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Journal of Medicinal Plants Research

Full Length Research Paper

Elicitation induced flavonoids, phenolic constituents, antioxidant and cytotoxic activities of *Artemisia monosperma* callus cultures

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***Artemisia monosperma* (Delile) callus was induced using seedling explant cultured on Murashige and Skoog solid medium (M&S) supplemented with 1 mg/L naphthalene acetic acid (NAA) and 1 mg/L kinetin (Kn). Cultures were maintained on 2 mg/L 2,4-dichlorophenoxy acetic acid (2, 4- D) and 1 mg/L Kn and treated with different elicitors. Total flavonoids and phenolics were determined by aluminum chloride-potassium acetate and Folin-Ciocalteu colorimetric methods, respectively. Yeast extract 10 mg/L (Y2) showed higher productivity and viability of callus cultures than *Fusarium oxysporum* and calcium chloride elicitors. Y2 shows an increase of 2.4 and 1.5 times non-elicited calli for flavonoids and phenolic compounds production, respectively. Pockets of embryogenic calli were transferred to M&S solid media supplemented with 0.5 mg/L NAA and 0.5 mg/L benzyl aminopurine (BAP), followed by hormonal free media where different stages of embryos were monitored, giving regenerated plantlets. HPLC analysis of methylene chloride and ethyl acetate fractions of parent plant (P), Y2 elicited embryogenic callus (EC.Y2) and Y2 elicited non-embryogenic callus (NEC.Y2) showed that, quercetin content in methylene chloride fraction of NEC.Y2 is 7.2 times the same fraction of P, while vanillic acid ethyl ester content in ethyl acetate fraction of EC.Y2 (EEC.Y2) is 8.6 times the same fraction of P. EEC.Y2 showed highest anti-oxidant activity with IC_{50} 7.22±0.14 µg/mL compared with ethyl acetate fraction of P with IC_{50} 20.01±0.82 µg/mL (IC_{50} of L-ascorbic acid = 1.24±0.07 µg/mL). Using MTT assay, EEC.Y2 exhibited potent cytotoxic activity against colon carcinoma cells and moderate activity against hepatocellular and lung carcinoma cells with IC_{50} 18.9±1.4, 22.3±0.9 and 41.6±1.2 µg/mL, respectively; compared with doxrubcin as reference standard.**

Key words: *Artemisia monosperma*, callus cultures, flavonoids, phenolic compounds, anti-oxidant, cytotoxicity.

INTRODUCTION

Flavonoids are one of the largest groups of secondary metabolites and widely distributed in leaves, seeds, barks and flowers of plants with more than 4000 different structures. Most of flavonoids possess coronary heart

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disease prevention, hepatoprotective, anti-inflammatory, anti-oxidative and anticancer activities, while some flavonoids exhibit potential antiviral activities (Kumar and Pandey, 2013). The evolving commercial importance of flavonoids and a need for renewable resources of valuable chemicals has led to attempts in developing alternative systems for their production e.g. callus culture (Jedinák et al., 2004). Phenolic compounds also have been reported to have multiple biological effects including antioxidant, anti-inflammatory, chemopreventive and anti-cancer activities (Servili et al., 2014).

The application of elicitors, which is currently the focus of researches, has been considered as one of the most effective methods to improve the synthesis of secondary metabolites in medicinal plants (Patel and Krishnamurthy, 2013). Yeast extract and *Fusarium oxysporum* (Azeez and Ibrahim, 2013) as biotic elicitors and calcium chloride (Rashid et al., 2011) as abiotic elicitor are suitable elicitors for induction of secondary metabolites production.

Artemisia monosperma (Delile), family Asteraceae, is reputed in traditional medicine for its antispasmodic, anthelmintic and anti-hypertensive activities (Wagner and Wolff, 1977). Volatile oils (Saleh, 1985), flavonoids (Elgamal et al., 1997), alkaloids (Zaki et al., 1984) and coumarins (Hammoda et al., 2008) were previously isolated and identified from *A. monosperma* parent plant. To the best of our knowledge, there is no report for any tissue culture studies on this plant. So, the aim of our study is to establish a stable callus culture to investigate the effect of biotic and abiotic elicitors on the flavonoids and phenolic contents production, as well as its anti-oxidant and cytotoxic activities compared with the parent plant.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. monosperma*, Delile (Athir) plant, family Asteraceae (Compositae) were collected from Bir El-Abd road, North Sinai, Egypt, in April 2013 during the flowering and fruiting stages. The plant was identified by Dr. Abdel-Halim Abdel-Mogaly, Herbarium of Horticultural Research Institute, Agricultural Research Centre, Ministry of Agriculture, Dokki, Giza, Egypt, where voucher specimen No. 1814-CAIM is kept there. Seeds were collected by rubbing out the capitulum of the shade dried parent plant.

In vitro seed germination

Seeds of *A. monosperma* were surface sterilized by immersion in 70% ethanol for 1 min followed by 2 min in 30% hydrogen peroxide and rinsed three times with sterile distilled water. The sterilized seeds were cultured on sterile Whatman No.1 filter paper in sterile Petri dishes, containing liquid M&S medium (Murashige and Skoog, 1962), supplemented with 30 g/L sucrose and adjusted to pH 5.8 before autoclaving. The seeds were incubated at 25±2°C under continuous light using fluorescent white lamps. Germination of seeds was evaluated using seed germination percentage according to the following equation:

$$\text{Seed germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of cultured seeds}} \text{x}100$$

Callus induction and maintenance

Non-embryogenic callus

Callus induction was carried out using M&S media supplemented with 1 mg/L NAA, 1 mg/L Kn and incubated in darkness at 25±2°C. Most seedlings produced sufficient calli for subculture within 5 to 8 weeks. Callus maintenance was carried out using M&S media supplemented with different hormonal combinations as 0.5 mg/L NAA and 0.5 mg/L Kn; 1 mg/L NAA and 1 mg/L Kn; 0.5 mg/L NAA and 0.5 mg/L BAP; 1 mg/L 2,4-D and 0.2 mg/L Kn. Yellowish, friable and healthy callus was maintained on two types of M&S solid media; the first supplemented with 2 mg/L 2,4-D and 1 mg/L Kn (M&S1) and the second supplemented with 1 mg/L 2,4-D & 0.5 mg/L Kn (M&S2). All media were supplied with 30 g/L sucrose and solidified with 8 g/L agar. Callus was sub-cultured into fresh medium every four weeks and incubated at 25±2°C with 12 h, photoperiod.

Growth parameters

Growth curves were carried out for calli growing on M&S1 and M&S2 media according to Godoy-Hernández and Vázquez-Flota (2006). Growth dynamics in callus cultures were calculated as follow:

Growth index (GI) = (Ge - G start)/G start (Verpoorte et al., 1998).

Where Ge = Weight of biomass at the end of generation (final weight); G start = Weight of biomass at zero time (initial weight).

Specific growth rate (μ):

$\mu = (\ln x - \ln x_o)/t$ (Godoy-Hernández and Vázquez-Flota, 2006).

Where x_o is the initial biomass and x is the biomass at time t.

Doubling time (dt) which is the time required for the biomass of a population of cells to double where dt = ln (2)/ μ (Godoy-Hernández and Vázquez-Flota, 2006).

Somatic embryogenesis

After 3 months of culture on M&S media supplemented with 1 mg/L NAA and 1 mg/L Kn, pockets of yellowish green embryogenic calli with nodular structures were transferred into M&S solid media supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP for 3 to 4 months where different stages of embryos were monitored by using microscope. The well proliferated cultures were further kept on hormonal free medium solidified with 6 g/L agar for five months. The developed undifferentiated plantlets were cultured on M&S media supplemented with different combinations of thidiazuron (TDZ), 2,4-D, kn and indole acetic acid (IAA).

Quantitative determination of total flavonoids and total phenolic contents of parent plant and callus cultures

Total flavonoids

The powdered parent plant (1 g) and 40 g of the fresh weight of callus (equivalent to 2 g dry weight) were extracted according to Al-

Gendy et al. (2015). Total flavonoids of both parent plant and callus extracts were determined according to aluminium chloride-potassium acetate method (Woisky and Salatino, 1998). The absorbance of the reaction mixture was measured at λ_{max} 415 nm by using 6715 UV/VIS (SENWAY) spectrophotometer. Flavonoids were expressed as rutin and quercetin equivalent. The calibration curves of rutin and quercetin were done by using standard solutions of 5, 10, 20, 40 and 80 µg/mL in 80% ethanol (v/v) and 12.5, 25, 50, 66.6 and 100 µg/mL in 80% ethanol (v/v), respectively and treated similarly (Hung and Morita, 2008).

Total phenolic contents

Total phenolics of parent plant and callus cultures were determined using Folin-Ciocalteau colorimetric method (Sellappan et al., 2002). The absorbance was measured at λ_{max} 765 nm. Concentration of phenolic contents was expressed as gallic acid equivalent. The calibration curve of gallic acid was constructed by using standard solutions of 12.5, 25, 33.3, 50, 66.6 and 100 µg/mL in 80% ethanol (v/v) (Al-Gendy et al., 2013).

Elicitation of callus cultures

Dried commercial yeast extract at different conc. 5, 10 and 15 mg/L (Ahmed and Baig, 2014) and calcium chloride obtained from El-Nasr Pharmaceutical Chemicals Company (0.2, 0.4 and 0.6 mg/L) were added as dried powder separately to M&S1 media before autoclaving (Baldi and Dixit, 2008). *Fusarium oxysporum* hyphae, supplied from Faculty of Agriculture, Zagazig University was washed with distilled water, filtered, dried at 40°C till constant weight and finely powdered before addition to M&S1 media at 1, 2 and 3 mg/L (Patel and Krishnamurthy, 2013). M&S1 media was adjusted to pH 5.8 after addition of these elicitors and then autoclaved. Control media was prepared by substituting the elicitor with distilled water. The embryogenic callus was similarly elicited with 10 mg/L yeast extract.

Extraction and fractionation

The dried powdered (25 g) parent plant (P) was cold macerated with 70% ethyl alcohol till exhaustion, concentrated at 50°C to yield 3.4 g of a sticky dark green residue. Additionally, 230 g of fresh weight non-embryogenic callus elicited with 10 mg/L yeast extract (NEC.Y2) and 330 g of fresh weight embryogenic callus elicited with 10 mg/L yeast extract (EC.Y2) were extracted according to Al-Gendy et al. (2015) as mentioned above to yield 5.3 g and 6.7 g of a sticky dark brown residue of NEC.Y2 and EC.Y2, respectively. The alcoholic callus extracts were dissolved in excess amount of methyl alcohol, filtered and concentrated at 50°C to yield 2.1 and 3.6 g of sticky dark brown residue of NEC.Y2 and EC.Y2, respectively. The dried alcoholic extracts of P, NEC.Y2 and EC.Y2 were defatted with hexane and fractionated by methylene chloride and ethyl acetate. Each fraction was dried over anhydrous sodium sulfate and concentrated under vacuum to afford methylene chloride fractions (0.6, 0.25 and 0.11 g) and ethyl acetate fractions (0.7, 0.2 and 0.19 g) for P, NEC.Y2 and EC.Y2, respectively. All fractions were stored at -20°C until use. Methylene chloride and ethyl acetate fractions (20 mg, each) were dissolved in 5 mL of 95% ethyl alcohol and quantitatively estimated for their flavonoids and phenolic contents as mentioned above.

HPLC analysis

Methylene chloride and ethyl acetate fractions of P, NEC.Y2 and EC.Y2 were investigated according to the method described by Mattila et al. (2000) and analyzed for their flavonoids and phenolic constituents by HPLC (Hewlett Packard, series 1050) using C18 hypersil BDS column with particle size 5 µm. The separation was carried out with methanol and acetonitrile as a mobile phase, flow with 1 mL/min. The identification depended on diode-array and electro-array detectors and based upon comparison of retention time with the available authentics (Dvořáková et al., 2008).

Antioxidant assay by DPPH method

The antioxidant activities of total extract, methylene chloride and ethyl acetate fractions of P, NEC.Y2 and EC.Y2 in addition to total extract of NEC were evaluated according to DPPH free radical scavenging activity method (Ratty et al., 1988; Sahu et al., 2013). Briefly, 2 mL of 0.1 mM solution of DPPH in methanol was added to 2 mL of the serial dilutions of the samples at concentration of 10, 20, 40, 60, 80 and 100 µg/mL. L-ascorbic acid was used as standard at 0.25, 0.5, 0.75, 1, 2, 4, 6 and 8 µg/mL. After incubation for 30 min at room temperature, the absorbance was measured at λ_{max} 517 nm and the activity is expressed as percentage DPPH-radical scavenging that is calculated according to the following equation:

$$\%\ \text{DPPH radical} - \text{scavenging} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{x}100$$

Where A_C is the absorbance of the control solution, A_S is the absorbance of sample in DPPH solution. The percentage of DPPH radical-scavenging was plotted against the different extract concentrations to determine the concentration (µg/mL) of extract required to scavenge DPPH by 50% (IC_{50}).

