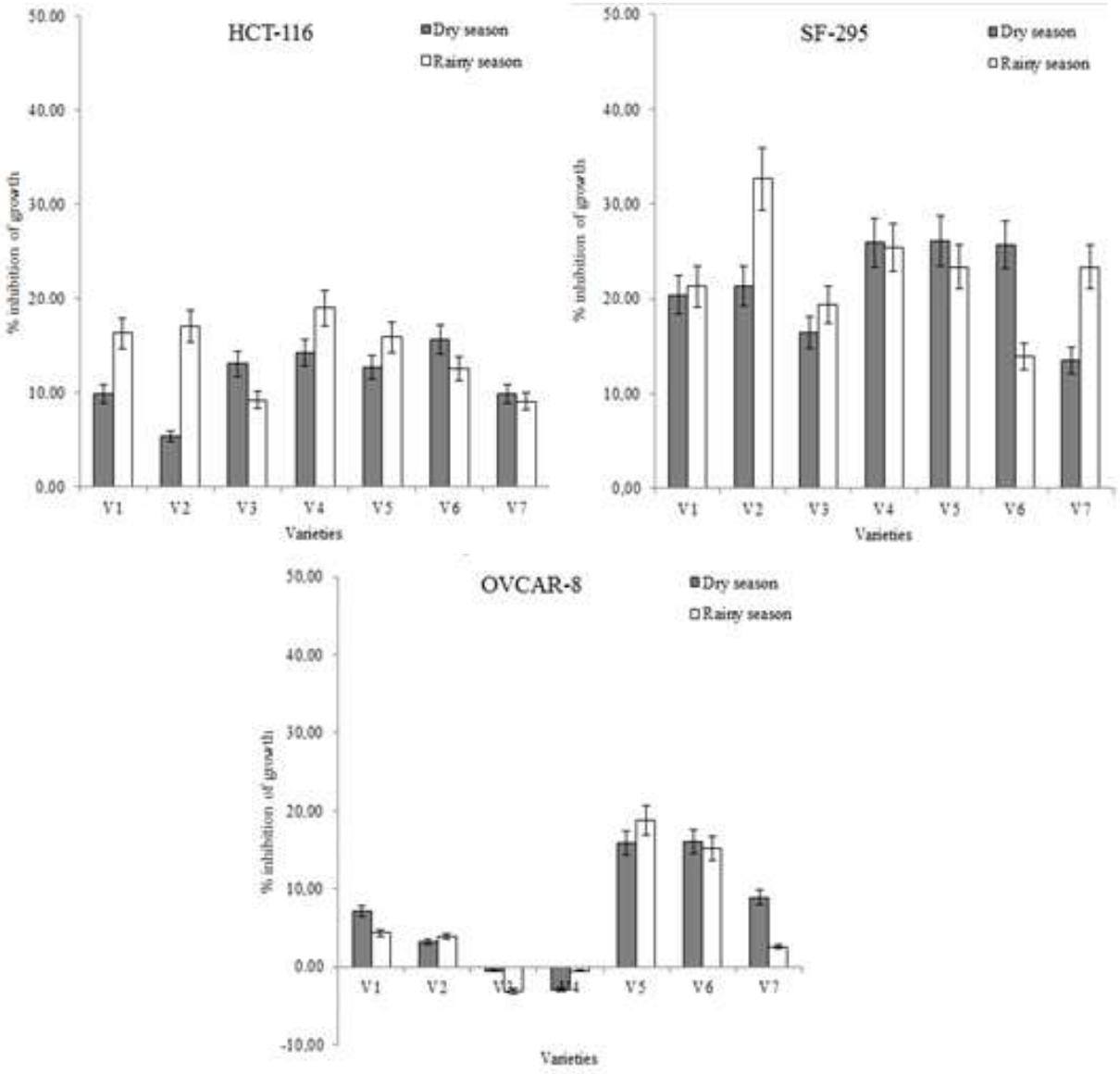


Figure 7. Cytotoxic activity against human cancer cells (a) HCT-116 (human colorectal), (b) SF-295 (human glioblastoma) and (c) OVCAR-8 (human ovary) of crude ethanol extract of cactus pear varieties (*Opuntia* and *Nopalea*), collected in the dry and rainy season. (V1) IPA-100003 (*O. ficus indica*), (V2) IPA-200016 (*O. stricta*), (V3) IPA-200008 (*O. atropes*), (V4) IPA-100004 (*N. cochenillifera*) (V5) IPA-200021 (*N. cochenillifera*) (V6) IPA-200205 (*N. cochenillifera*) and (V7) IPA-200149 (*O. larreri*). The bars represent the mean (n = 3) ± standard deviation

(Caco-2), and liver (HepG2). The researchers reported cactus pear variation between species compared to cytotoxic activity and variation in sensitivity among the types of cancer cells to the cactus pear extracts (0.5%) incubated for 48 hours. The most sensitive cancer cells were the colon (Caco-2) and prostate (PC3), with mean reduction in growth of around 15% and 23%, respectively. In this study the response of cactus pear species showed little activity (inhibition of cell growth less than 50%) against cancer cell lines used in the study.

