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Bioactive compounds and antioxidant capacity of fruit on *Sechium edule*

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ABSTRACT

Vegetables and fruits contain bioactive compounds with antioxidant activity such as phenols and flavonoids, carotenoids, ascorbate, alimentary fiber, selenium and many substances with antiatherogenics and anticarcinogenics properties. The purpose of this work is to assess some bioactive constituents and the antioxidant activity of the *Sechium edule* (Jacq) Swartz (Cucurbitaceae) fruit. Total phenol content was higher ($p < 0,001$) in pulp (*Sep*):124.83mg/100g than seed (*Ses*): 86.36mg/100g. The antioxidant activity were estimated by: nitric oxide scavenging activity (NO), DPPH radical scavenging activity (DPPH) and β-carotene bleaching method (β-carotene). The values expressed as percentage (%) corresponds to *Ses* and *Sep* respectively: NO 47.51 ± 1.9 and 55.62 ± 1.3; DPPH: 88.87 ± 1.1 and 89.13 ± 1.5 and β-carotene, 63.32 ± 1.5 and 53.15 ± 1.4. The studied samples have important radical scavenging activity and inhibited lipid peroxidation *in vitro*. We concluded that the present study provides information that *Sechium edule* can be a source of natural antioxidants.

KEYWORDS

Sechium edule
(Cucurbitaceae);
Total phenols;
Vitamins;
Minerals;
Antioxidant capacity.

INTRODUCTION

The concept of food and nutrition is currently changing. In fact, besides their nutritional and sensory properties, the role of certain foods as health protective agents is being recognized[1]. Therefore, not only is intake of nutrients such as lipids, sugars, proteins and mineral compounds being recommended; it is also becoming increasingly evident that certain foods may contain a number of compounds with a health protective action.

Recent epidemiological studies have indicated that high consumption of fruits and vegetables is related with a reduced risk of certain chronic disorders such as coronary disease and some forms of cancer. This is attributed to the fact that these foods provide an optimum combination of natural antioxidants such as polyphenols, flavonoids and alimentary fiber[2] and antioxidant micronutrients: vitamin A, carotenoids, vitamin C, vitamin E, selenium, zinc, copper, iron and manganese. These components collaborate with defense system against free radicals and exert their protective activity through of different mechanisms. A large number of beneficial components are currently being reported for fruit and vegetable foods, and their consumption is being valued and recommended[3].

The important role played by these foods enhances the human antioxidant system because the non enzymatic compounds of vegetables can be used as such by animal cells. The results of the "Lyon Diet Heart" study

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TABLE 1 : Contents of total phenol, total flavonoids and total anthocyanins methanolic extracts of S. edule

Sample	Total phenols[1]	Flavonoids[2]	Anthocyanins[3]
Ses	86.36 ± 1.55	62.38 ± 2.60	93.82 ± 1.30
Sep	124.83 ± 1.23	85.83 ± 2.13	119.30 ± 1.70
Sefc	138.52 ± 1.20	95.52 ± 1.92	137.19 ± 1.52
One-way Analysis of Variance (ANOVA)			
Ses vs Sep	P< 0.001	P< 0.001	P< 0.001
Ses vs Sefc	P< 0.001	P< 0.001	P< 0.001
Sep vs Sefc	P< 0.001	P< 0.01	P< 0.001

Values are mean ± SD (Standard Deviation) of three measurements. [1]expressed as mg/100g DW of gallic acid equivalent. [2]expressed as mg/100g DW of catechin equivalent. [3]expressed as mg/100g DW in cyanidin-3-glucoside

TABLE 2 : Content of microelements in flour of S. edule (ppm)

Sample	Zinc	Selenium	Copper
Ses	86.17 ± 2.34	1.02 ± 0.01	4.83 ± 0.32
Sep	37.47 ± 0.92	< 0.5	16.60 ± 0.93
Sefc	94.70 ± 1.83	< 0.5	6.30 ± 0.45
One-way Analysis of Variance (ANOVA)			
Ses vs Sep	P<0.001	---------	P<0.001
Ses vs Sefc	P<0.001	---------	NS
Sep vs Sefc	P<0.001	---------	P<0.001

Values are mean ± SD (Standard Deviation) of three measurements. NS: No significative

indicated that a vegetable based diet reduced the rate of recurrence after a first episode of myocardial infarction. These authors claim that the antioxidant properties of vegetable foods could be the mediators of the beneficial effects of this diet.

Sechium edule is a crop of Meso American origin, where the widest genetic diversity is found. It has been cultivated since pre-Columbian times in Mexico, where its edible fruit is popularly known as "chayote", but its rustic cultivation is practiced in many regions of the world, constituting an accessible source of vegetable food for low-income people[4]. In Argentina, the fruit is popularly known as "papa del aire".

The medical properties of the leaves, fruit and seeds of *Sechium edule* as a diuretic, antihypertensive, cardiovascular and anti-inflammatory have been confirmed by pharmacological studies[5].

Previously obtained data in our laboratory on the chemical composition and nutritional value of S. edule[6] are very similar to those reported by other researchers[7-9]. However, there is insufficient information on its antioxidant capacity. Therefore, the purpose of this work was to assess some bioactive constituents and the antioxidant activity of the Sechium edule (Jacq) Swartz (Cucurbitaceae) fruit.