Evaluation of cytotoxic activity

Cytotoxic activity of ethyl acetate fraction of EC.Y2 against colon (HCT-116), hepatocellular (HepG-2) and lung (A-549) carcinoma cell lines was detected using MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) where doxrubcin was used as standard. Serial dilutions of samples and standard were used at concentration of 0, 1.56, 3.125, 6.25, 12.5, 25 and 50 µg/mL (Mosmann, 1983; Elaasser et al., 2011).

Statistical analysis

All the results in the current study were calculated using (Microsoft Excel 2010) and recorded in triplicate. Each value represents the mean ± S.D of three samples and all bars in the figures represent S.D. The IC_{50} was determined as the drug conc. which resulted in 50% reduction in cell viability or inhibition of the biological activity

RESULTS AND DISCUSSION

Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavors, fragrances and colorants, which can not be produced by microbial cells or chemical synthesis (Mulabagal and Tsay, 2004).

Induction and maintenance of callus cultures

The germination percentage of sterilized seeds was

30.9%. Induction of *A. monosperma* non-embryogenic calli sufficient for subculture, was successful on M&S media supplemented with 1 mg/L NAA and 1 mg/L Kn. After 5 weeks, the cultures were maintained on M&S1 and M&S2 and the best growing calli were followed for 31 days. Fresh and dry weights were determined every 3 days (Figure 1). Both M&S1 and M&S2 produced yellowish, friable and healthy callus but M&S1 media showing higher growth parameters (GI and μ) and lower dt as shown in Table 1.

Somatic embryogenesis and plantlets regeneration

Pockets of yellowish green embryogenic calli with nodular structures appeared on the surface of the non-embryogenic callus after 3 months of culture on M&S medium supplemented with 1 mg/L NAA and 1 mg/L Kn (Figure 2a). Embryogenic callus was maintained on M&S solid media supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP. Somatic embryos as globular (G: 60-120 μm), heart-shaped (H: 100-295 μm) and torpedo-shaped (T: 270-440 μm) were monitored (Figure 2b, c and d). Mature embryos successfully germinated into cotyledonary embryo, which further developed into cotyledonary leaves of 0.4 cm height (Figure 2e). When proliferated calli were moved to hormonal free medium, they kept the embryogenic potential and showed further embryo development giving undifferentiated plantlets of nearly 2 cm height (Figure 2f). When the embroids were transferred to M&S solid media supplemented with 0.5 mg/L TDZ, 1 mg/L 2,4-D and 0.1 mg/L Kn in a trial for regeneration of the whole plant, browning of callus was observed. Also, other trials were done by culturing on M&S solid media supplemented with 2 mg/L IAA alone or in combination with 0.5 mg/L Kn, where no change in behavior was observed but abnormal embryos as fused globular and torpedo shaped were detected (Figure 3). No further differentiation of the whole plant occurred even after culturing on M&S solid media supplemented with different hormonal types and concentrations may be due to the appearance of abnormal embroids. In a previous report, somatic embryos also failed to mature in *Stevia rebaudiana* (Pande and Gupta, 2013) which is in agreement with our results.

Quantitative determination of total flavonoids and total phenolics of parent plant and callus cultures

The quantitative determination of total flavonoids and phenolic contents of *A. monosperma* 28th day old of 4th generation calli growing on M&S media supplemented with different hormonal combinations was recorded as shown in Figure 4. M&S1 showed calli with the highest content of flavonoids (1002.8±20.1 and 1723±40.6 μg/g.DW) expressed as rutin and quercetin equivalent, respectively which is nearly 2.3 times its content on M&S media supplemented with 0.5 mg/L NAA and 0.5 mg/L Kn. Also M&S1 showed calli with the highest phenolic contents (3561.1±50.1 μg/g.DW) expressed as gallic acid equivalent which is 1.26 times its content on M&S media supplemented with 0.5 mg/L NAA & 0.5 mg/L BAP. Callus maintained on M&S1 media was followed every 3 days for its flavonoids and phenolic contents expressed as rutin, quercetin and gallic acid equivalent, showed that, the 28th day old calli have highest content of flavonoids while 18^{en} day old calli have highest content of phenolics (Figure 5).

Elicitation of callus culture

M&S1 was treated with different elicitors as yeast extract, *Fusarium oxysporum* and calcium chloride because this media showed the best growth parameters of callus cultures in addition to its highest flavonoids and phenolic contents. These elicitors were previously reported to increase the concentrations of some metabolites.

Yeast (Y)

Y2 (10 mg/L) resulted in maximum increase in flavonoids content (2452±85.7 and 4226±92.4 μg/g.DW) expressed as rutin and quercetin equivalent, respectively after 24 days of subculture representing 2.4 times control (Y0). Flavonoids content increased gradually from zero time till the stationary phase after 24 days (Figure 6a and b). Also Y2 showed highest phenolic contents (5401±95.3 μg/g.DW) expressed as gallic acid equivalent at 28th day which is nearly 1.51 times Y0 (Figure. 6c). Y1 (5 mg/L) and Y3 (15 mg/L) increased flavonoids and phenolic contents, which is nearly 1.46 and 1.42 times Y0, respectively (Figure 6a, b and c). Different flavonoids and phenolic compounds were enhanced through the addition of yeast extract in callus and suspension cultures of *Iphiona mucronata* (Al-Gendy et al., 2015), flavonolignans in hairy root cultures of *Silybum marianum* (Hassanloo et al., 2008) and artemisinin in callus culture of *Artemisia. annua* (Baldi and Dixit, 2008).

F. oxysporum **fungi (F)**

F2 (2 mg/L) resulted in maximum flavonoids content (1980±80.5 and 3412±87.5 μg/g.DW) expressed as rutin and quercetin equivalent, respectively after 24 days of subculture (Figure 7a and b), which is nearly 1.98 times control (F0) and also 1.94, 1.98 times F1 (1 mg/L) and F3 (3 mg/L), respectively. Also F2 gives maximum content of phenolics at 24th day of subculture (5048±91.4 μg/g.DW) expressed as gallic acid equivalent, representing 1.65 times F0, 1.37 and 1.31 times F1 and F3, respectively (Figure 7c). Application of fungal preparations as elicitors

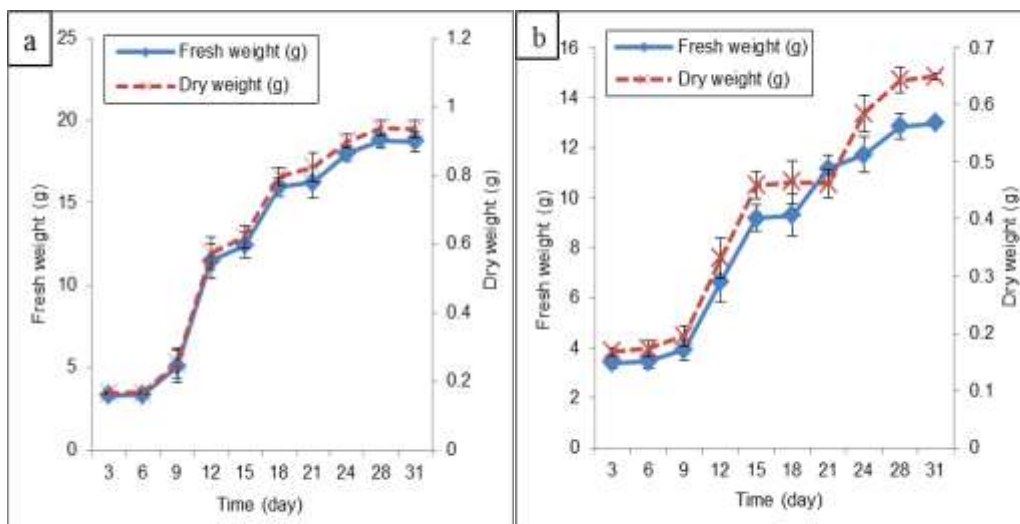

Figure 1. Growth curves of *A. monosperma* calli for 31 days on M&S medium supplemented with **(a)** 2 mg/L 2,4-D & 1 mg/L kn (M&S1), **(b)** 1 mg/L 2,4-D & 0.5 mg/L kn (M&S2).

Table 1. Growth dynamics in callus cultures on M&S1 and M&S2 media.

Growth parameter	Growth media	
	M&S1	**M&S2**
Growth index (GI)	4.61	2.85
Specific growth rate (µ)	0.055 gm/day	0.043 gm/day
Doubling time (dt)	12.4 day	15.9 day

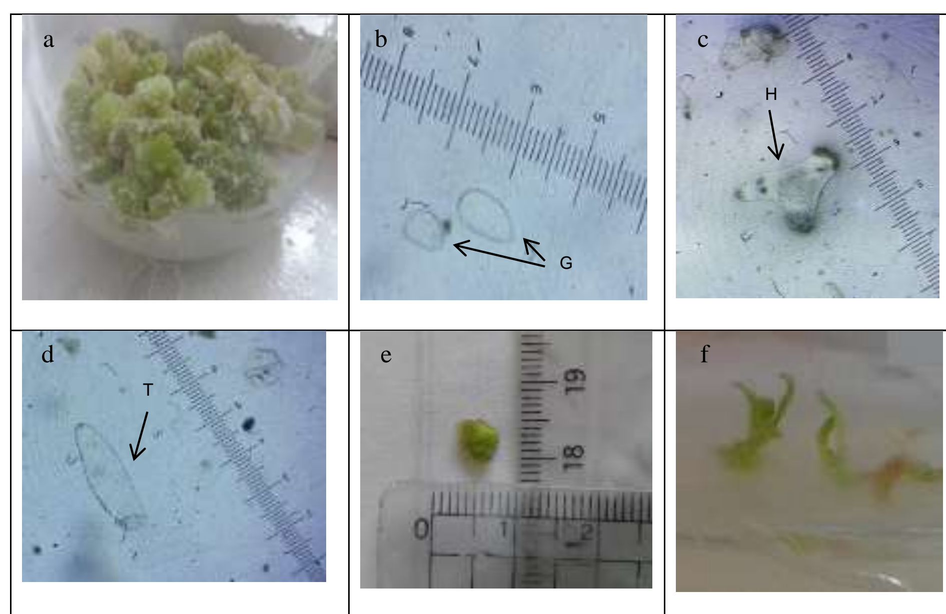

Figure 2. Stages of embryogenic callus of *A. monosperma* parent plant. (a) Pockets of embryogenic calli; (b) Globular "G" embryo (85.12 µm); (c) Heart shaped "H" embryo (266 µm); (d) Torpedo shaped "T" embryo (308.56 µm); (e) Cotyledonary leaves with 0.4 cm height; (f) Undifferentiated plantlets with 2 cm height..

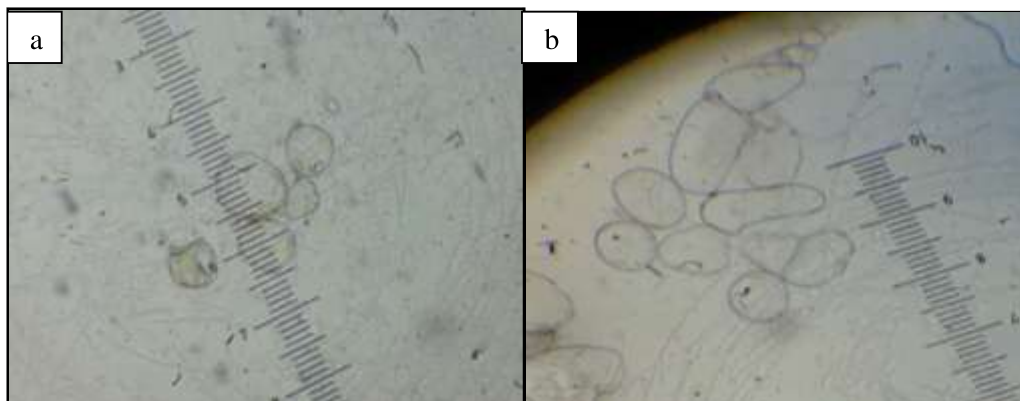

Figure 3. Fused embryos in callus culture of *A. monosperma* parent plant. (a) Fused globular (188-192 µm), (b) Fused globular and heart shaped embryos (425-431 µm).