Zou et al. (2005) studied the aqueous extract effect of fruit and cactus pear seeds (*Opuntia* spp.) on the proliferation of cervical cancer cells (TCL1, HeLa and Me180), ovary (IOSE, OVCA420 e SKOV3) and bladder (UM-UC6, T24 e UM-UC9), reported a difference between the sensitivity of cancerous cells used extract concentrations (0.5, 1, 5, 10 and 25%) and exposure time (1, 3, and 5 days). The authors reported that the concentration of the aqueous extract from cactus pear fruits (1%) is effective in 40-60% inhibition of growth of cervical cancer cells and immortalized cervical epithelium; and the inhibition of growth may get close to

100%, depending on the cancer cell, the dose and time of exposure to the extracts. Naselli et al. (2014) studying the anticancer activity of *O. ficus* indicates fruit extracts on the proliferation of colorectal cancer carcinoma cells (Caco-2), have reported that inhibition of growth of cancer cells depends on the concentration of extract used. The concentration of 400,000 µg.mL^{-1} extract inhibited 50% of Caco-2 growth inhibition reaches 100% the concentration (750,000 µg.mL^{-1}), when incubated for 48 hours. These studies corroborate the results presented in this study, and enhance the cytotoxic activity of cactus pear extracts against some cells of human cancer, where this activity depends on the concentration and time of exposure to the extract.

Kim et al. (2013) studied the anticancer activity of cladodes extracts of *O. humifusa* and reported growth inhibition of 80.2% for SW480 cells (cervical cancer) and 54.4% for MCF7 cells (breast cancer). The researchers report differences in anticancer activity among the extracts (hexane, ethyl acetate, acetone, methanol and methanol/water) concentrations (6.25, 12.5, 25, 50 and 100 µg ml^{-1}) and the test time (24, 48 and 72 hours). Overall inhibition of growth of cancer cells of extracts from cladodes does not reach 50% when used at concentrations (50 µg ml^{-1}) for 72 h, confirming the results of our work.

The anti-cancer property of cactus pear extracts have been attributed to the antioxidant properties of phenolic compounds, mainly phenolic acids, flavonoids, betacyanins and betaxantins (Dhaouadi et al., 2013; Kim et al., 2013; Serra et al., 2013; Zou et al., 2005). Although the chemicals responsible for cytotoxic activity in this work have not been isolated, it is certainly in the class of flavonoids.

The low percentage of inhibition of cancer cell growth found in this study is probably related to most glycosidic flavonoids present in the extract with sugar moieties attached to the hydroxyl at C-3, thus losing its antioxidant capacity (Santos-Zea et al., 2011). Furthermore, it can be attributed to sub-dose (50 µg ml^{-1} or 0.005%), since most of the studies in the literature reports close to 100% inhibition at higher doses. Also, most studies were conducted with fruit extracts (pericarp + seeds) and this work with cladodes.

Furthermore, the limited cytotoxic action of ethanolic extract of cactus pear against cancer cells (HCT-116, SF-295 and OVCAR-8) in the concentration 50 µg ml^{-1} may be due limited interaction between the phenolic compounds present in extract and cancer cells (Peixoto et al., 2014). Despite the cytotoxic activity of cactus pear cladodes extracts having shown low values against cancer cell lines studied in this research, the results are important for the use of these materials as preventative natural dietary supplements against cancer in normal populations at risk. Additional precision research needs to be carried out to identify and quantify the bioactive compounds present in the cladodes and other plant organs.

Conclusions

The cactus pear varieties (IPA-100003, IPA-100004, IPA-200021, IPA-200205, IPA-200008, IPA-200149 and IPA-200016) feature seasonal and genetic variability of the chemical compounds. The cytotoxic activity of cactus pear cladodes (*Opuntia* and *Nopalea*) against human cancer cell lines is attributed to phenolic compounds, especially the flavonoids and/or steroids.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the support of doctoral student, Otemberg Souza Chaves, and graduate student Maria Isabel Barros Fernandes, for the support in the analysis and acquisition of laboratory data.

REFERENCES

Araújo L de F, Oliveira L de SC, Perazzo-Neto A, Alsina OLS de, Silva FLH da (2005). Equilíbrio higroscópico da palma forrageira: Relação com a umidade ótima para fermentação sólida. Rev. Bras. Eng. Agr. Amb. 9(3):379-384.