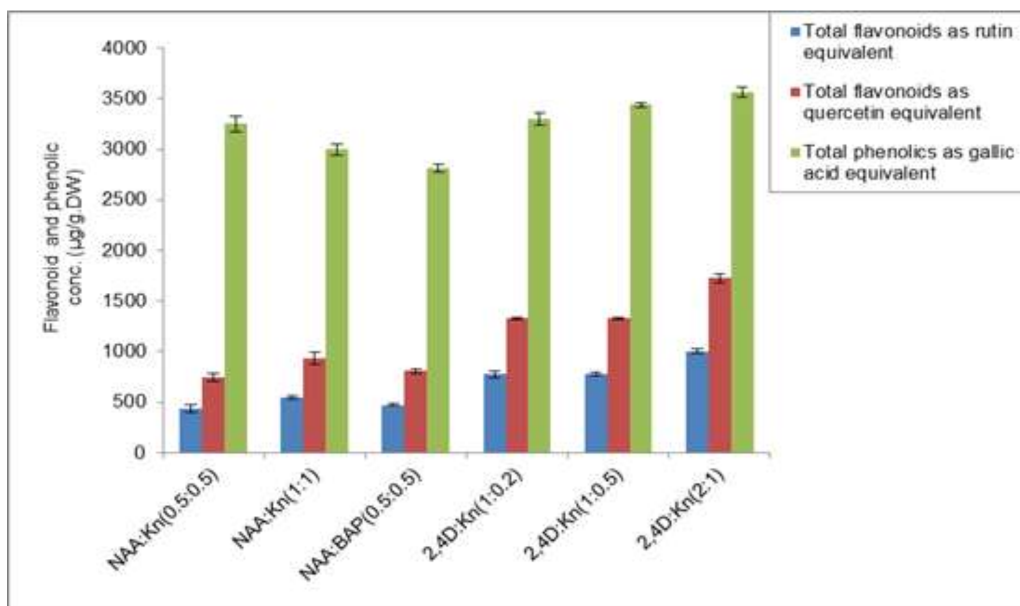

Figure 4. Total flavonoids and phenolic contents of *A. monosperma* 4th generation calli growing on M&S media supplemented with different hormonal combinations expressed as rutin, quercetin and gallic acid equivalent, respectively.

has become one of the most important and successful measures to enhance secondary metabolites production in plant cell cultures as phenolic acids and diterpenes from Rosemary (*Rosmarinus officinalis* L.) leaf and callus cultures (Rashid et al., 2011), hyoscyamine and scopolamine in root cultures of *Hyoscyamus niger* and *H. muticus* (Namdeo, 2007) and four major isomers of boswellic acid in callus culture of *Boswellia serrata* (Ghorpade et al., 2011).

Calcium chloride (C)

Different concentrations of C were used but C2 (0.4 mg/L) showed maximum concentration of flavonoids at 28th day of subculture (1465±70.5 and 2545±77.1 µg/g.DW) expressed as rutin and quercetin equivalent, respectively (Figure 8a and b) which is nearly 1.48 times control (C0). Only C2 increased phenolic contents (4802±80.9 µg/g.DW) after 24 days representing nearly 1.47 times C0 but C1 and C3 was not successful as it decreased phenolic contents to give 90 and 60% of C0, respectively (Figure 8c). The effect of calcium chloride as abiotic elicitor was extensively studied as enhancement of phenolic acids production from leaf and callus cultures of *R. officinalis* (Rashid et al., 2011) and artemisinin production in hairy roots culture of *A. annua* (Patra et al.,

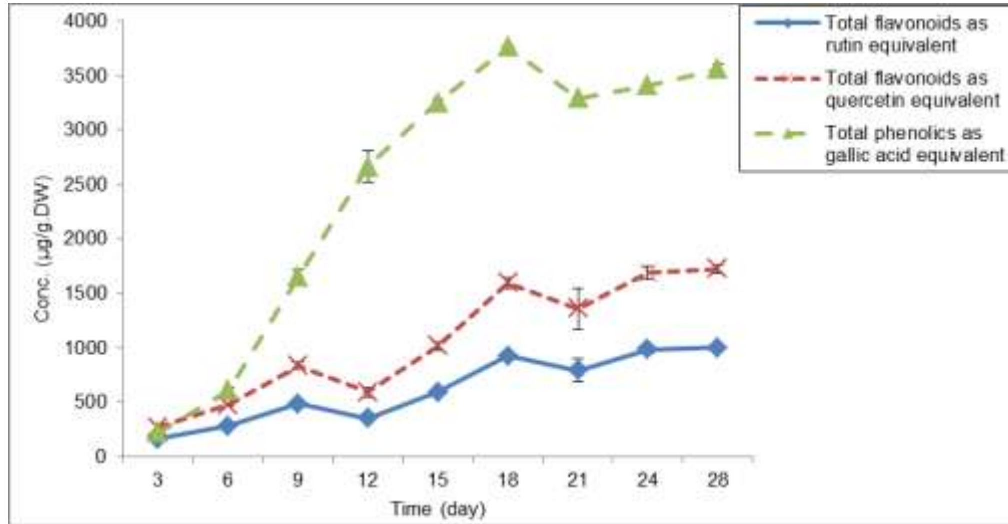

Figure 5. Quantitative determination of total flavonoids and phenolics of *A. monosperma* calli growing on M&S1 expressed as rutin, quercetin and gallic acid equivalent, respectively

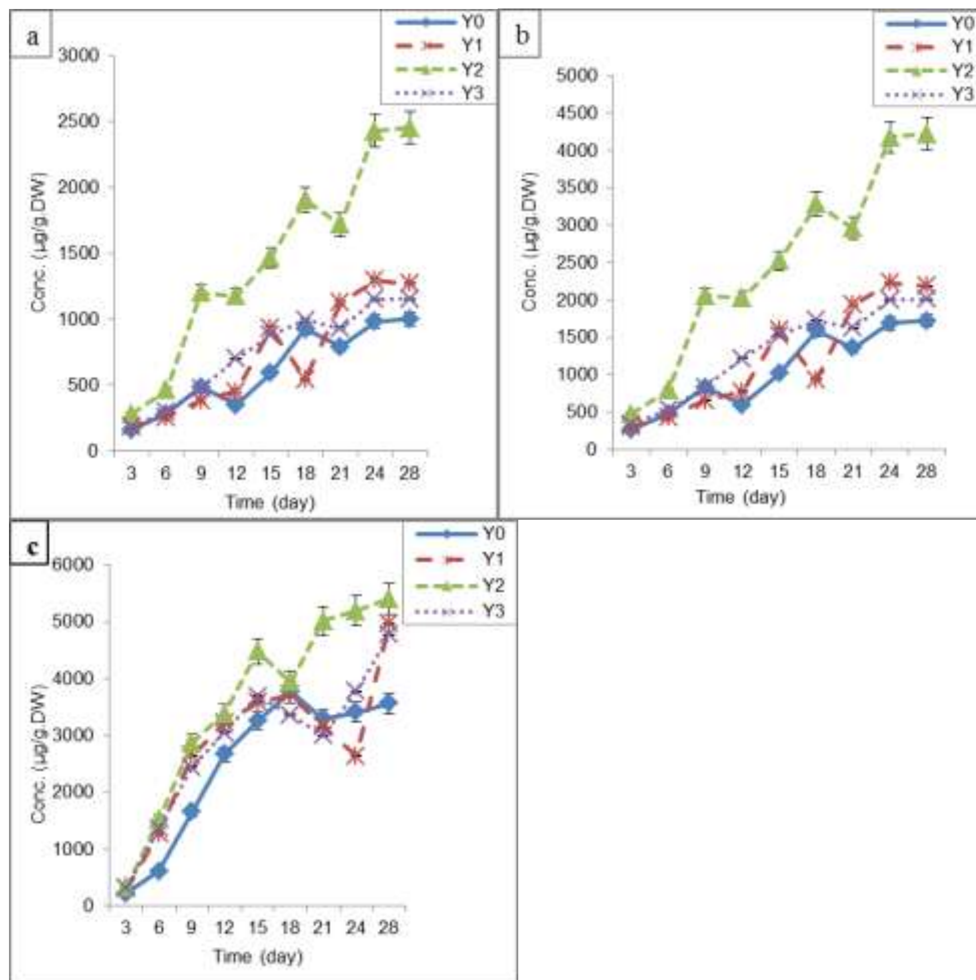

Figure 6. Effect of yeast elicitation on flavonoids and phenolic contents of *A. monosperma* callus cultures expressed as (a) rutin equivalent, (b) quercetin equivalent, (c) gallic acid equivalent, respectively (Y0: control, Y1: 5 mg/L, Y2: 10 mg/L, Y3: 15 mg/L yeast extract).

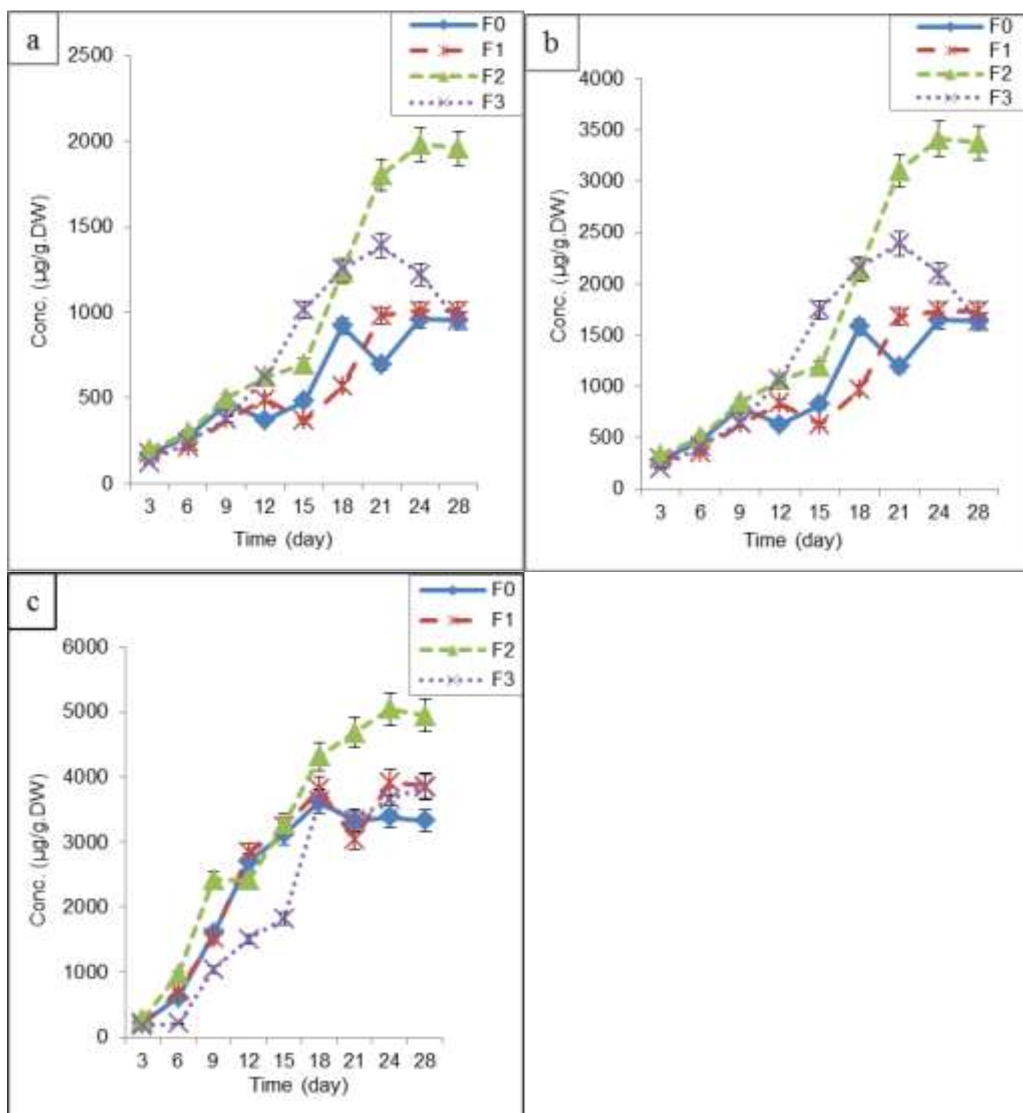

Figure 7. Effect of *Fusarium oxysporum* fungi elicitation on flavonoids and phenolic contents of *A. monosperma* callus cultures expressed as (a) rutin equivalent, (b) quercetin equivalent, (c) gallic acid equivalent, respectively (F0: control, F1: 1 mg/L, F2: 2 mg/L, F3: 3 mg/L *F. oxysporum*).

2013).

Through the use of different elicitors added at zero time of subculture and measuring flavonoids and phenolic contents expressed as rutin, quercetin and gallic acid equivalent, respectively (Figure 9a, b and c); it is clear that Y2 gave maximum flavonoids and phenolic contents at 28th day of subculture compared with F2 and C2 elicitors.

Comparative study of total extract, methylene chloride and ethyl acetate fractions obtained from P and different callus cultures for their flavonoids and phenolic contents revealed that, the ethyl acetate fraction of Y2 elicited embryogenic calli (EEC.Y2) represents 1.09, 1.1 and 3.2 times the ethyl acetate fraction of P (EP) for the total flavonoids and phenolic contents expressed as rutin, quercetin and gallic acid equivalent, respectively (Figure 10).

HPLC analysis of flavonoids and phenolic compounds

Quantitative analysis of *A. monosperma* parent plant confirmed the high flavonoids and phenolic contents and the obtained results encourage us to identify these contents in the ethyl acetate fractions of Y2 elicited embryogenic callus (EEC.Y2), Y2 elicited non-embryogenic callus (ENEC.Y2) and the parent plant (EP) in addition to methylene chloride fractions of Y2 elicited

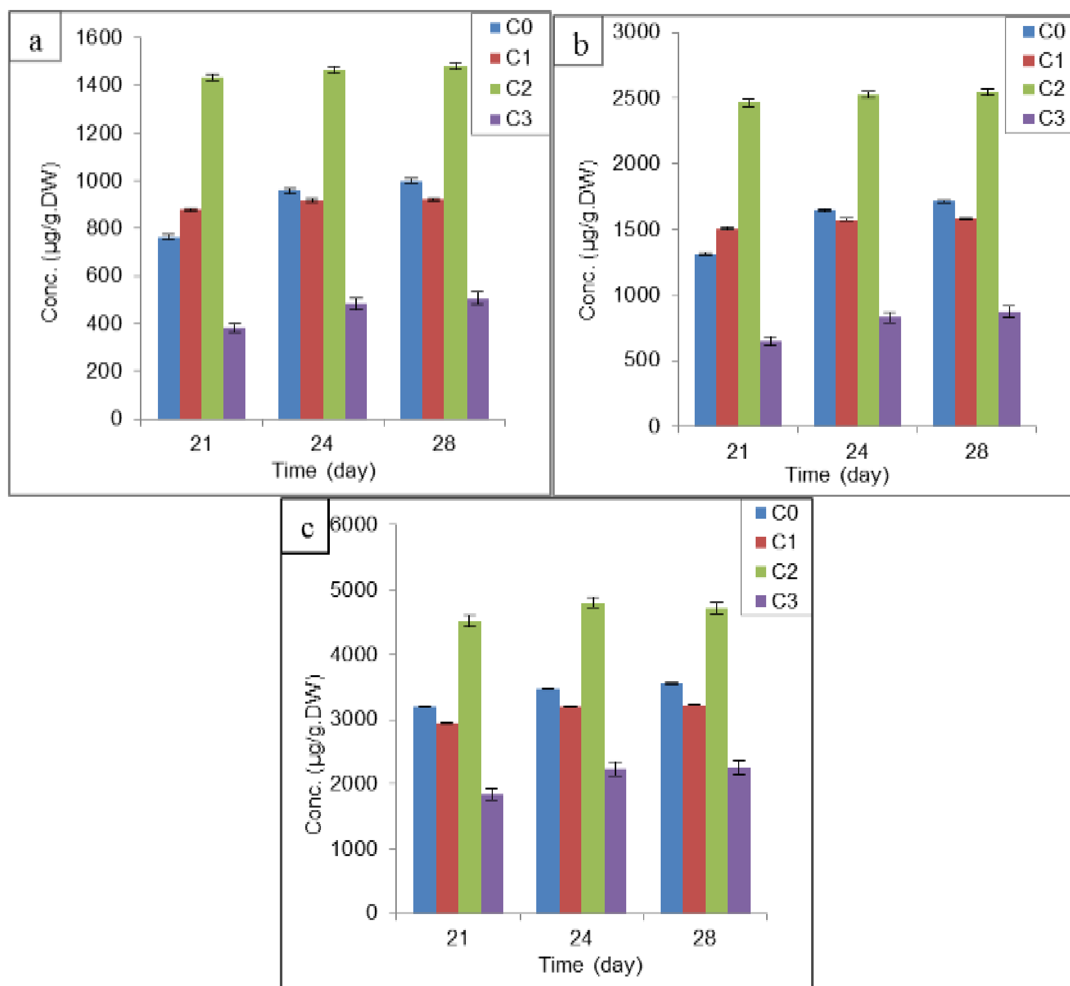

Figure 8. Effect of calcium chloride elicitation on flavonoids and phenolic contents of *A. monosperma* callus cultures expressed as (a) rutin equivalent, (b) quercetin equivalent, (c) gallic acid equivalent, respectively (C0: control, C1: 0.2 mg/L, C2: 0.4 mg/L, C3: 0.6 mg/L calcium chloride).

embryogenic callus (MEC.Y2), Y2 elicited non-embryogenic callus (MNEC.Y2) and the parent plant (MP) using HPLC technique against the available authentics of flavonoids and phenolic compounds. Eleven flavonoidal compounds (Table 2) and twenty three phenolic compounds (Table 3) were identified by comparing the retention time of their peaks with that of the available authentic flavonoids and phenolics injected under the same conditions of the experiment (Karimi et al., 2012). It is worthy to note that some peaks can't be identified due to the low concentration and/or the limited number of available authentics.

HPLC analysis of *A. monosperma* parent plant and the elicited calli fractions showed that some flavonoids were found in callus in higher concentration than parent plant as hesperidin content in ENEC.Y2 is 5.82 times EP and kaempferol in EEC.Y2 is 1.5 times EP. Additionally, the highest conc. of quercetin in MNEC.Y2 and MEC.Y2 is nearly 7.2 times more than MP. Some phenolics were found in callus in higher concentration than plant as vanillic acid ethyl ester and pyrogallol in EEC.Y2, which are 8.6 and 6.5 times EP, respectively. Also, MNEC.Y2 has the highest concentration of both ferulic acid and cinnamic acid, which are 3.2 and 3.5 times MP, respectively. On the other hand, some flavonoids and phenolic compounds were found in the parent plant in equal or higher concentration than callus as quercetrin in both EP and EEC.Y2 is 8.3 times ENEC.Y2. Additionally, *iso*-ferulic acid of EP is 1.5 and 2.4 times EEC.Y2 and ENEC.Y2, respectively. All the identified compounds were not reported to be isolated from *A. monosperma* parent plant except quercetin (El-Toumy et al., 2011) and *p*-coumaric acid only (Abdel-Mogib et al., 1990). Different flavonoids were determined by HPLC analysis of *A. vulgaris* and *A. annua* where quercetin and its derivatives was dominant (Nikolova et al., 2004).

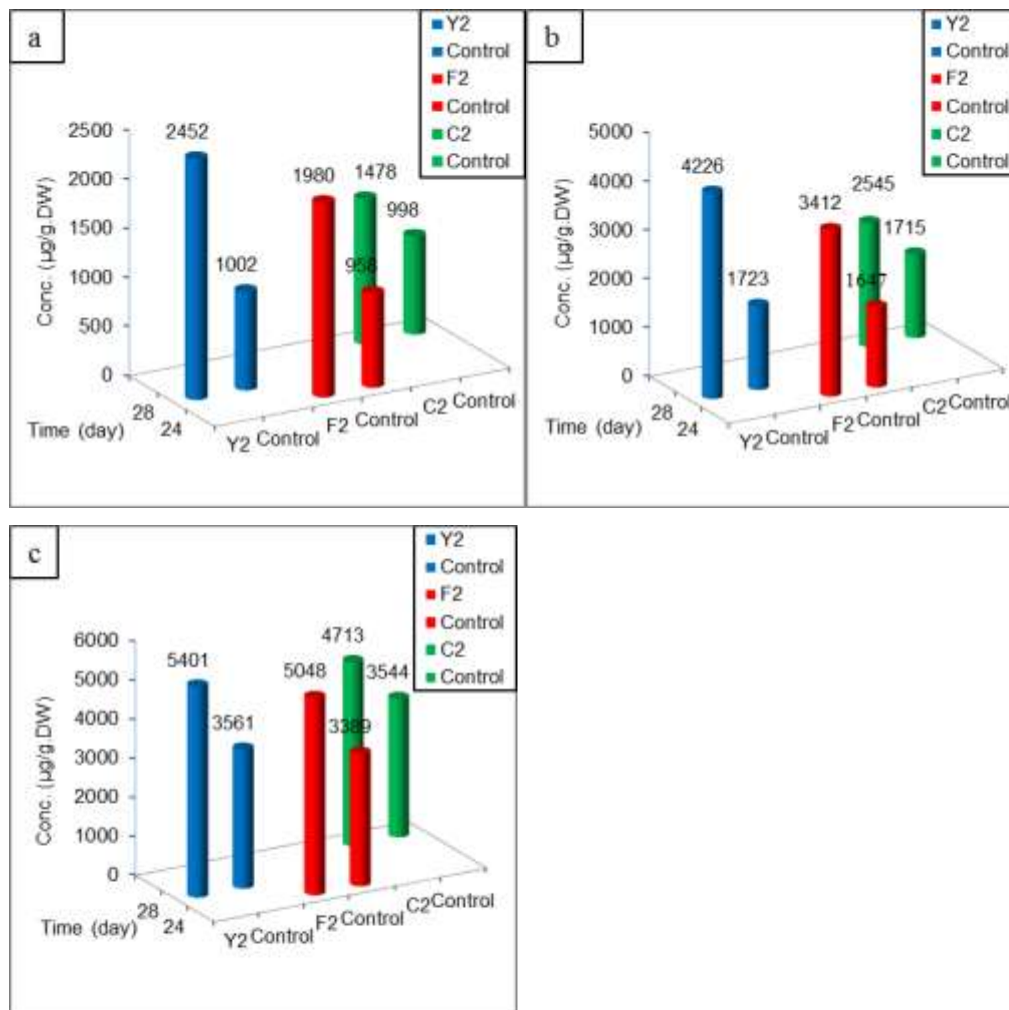

Figure 9. Effect of best elicitors concentrations on flavonoids and phenolic contents of *A. monosperma* callus cultures expressed as **(a)** rutin equivalent, **(b)** quercetin equivalent, **(c)** gallic acid equivalent, respectively (Y2: 10 mg/L yeast extract, F2: 2 mg/L *F. oxysporum* fungi, C2: 0.4 mg/L calcium chloride).

Biological Activitities

Anti-oxidant activity

DPPH radical scavenging activity showed that EEC.Y2 has higher anti-oxidant activity (IC_{50} 7.22±0.14 µg/mL) than EP (IC_{50} 20.01±0.82 µg/mL) compared with L-ascorbic acid (IC_{50} 1.24±0.07 µg/mL) as showed in Figure 11. It was previously reported that some of the detected flavonoids and phenolic compounds showed high anti-oxidant activities as hesperidin, hesperetin (Parhiz et al., 2015) and *p*-coumaric acid (Kiliç and Yeşiloğlu, 2013). Skowyra et al. (2014) reported the high antioxidant activity of *A. annua* species.