Bari MN, Zubair M, Rizwan K, Rasool N, Bukhari IH, Akram S, Bokhari TH, Shahid M, Hameed M, Ahmad VU (2012). Biological activities of *Opuntia monacantha* cladodes. J. Chem. Soc. Pak. 34(4):990-995.

Bourgou S, Bettaieb I, Saidani M, Marzouk B (2010). Fatty acids, essential oil, and phenolics modifications of black Cumin fruit under NaCl stress conditions. J. Agric. Food Chem. 58(23):12399-12406.

Brás AAQ (2011). Caracterização do extrato de *Opuntia ficus indica* (L.) Mill e avaliação de sua atividade fotoprotetora. (Monografia, Universidade Estadual da Paraíba). Available at: http://dspace.bc.uepb.edu.br/jspui/handle/123456789/395

Buchanan BB, Gruissem W, Jones RL (2009). Biochemistry & Molecular Biology of Plants, American Society of Plant Biologists, Rockville. 1367 p.

Chavez-Santoscoy RA, Gutierrez-Uribe JA, Serna-Saldívar SO (2009). Phenolic composition, antioxidant capacity and *in vitro* cancer cell cytotoxicity of nine prickly pear (*Opuntia* spp.) juices. Plant Foods Hum. Nutr. 64(2):146-152.

Cruz CD (2001). Programa GENES: Aplicativo computacional em genética e estatística, Editora UFV, Viçosa. P 648.

Cruz CD, Regazzi AJ, Carneiro PCS (2012). Modelos biométricos aplicados ao melhoramento genético, Editora UFV, Viçosa P 514.

Desoti VC, Maldaner CL, Carletto MS, Heinz AA, Coelho MS, Piati D, Tiuman TS (2011). Triagem fitoquímica e avaliação das atividades antimicrobiana e citotóxica de plantas medicinais nativas da região oeste do estado do Paraná. Arq. Ciên. Saúde UNIPAR 15(1):3-13.

Dhaouadi K, Raboudi F, Funez-Gomez L, Pamies D, Estevan C, Hamdaoui M, Fattouch S (2013). Polyphenolic extract of Barbary-Fig (*Opuntia ficus-indica*) syrup: RP–HPLC–ESI–MS analysis and determination of antioxidant, antimicrobial and cancer-cells cytotoxic potentials. Food Anal. Methods 6(1):45-53.

El-Kaoua M, Serraj R, Benichou M, Hsissou D (2006). Comparative sensitivity of two Moroccan wheat varieties to water stress: the relationship between fatty acids and proline accumulation. Bot. Stud. 47:51-60.

Iulianelli GCV, Tavares MIB (2011). Caracterização de diferentes amostras de mandioca por espectroscopia de ressonância magnética nuclear. Polímeros 21(2):131-136.

Kim J, Jho KH, Choi YH, Nam S-Y (2013). Chemopreventive effect of cactus (*Opuntia humifusa*) extracts: radical scavenging activity, pro-apoptosis, and anti-inflammatory effect in human colon (SW480) and breast cancer (MCF7) cells. Food Funct. 4(5):681-688.

Matos FJA (2009). Introdução à fitoquímica experimental, EdUFC, Fortaleza. P 150.

Medeiros S de S, Cecílio RA, Melo-Júnior JCF, Silva-Júnior JLC da (2005). Estimativa e espacialização das temperaturas do ar mínimas, médias e máximas na Região Nordeste do Brasil. Rev. Bras. Eng. Agríc. Amb. 9(2):247-255.

Mendez M, Rodríguez R, Ruiz J, Morales-Adame D, Castillo F, Hernández-Castillo FD, Aguilar CN (2012). Antibacterial activity of plant extracts obtained with alternative organics solvents against food-borne pathogen bacteria. Ind. Crop. Prod. 37(1):445-450.

Mondragón-Jacobo C, Pérez-González S (2001). Cactus (*Opuntia* spp.) as forage, Food and Agriculture Organization of the United Nations, Roma. P 146.

Morales P, Ramírez-Moreno E, Sanchez-Mata M de C, Carvalho AM, Ferreira ICFR (2012). Nutritional and antioxidant properties of pulp and seeds of two xoconostle cultivars (*Opuntia joconostle* F.A.C. Weber ex Diguet and *Opuntia matudae* Scheinvar) of high consumption in Mexico. Food Res. Int. 46:279-285.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65(1-2):55-63.

Nascimento CCHC, Vasxoncelos SDDde, Nascimento SF, Oliveira JFF, Nogueira RI, Barreto AS, Dire GF (2016). Analysis of measurement of weight of animals treated with a freeze-dried aqueous extract of *Costus spicatus*. World J. Pharm. Med. Res. 2(4):190-195.