Cytotoxic activity

Due to the high flavonoids and phenolic contents and also antioxidant activity of EEC.Y2, it was evaluated for its cytotoxicity against colon (HCT-116), hepatocellular (HepG-2) and lung (A-549) carcinoma cell lines *in vitro* using doxrubcin as standard (Figure 12). The criteria used to categorize the activity of EEC.Y2 against human cancer cell lines based on the US National Cancer Institute (NCI) which proposed that crude extracts with potential cytotoxic activity are those presenting IC_{50} of ≤ 30 µg/mL (Baravalia et al., 2012; Moo-puc et al., 2013). The criteria was modified from these of Geran et al. (1972) as follows: IC_{50} ≤ 20 µg/mL is highly active, IC_{50} = 21 - 200 µg/mL is moderately active, IC_{50} = 201 - 500 µg/mL is weakly active and IC_{50} > 501 µg/mL is inactive (Srisawat et al., 2013). So, EEC.Y2 (Figure 13) is highly active against HCT-116 (IC_{50}= 18.9±1.4 µg/mL) and moderately active against HepG-2 cell lines (IC_{50}= 22.3±0.9 µg/mL) and A-549 cell lines (IC_{50}= 41.6±1.2 µg/mL). The cytotoxic activity of this fraction may be

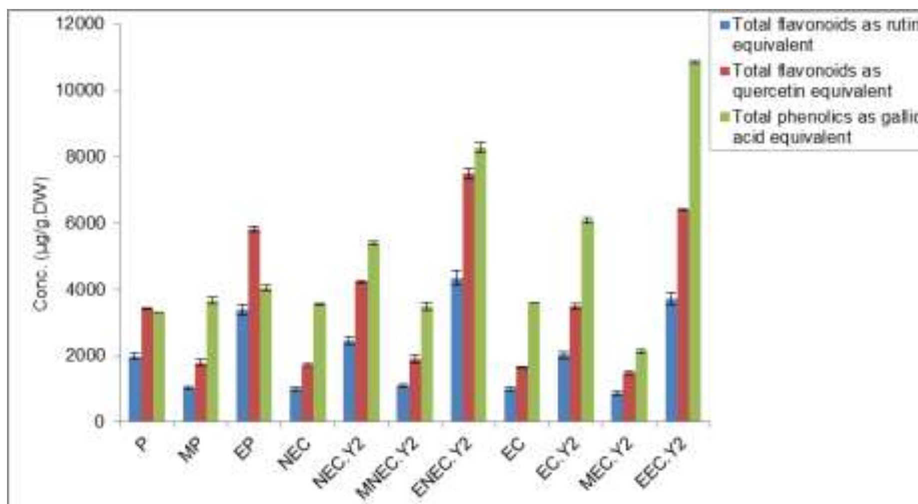

Figure 10. Total flavonoids and phenolic contents of *A. monosperma* parent plant and calli extracts (µg/g.DW) expressed as rutin, quercetin and gallic acid equivalent, respectively (P, Parent plant extract; MP, methylene chloride fraction of parent plant; EP, ethyl acetate fraction of parent plant; NEC, non-embryogenic callus extract; NEC.Y2, 10 mg/L yeast extract elicited non-embryogenic callus; MNEC.Y2, methylene chloride fraction of Y2 elicited non-embryogenic callus; ENEC.Y2, ethyl acetate fraction of Y2 elicited non-embryogenic callus; EC, embryogenic callus extract; EC.Y2, Y2 elicited embryogenic callus; MEC.Y2, methylene chloride fraction of Y2 elicited embryogenic callus; EEC.Y2, ethyl acetate fraction of Y2 elicited embryogenic callus) Y2= 10 mg/L yeast extract.

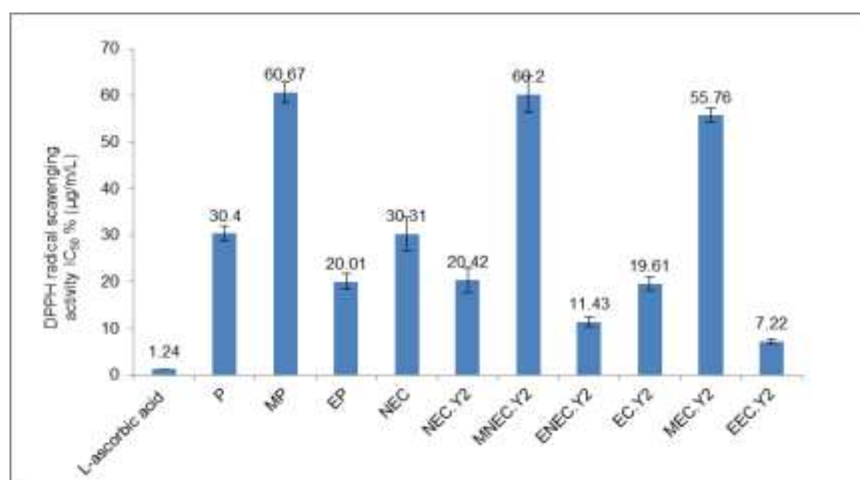

Figure 11. DPPH radical scavenging activity IC50 % (µg/mL) of the different fractions of *A. monosperma* parent plant and callus in comparison with L-ascorbic acid (P, total extract of parent plant; MP, methylene chloride fraction of parent plant; EP, ethyl acetate fraction of parent plant; NEC, non-embryogenic callus extract; NEC.Y2, Y2 elicited non-embryogenic callus; MNEC.Y2, methylene chloride fraction of Y2 elicited non-embryogenic callus; ENEC.Y2, ethyl acetate fraction of Y2 elicited non-embryogenic callus; EC, embryogenic callus extract; EC.Y2, Y2 elicited embryogenic callus; MEC.Y2, methylene chloride fraction of Y2 elicited embryogenic callus; EEC.Y2, ethyl acetate fraction of Y2 elicited embryogenic callus). Y2= 10 mg/L yeast extract.

attributed to its content of some flavonoids which previously reported to have high cytotoxic activity (Febriansah et al., 2014). It was previously reported that, polyacetylene capillin, a constituent of *A. monosperma*, inhibit proliferation of colon, larynx, lung and pancreatic carcinoma cell lines (Whelan and Ryan, 2004).

Table 2. HPLC analysis of flavonoids of *A. monosperma* parent plant and calli elicited with 10 mg/L yeast extract (Y2).

Flavonoids standard	RT (min.)	Flavonoids contents (mg/g extract)					
		EEC.Y2	ENEC.Y2	EP	MEC.Y2	MNEC.Y2	MP
Naringin	12.73	1.72	3.44	18.69	0.46	1.43	0.88
Rutin	12.91	1.03	1.85	6.07	0.28	1.01	0.13
Hesperidin	12.97	54.92	69.91	12.01	2.25	2.48	2.19
Rosmarinic	13.43	1.7	1.51	3.95	1.36	1.11	0.68
Quercetrin	13.89	4.6	0.55	5.26	2.74	0.3	2.7
Quercetin	15.48	1.01	0.85	1.2	4.23	6.66	0.92
Narengenin	15.77	0.23	0.21	1.33	0.39	0.26	0.15
Kaempferol	16.00	1.93	0.76	1.25	0.15	0.37	0.26
Hesperetin	16.00	2.32	1.76	10.94	3.4	4.96	3.48
Apigenin	17.13	0.27	0.66	1.9	0.83	1.89	5.73
7-Hydroxyflavone	18.00	0.01	0.07	0.78	0.1	0.18	2.14
Identified flavonoids	--	69.82	81.57	63.38	16.19	20.65	19.26

EEC.Y2, Ethyl acetate fraction of 10 mg/L yeast elicited embryogenic callus; ENEC.Y2, ethyl acetate fraction of Y2 yeast elicited non-embryogenic callus; EP, ethyl acetate fraction of parent plant; MEC.Y2, methylene chloride fraction of Y2 yeast elicited embryogenic callus; MNEC.Y2, methylene chloride fraction of Y2 yeast elicited non-embryogenic callus; MP, methylene chloride fraction of parent plant; RT, retention time;--, not detected.

Table 3. HPLC analysis of phenolic compounds of *A. monosperma* parent plant and calli elicited with 10 mg/L yeast extract (Y2).

Phenolic standard	RT (min.)	Phenolic contents (mg/g extract)					
		EEC.Y2	ENEC.Y2	EP	MEC.Y2	MNEC.Y2	MP
Gallic acid	7.22	0.12	0.36	0.01	0.01	0.01	0.02
Pyrogallol	7.27	5.05	0.77	0.76	0.2	0.26	0.64
3-Hydroxytyrosol	8.41	2.77	0.73	0.45	0.24	0.12	0.37
4-Aminobenzoic acid	8.50	0.33	0.25	0.13	0.02	0.05	0.04
Protocatchuic acid	8.61	0.55	0.26	10.2	0.22	0.34	0.12
Chlorogenic acid	9.51	0.54	0.85	0.44	--	0.18	0.12
Catechol	9.59	1.44	0.29	2.21	0.12	0.4	0.9
Epicatechein	10.01	1.33	0.42	0.97	1.6	1.91	0.7
Catechein	10.03	3.49	0.53	--	0.06	0.21	0.19
p-Hydroxybenzoic acid	10.21	0.51	0.35	0.5	0.14	0.33	0.25
Caffeic acid	10.55	1.05	1.61	0.88	0.08	0.24	1.06
Vanillic acid	10.59	--	0.33	1.39	--	--	--
Ferulic acid	12.33	3.97	3.77	3.54	4.45	6.32	1.92
Iso-ferulic acid	12.70	1.83	1.16	2.8	0.22	0.21	0.5
Vanillic acid ethyl ester	13.05	149.1	128.7	17.3	7.12	27.04	8.5
Reversetrol	13.30	3.25	0.87	1.12	0.52	0.34	--
Ellagic acid	13.72	3.34	2.62	7.48	1.93	5	10.6
α-Coumaric acid	13.87	0.55	0.49	1.83	0.34	0.28	0.76
Benzoic acid	13.96	11.64	6.45	9.43	19.55	19.4	31.08
3,4,5-Trimethoxy-cinnamic acid	14.62	8.85	--	4.78	0.97	0.66	2.81
Salycilic acid	14.68	4.96	2.83	3.16	1.98	--	2.07
p-Coumaric acid	*15.34*	0.51	0.22	0.34	0.22	0.28	0.54
Cinnamic acid	15.92	0.15	0.11	0.19	0.65	1.39	0.39
Identified phenolic compounds	--	205.33	153.97	69.91	40.64	64.96	63.58

EEC.Y2, ethyl acetate fraction of Y2 elicited embryogenic callus; ENEC.Y2, ethyl acetate fraction of Y2 elicited non-embryogenic callus; EP, ethyl acetate fraction of parent plant; MEC.Y2, methylene chloride fraction of Y2 elicited embryogenic callus; MNEC.Y2, methylene chloride fraction of Y2 elicited non-embryogenic callus; MP, methylene chloride fraction of parent plant; RT, retention time; --, not detected.

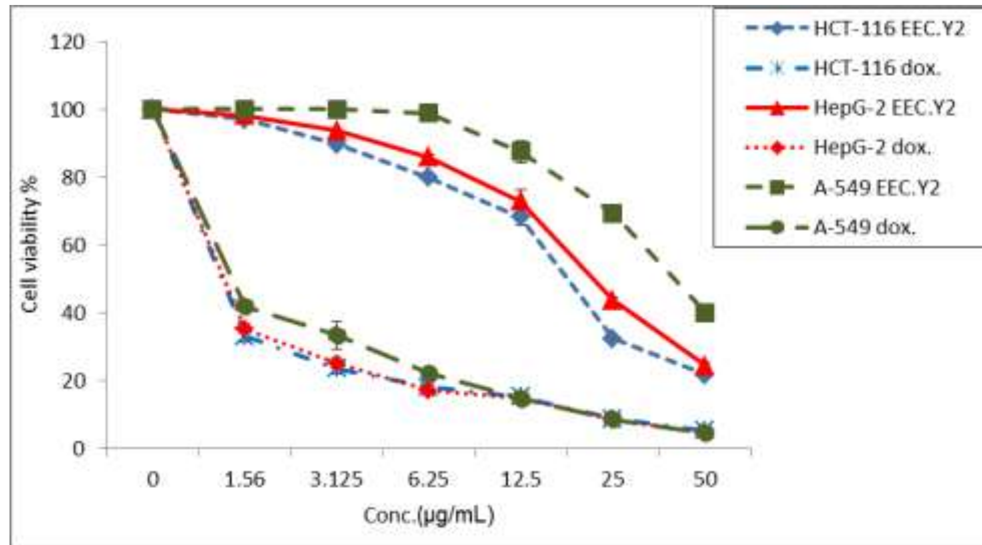

Figure 12. Inhibitory activity of ethyl acetate fraction of 10 mg/L yeast elicited embryogenic callus (EEC.Y2) against colon, hepatocellular and lung carcinoma cell lines (HCT-116, colon carcinoma cell line; HepG-2, hepatocellular carcinoma cell line; A-549, lung carcinoma cell line; dox., doxrubcin

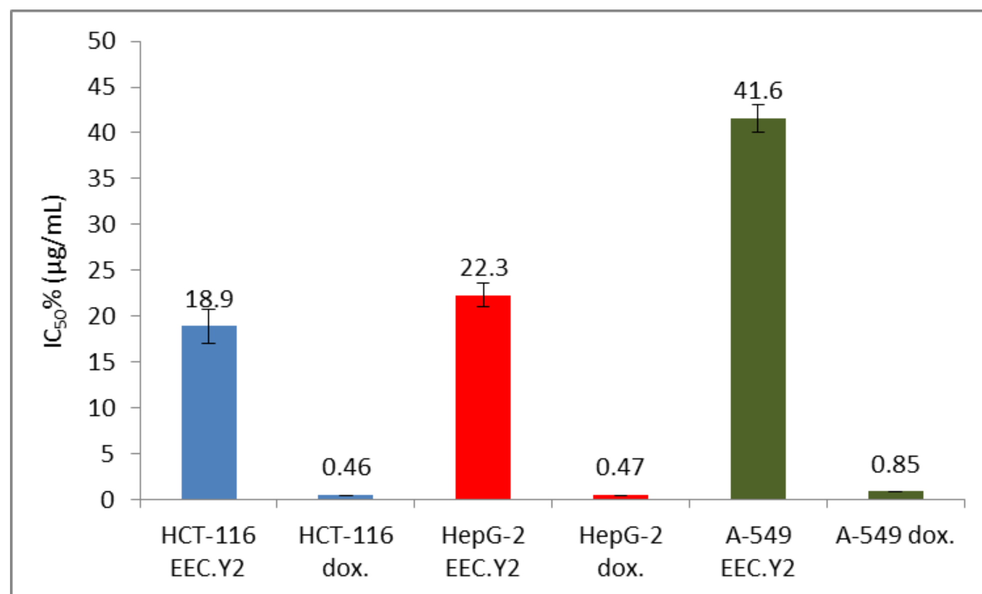

Figure 13. IC_{50} of ethyl acetate fraction of 10 mg/L yeast elicited embryogenic callus (EEC.Y2) against colon, hepatocellular and lung carcinoma cell lines (HCT-116, colon carcinoma cell line; HepG-2, hepatocellular carcinoma cell line; A-549; lung carcinoma cell line).

Conclusion

Elicitation of *A. monosperma* callus, using yeast and *F. oxysporum* fungi as biotic elicitors and calcium chloride as abiotic elicitor increased the flavonoids and phenolic compounds production, compared to the parent plant. The results favored the use of yeast extract compared with other elicitors. Embryogenic callus cultures afforded small non differentiated plantlets without further proliferation. The ethyl acetate fraction of yeast elicited embryogenic callus shows the highest antioxidant activity. Moreover, it has also high *in vitro* cytotoxic activity against colon carcinoma cells and moderate activity against hepatocellular and lung carcinoma cells.

Conflicts of Interests

The authors have not declared any conflict of interests.

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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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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).

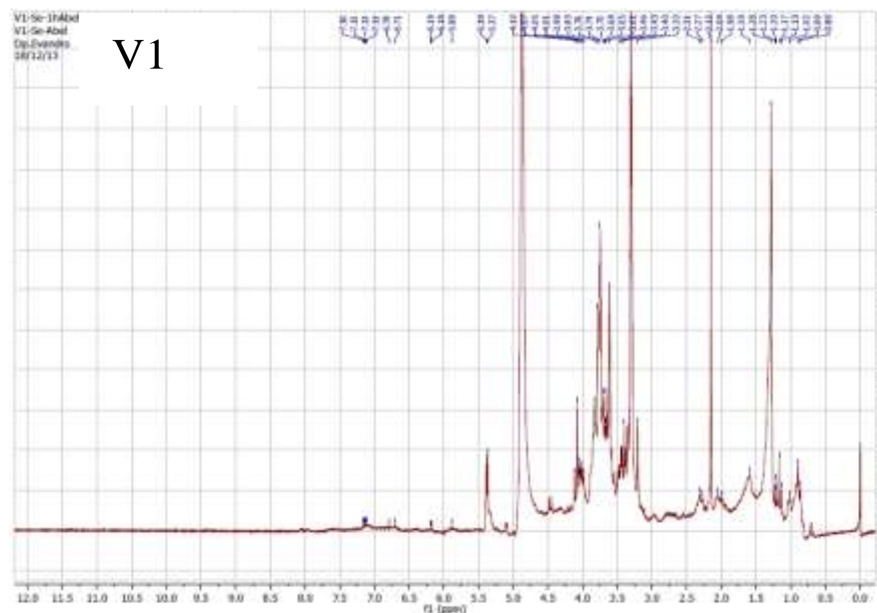
Thus, the storage of carbohydrates, glucosides substances and phenolic compounds in the rainy season will serve as molecules, that will act against water stress during the dry season (Tao et al., 2015; Zhong et al., 2010). In addition, the accumulation of some fatty acids and steroids in the dry season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

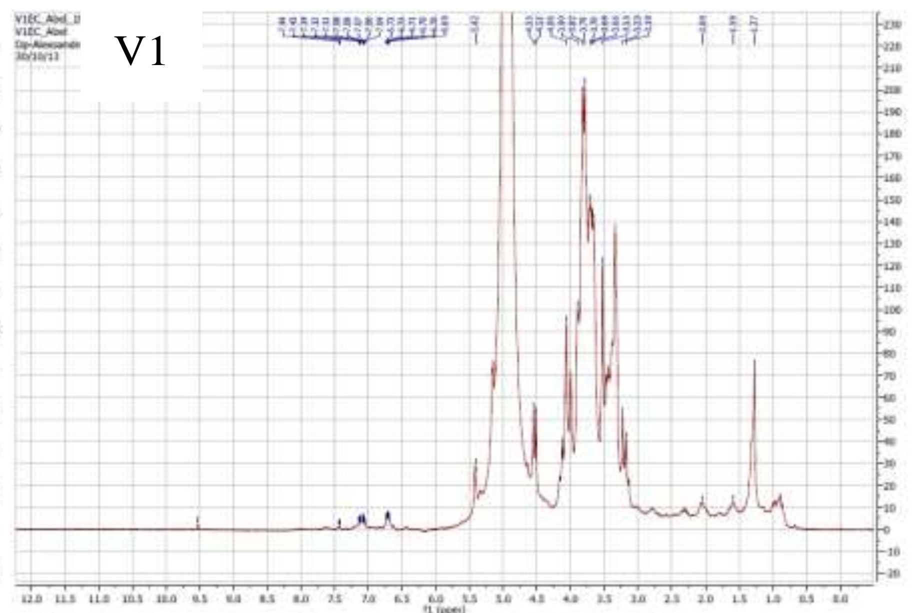
Genetic diversity through ^{1}H NMR

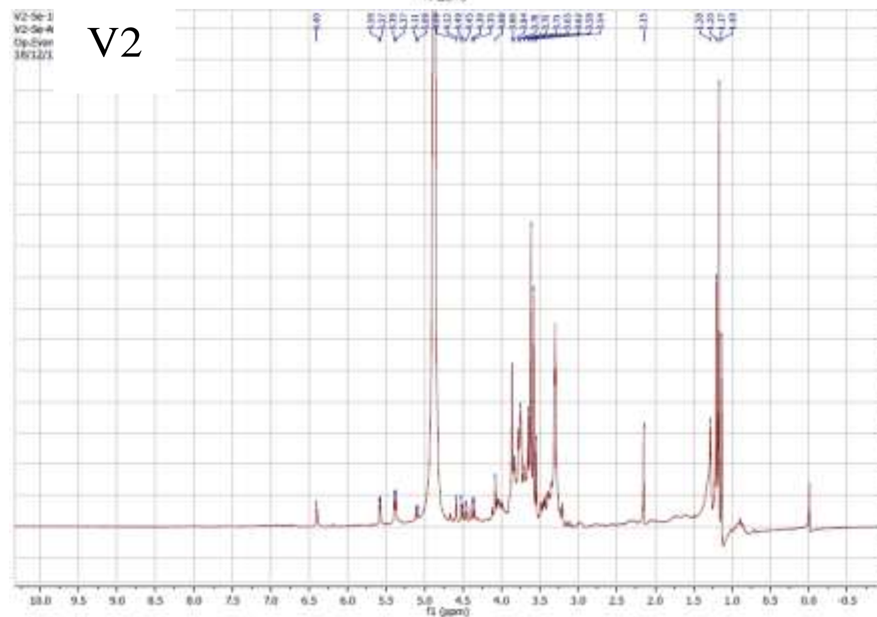
The analysis of the ^{1}H NMR spectra of cactus pear varieties by methods of multivariate analysis found genetic diversity among the materials in the dry and rainy seasons. Furthermore, there was a difference in identifying groups of similar subjects in the two seasons (Tables 4 and 5, Figures 3 to 6). 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