Naselli F, Tesoriere L, Caradonna F, Bellavia D, Attanzio A, Gentile C, Livrea MA (2014). Anti-proliferative and pro-apoptotic activity of whole extract and isolated indicaxanthin from *Opuntia ficus-indica* associated with re-activation of the onco-suppressor p16INK4a gene in human colorectal carcinoma (Caco-2) cells. Biochem. Biophys. Res. Commun. 450(1):652-658.

Oliveira FT, Silva JS, Silva RP, Andrade-Filho FC, Pereira-Junior EB (2010). Palma forrageira: Adaptação e importância para os ecossistemas áridos e semiáridos. Rev. Verde Agroecol. Des. Sust. 5(4):27-37.

Peixoto AG, Nascimento CCHC, Azevedo LAC, Paim DRSF, Nogueira RI, Barreto AS, Diré GF (2014). Chemical analysis of biological effects of a hydroalcoholic extract *Punica granatum* (Pomegranate). Innovative J. Med. Health Sci. 4(6):172-179.

Prestes RA, Almeida DM, Barison A, Pinheiro LA, Wosiacki G (2012). Caracterização por ressonância magnética nuclear de sucos de maçã obtidos por preparações enzimáticas. Quím. Nova 35(6):1141-1145.

Ramakrishna A, Ravishankar GA (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6(11):1720-1731.

Rao CR (1952). Advanced statistical methods in biometric research, John Wiley & Sons, New York P 390.

Ribeiro CM, Souza NÂ de (2007). Esquema geral para elucidação de substâncias orgânicas usando métodos espectroscópico e espectrométrico. Quím. Nova 30(4):1026-1031.

Ribeiro EM de O, Silva NH da, Lima-Filho JL de, Brito JZ de, Silva M da PC da (2010). Study of carbohydrates present in the cladodes of *Opuntia ficus-indica* (fodder palm), according to age and season. Ciênc. Tecnol. Aliment 30(4):933-939.

Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014). Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol. Plant 36(1):1-19.

Sánchez-Rodríguez E, Moreno DA, Ferrenes F, Rubio-Wilhelmi M del M, Ruiz JM (2011). Differential responses of five cherry tomato varieties to water stress: Changes on phenolic metabolites and related enzymes. Phytochemistry 72(8):723-729.

Sánchez-Rodríguez E, Ruiz JM, Ferrenes F, Moreno DA (2012). Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. Food Chem. 134(2):775-782.

Santos-Zea L, Gutiérrez-Uribe JA, Serna-Saldivar SO (2011). Comparative analyses of total phenols, antioxidant activity, and flavonol glycoside profile of cladode flours from different varieties of *Opuntia* spp. J. Agric. Food Chem. 59(13):7054-7061.

Serra AT, Poejo J, Matias AA, Bronze MR, Duarte CMM (2013). Evaluation of *Opuntia* spp. derived products as antiproliferative agents in human colon cancer cell line (HT29). Food Res. Int. 54(1):892-901.

Shedbalkar UU, Adki VS, Jadhav JP, Bapat VA (2010). *Opuntia* and other cacti: Applications and biotechnological insights. Trop. Plant Biol. 3(3):136-150.

Silva NR da, Padovani CR (2006). Utilização de componentes principais em experimentação agronômica. Energ. Agric. 21(4):98-113.

Soares BSA (2012). Obtenção e caracterização do extrato nebulizador da *Opuntia fícus-indica* (L.) Mill e avaliação da sua atividade antimicrobiana e fotoprotetora. (Monografia, Universidade Estadual da Paraíba). Available at: http://dspace.bc.uepb.edu.br/jspui/bitstream/123456789/321/1/PDF%20%20Bruno%20Samid%20Arag%C3%A3o%20Soares.pdf

Tao R, Hao L, Jia S, Zheng X, Yu J, Jiang Q (2015). A comparative study on the antioxidant activity of two polysaccharides from *Ganoderma lucidum*. Adv. Appl. Biotechnol. 333:441-450.

Vizcaino RLM, Mendoza DM, Alcocer MSP, Hernandez R, Gonzalez AM, Contreras AMV (2007). Evaluación química del extracto total etanólico de las hojas y corteza fresca de *Muntingia calabura* (Elaeocarpaceae). Sci. Technol. 13(33):455-456.

Yeilaghi H, Arzani A, Ghaderian M, Fotovat R, Feizi M, Pourdad SS (2012). Effect of salinity on seed oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) genotypes. Food Chem. 130(3):618-625.

Zhong X-K, Jin X, Lai F-Y, Lin Q-S, Jiang J-G (2010). Chemical analysis and antioxidant activities *in vitro* of polysaccharide extracted from *Opuntia ficus indica* Mill. cultivated in China. Carbohydr. Polym. 82(3):722-727.

Zou Da-M, Brewer M, Garcia F, Feugang JM, Wang J, Zang R, Liu H, Zou C (2005). Cactus pear: a natural product in cancer chemoprevention. Nutr. J. 4(25):1-12.