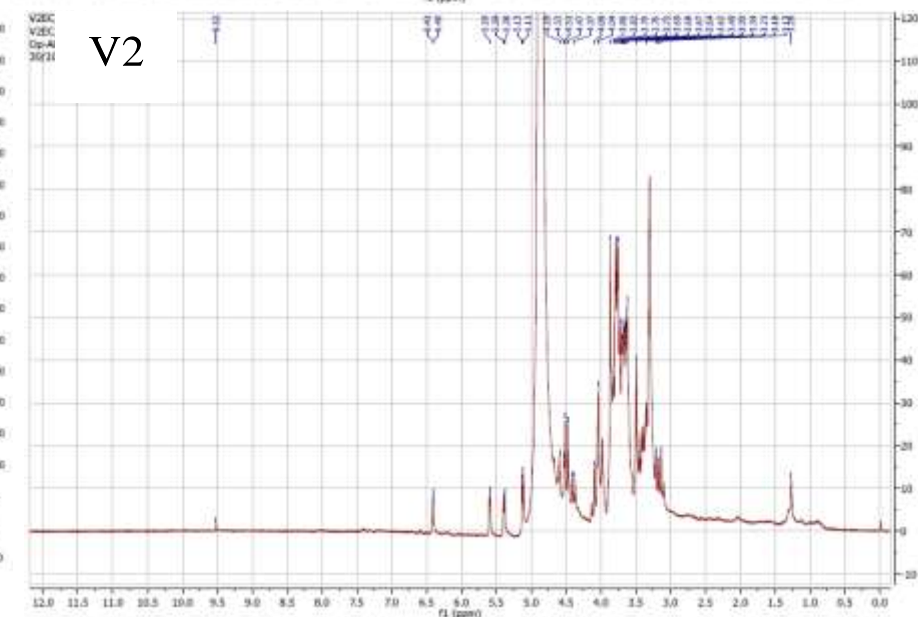
Dry season

Rainy season









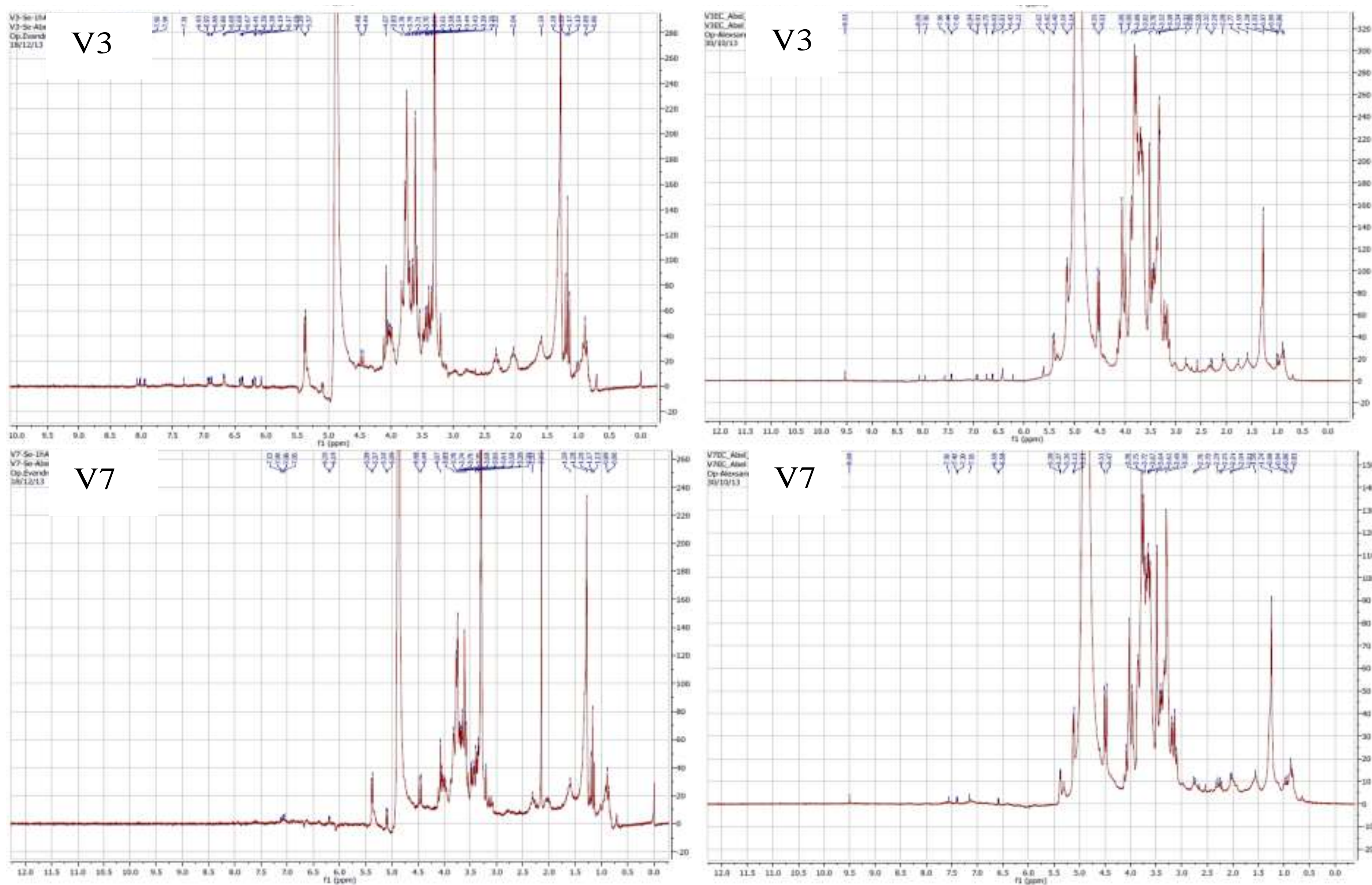
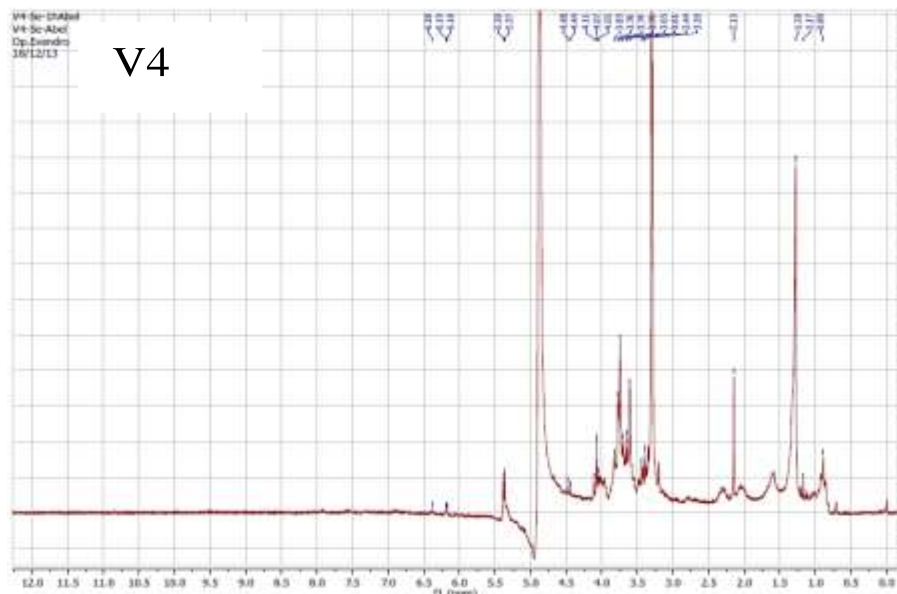
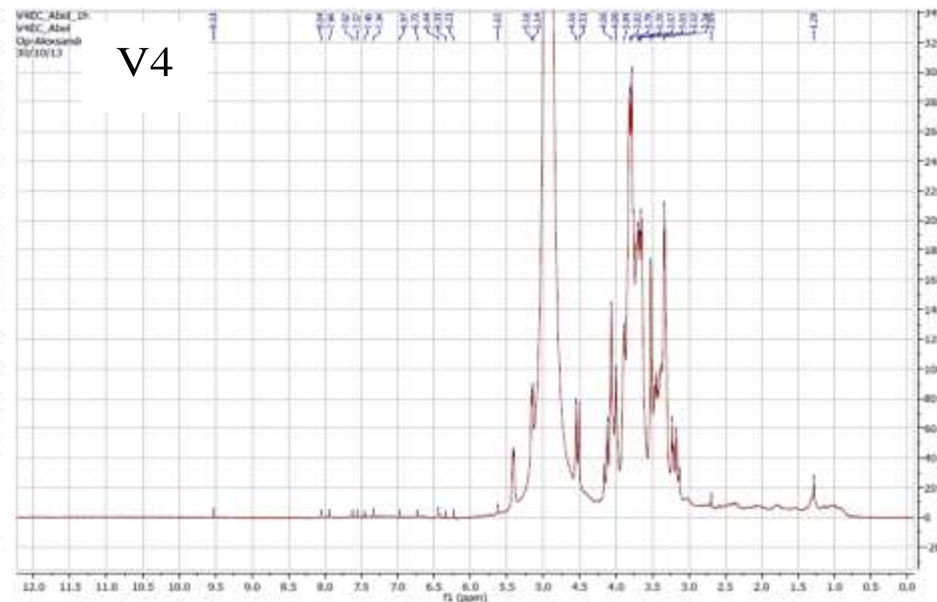


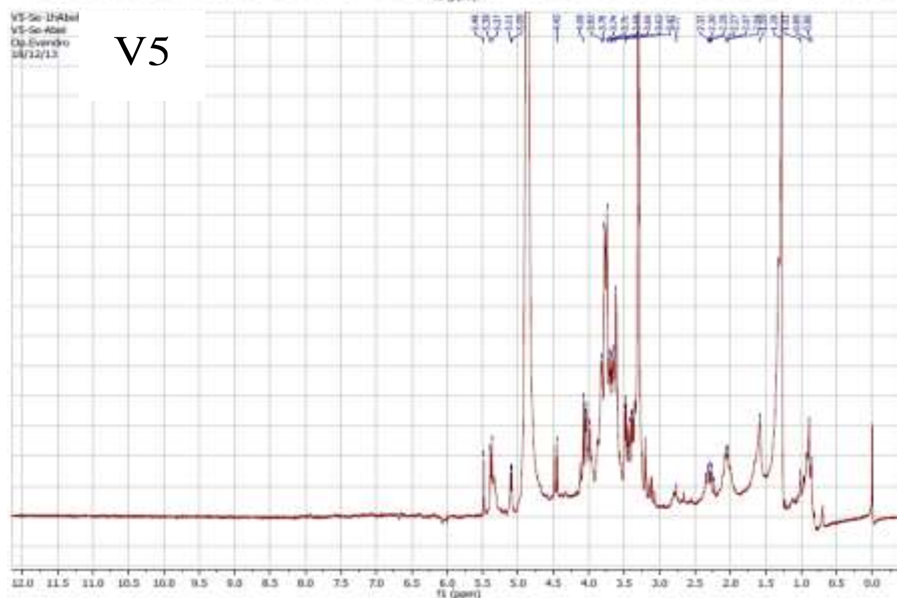
Figure 1. ^{1}H NMR spectrum of crude ethanol extract of varieties of cactus pear (*Opuntia*), collected in the dry and rainy seasons. (V1) IPA-100003 (*O. ficus indica*), (V2) IPA-200016 (*O. stricta*), (V3) IPA-200008 (*O. atropes*) and (V7) IPA-200149 (*O. larreri*)

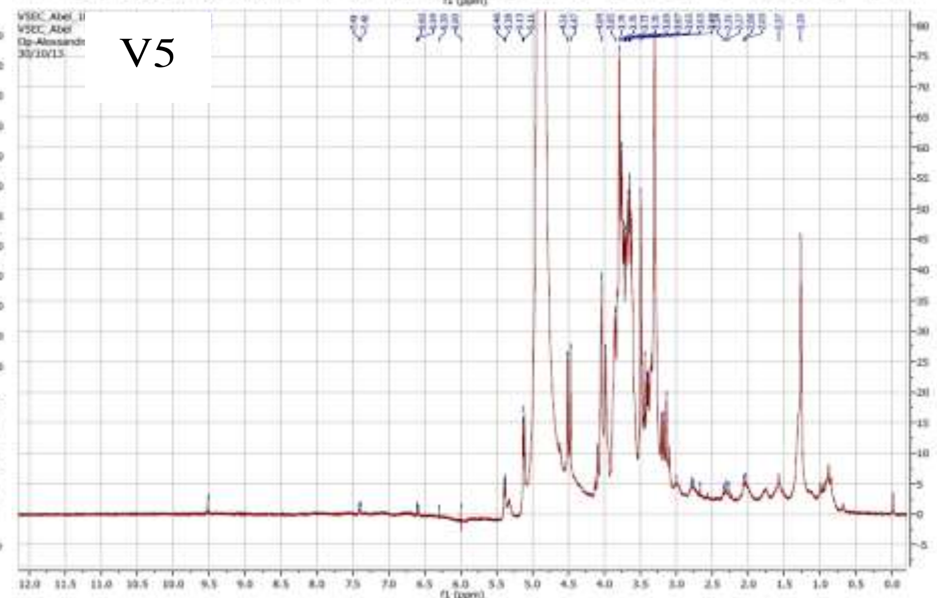
Dry season

Rainy season









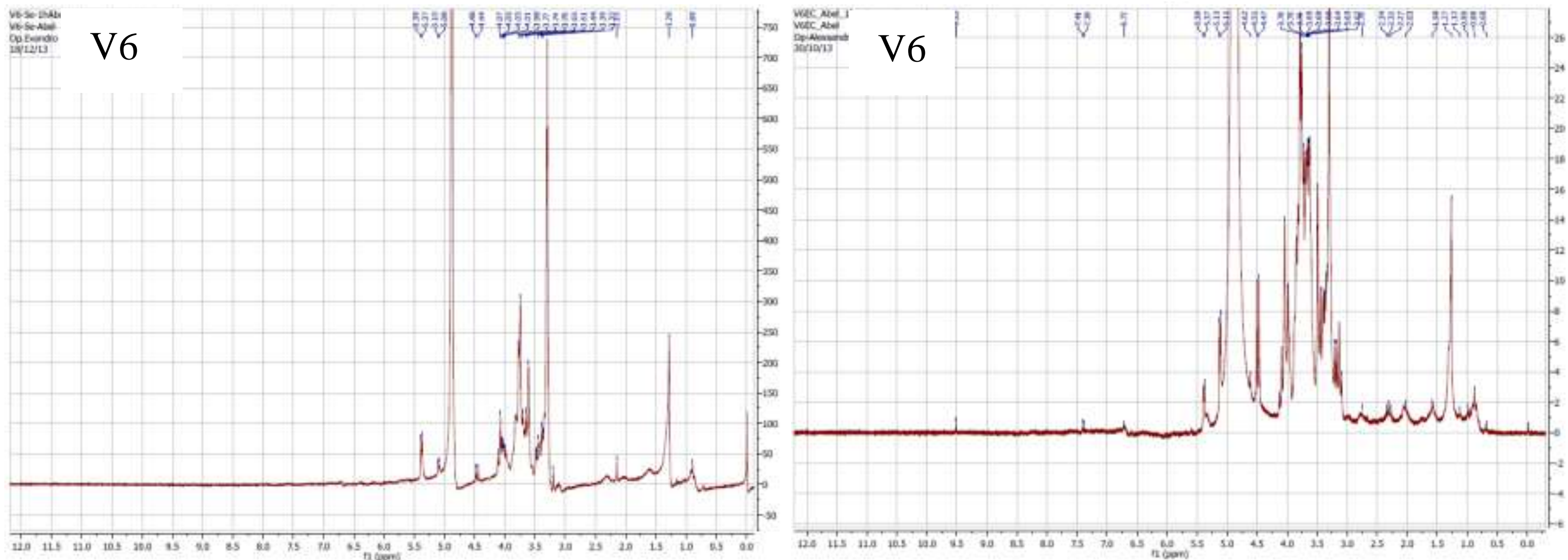


Figure 2. ^{1}H NMR spectrum of crude ethanol extract of varieties of cactus pear (*Nopalea*), collected in the dry and rainy seasons. (V4) IPA-100004 (*N. cochenillifera*), (V5) IPA-200021 (*N. cochenillifera*) and (V6) IPA-200205 (*N. cochenillifera*).

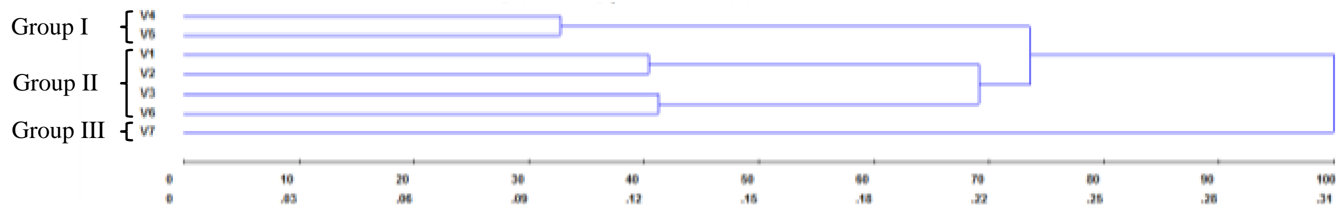


Figure 3. Representative dendrogram grouping by UPGMA seven varieties of cactus pear of *Opuntia* and *Nopalea* genres grown in the semiarid region of Pernambuco, based on the ^{1}H NMR spectrum in the dry season.

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

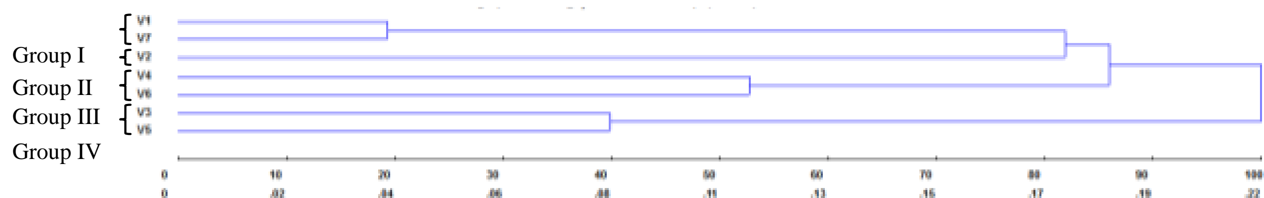


Figure 4. Representative dendrogram grouping by UPGMA seven varieties of cactus pear of *Opuntia* and *Nopalea* genres grown in the semiarid region of Pernambuco, based on the ^{1}H NMR spectrum in the rainy season

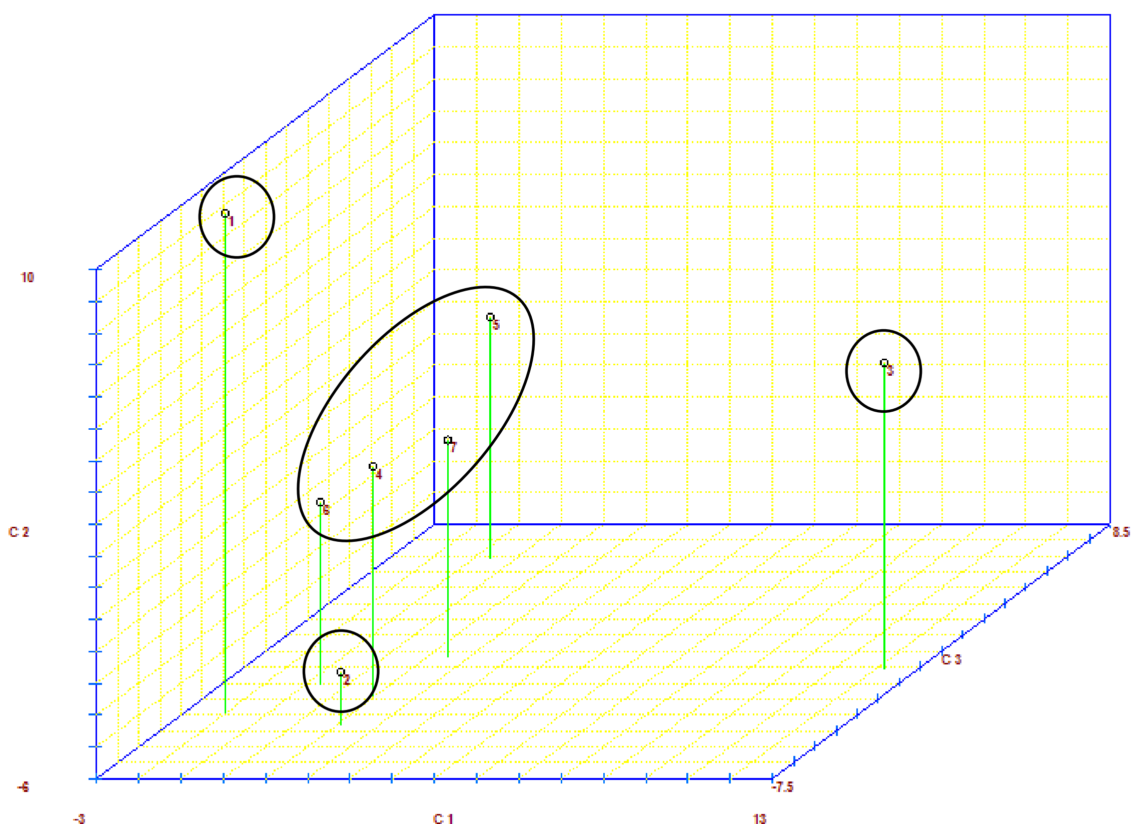


Figure 5. Graphical dispersion of the seven varieties of cactus pear *Opuntia* and *Nopalea* genres, from the first, second and third main component (C1, C2, C3) based on the ^{1}H NMR spectrum of the dry season

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

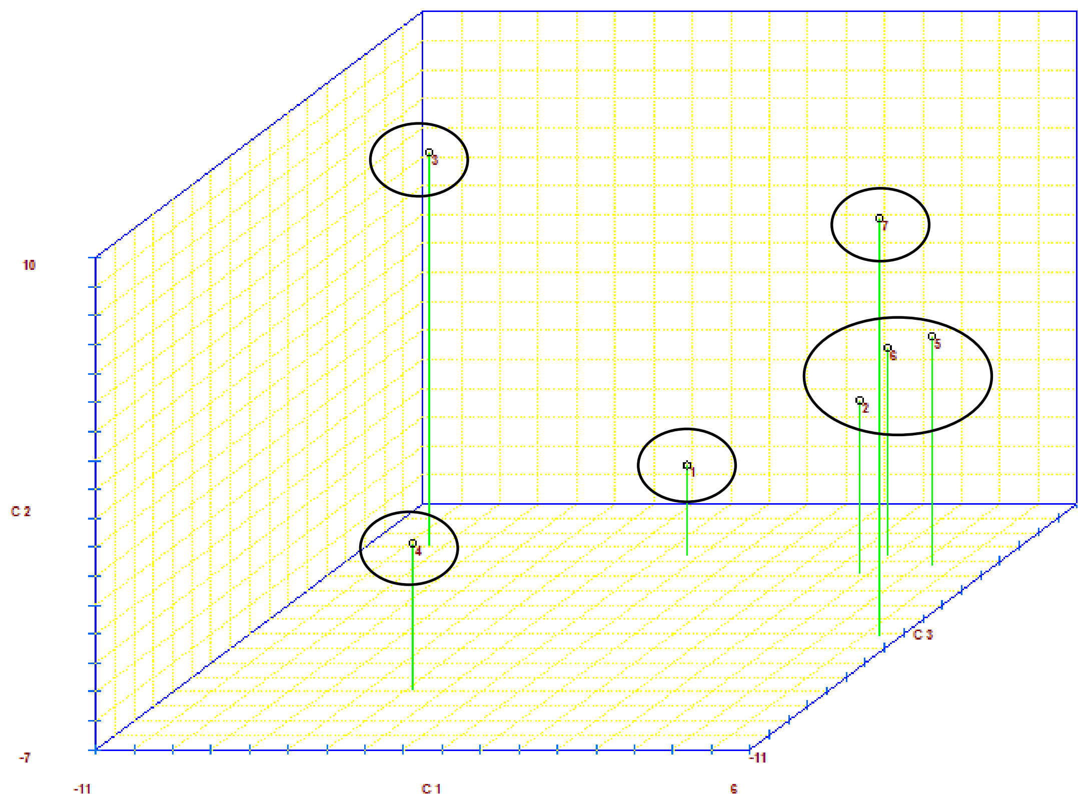


Figure 6. Graphical dispersion of the seven varieties of cactus pear *Opuntia* and *Nopalea* genres, from the first, second and third main component (C1, C2, C3) based on the ^{1}H NMR spectrum of the rainy season

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

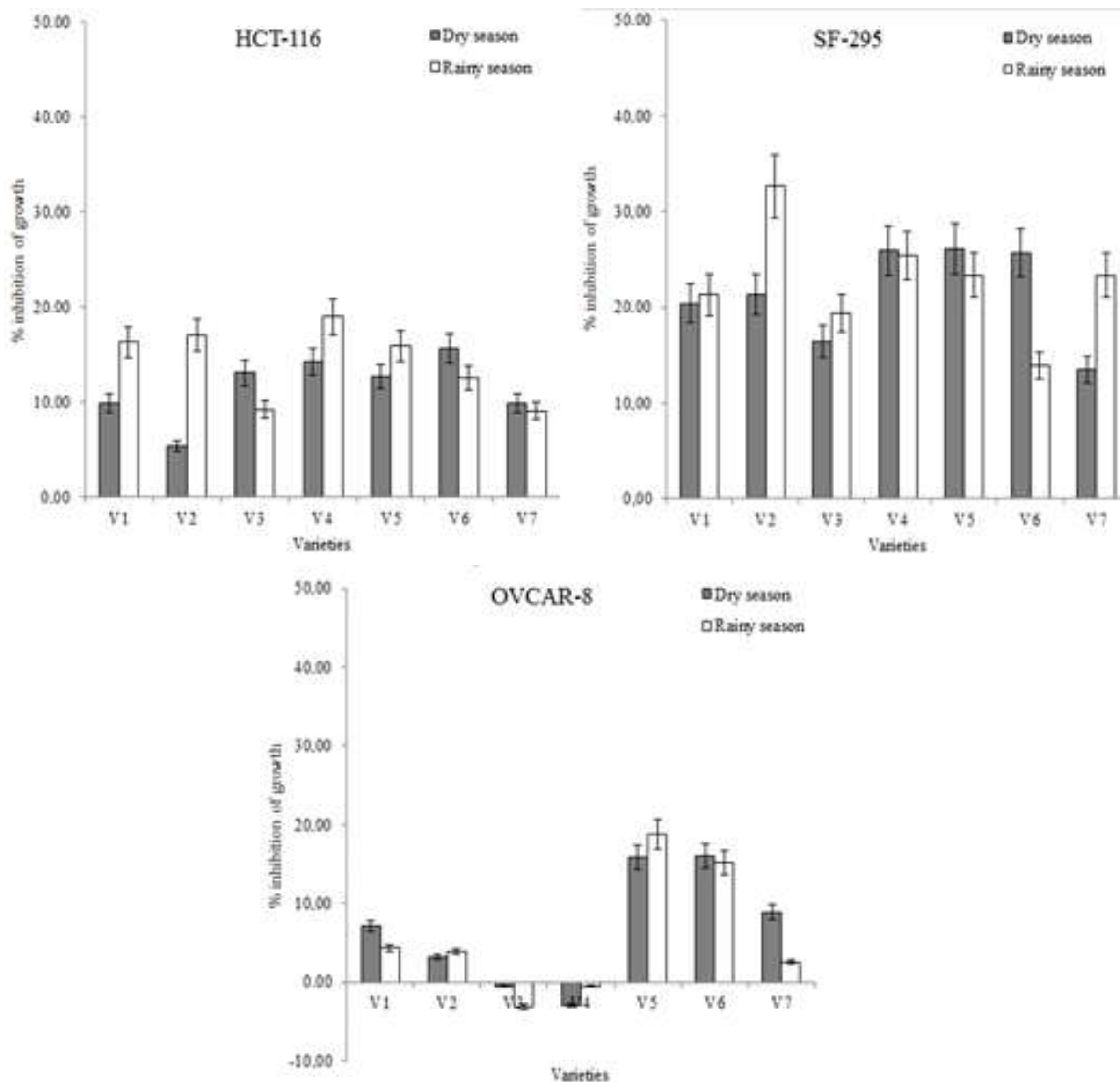


Figure 7. Cytotoxic activity against human cancer cells (a) HCT-116 (human colorectal), (b) SF-295 (human glioblastoma) and (c) OVCAR-8 (human ovary) of crude ethanol extract of cactus pear varieties (*Opuntia* and *Nopalea*), collected in the dry and rainy season. (V1) IPA-100003 (*O. ficus indica*), (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.

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