MATERIALS AND METHODS

Chemicals

Folin Ciocalteu reagent, gallic acid (Anedra, Buenos Aires, Argentina), Griess reagent (Britania, Argentina), buthylated hydroxy toluene (BHT) (Merck, USA), catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene and linoleic acid (Sigma, Aldrich, Argentina). Unless stated otherwise, all chemicals were from Sigma.

Plant materials

S. edule (Jacq) Swartz (Cucurbitaceae) fruits (*Se*) from an experimental crop carried out in San Luis, Argentina were used for this work. The mature spiny fruits were collected in May 2005 and fruits with an average weight of 500 g were selected.

Flour preparation

Removed the thorn and separate the different parts: pulp (*Sep*) and seed (*Ses*) were dried in forced air oven at 40°C-45°C for 72 hours, the product was subjected to dry grinding in electric grinder (CG-8 Stylo, 220 V-50 Hz 90 W, China) and sifted through sieve with 200μm nylon. In the same way it worked with the entire fruit (*Seef*). The flour was stored in sealed airtight container away from light and stored at 4°C.

Analyses were performed by triplicate and the mean value was expressed for dry matter.

Extraction of total phenols

The flours from different parts of *Se* were defatted. Lipid extraction was performed by refluxing the samples in hexane in a Soxhlet apparatus for 10 hours. The hexane was evaporated and then the samples were stored at 5°C. The extraction of total phenols was done from 50mg of defatted sample with 5ml of 1.2 mol/l HCl in 50% methanol: water. The sample was heated at 90°C for 3 h with vortexing every 30 min. Then the sample was cooled and diluted to 10ml with methanol and centrifuged for 5 min at 5000g. The supernatant was used for determination of phenols, flavonoids, anthocyanins

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TABLE 3 : Content of β-carotene and ascorbic acid of S. edule

Sample	β-carotene (mg/kg)	Acido ascórbico (mg/kg)
Ses	Nd*	123.24 ± 2.76
Sep	67.80 ± 1.56	342.76 ± 3.53[c]

Values are mean ± SD (Standard Deviation) of three measurements. Ses Sechium edule seed. Sep Sechium edule pulp. * LD (Límite de detección) = 10mg/kg. Means in columns with superscript letters differ significantly: [c] P< 0.001 using Student's test

and antioxidant activity[10].

Total phenols

The determination of total phenols was measured at 750 nm using Folin Ciocalteu reagent with gallic acid as a standard and was expressed as mg/100g of dry weight (DW) of gallic acid equivalent. Aliquots 0.5ml of standard, distilled water (blank) and methanolic extract were added to flasks containing 4.5ml of distilled water, later they were mixed with 0.5ml of the Folin-Ciocalteu reagent and 5ml of 7% sodium carbonate. The totals were made up to 12.5ml with distilled water. The mixture was allowed to stand for 90 min at room temperature before measuring the absorbance at 750nm (UV-Vis Beckman DK-2[a])[11].

Flavonoids

Total flavonoids content was determined by a colorimetric method. Al_3Cl was used as agent complex. First, 0.25ml of the sample extracts were diluted with 1.25ml of distilled water. Then 75µl of a 5% $NaNO_2$ solution was added to the mixture. After 6 min, 150µl of a 10% $AlCl_3x6H_2O$ solution was added, and the mixture was allowed to stand for 5 min. A 0.5ml quantity of 1 mol/l OHNa was added, and the total was made up to 2.5ml with distilled water. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm using a spectrophotometer (UV- Vis Beckman DK-2[a]) in comparison with the standards prepared similarly with known catechin concentrations.

The result was expressed as mg/100g of DW of catechin equivalent[12].

Anthocyanins

Anthocyanins were estimated by a pH differential method[13]. Anthocyanins are natural pigments which undergo from pH 1 to pH 4.5 reversible structural transformation. At pH 1.0, anthocyanins exist as colored oxonium or flavilium and at pH 4.5 the carbinol form, the one with less color, prevails. An aliquot for an aqueous anthocyanin solution is adjusted at pH 1.0 and another aliquot at pH 4.5. Difference in absorbance at 510nm is proportional to the anthocyanin content. The measuring at 700nm includes interfering substances and degraded anthocyaninic derivates. Two buffers systems are used: pH 1 chloride acid/potassium chloride and pH 4.5 acetic acid/ sodium acetate. The first buffer system is prepared with KCl 0.025 M and it is adjusted at pH 1.0 with HCl; the second buffer system consists in an acetic acid/sodium acetate 0.4 M solution. Methanolic extract (1mL) from the sample was added with 2mL of the buffer 1, at another aliquot the sample is added with 2mL of the buffer 2. Absorbance was measured in spectrophotometer (UV-Vis Beckman DK-2[a]) at 510nm and at 700nm in buffers at pH 1.0 and 4.5, using:

$$\mathbf{A = [(A_{510}–A_{700})\ pH\ 1.0 - (A_{510}–A_{700})\ pH\ 4.5]}$$

Monomeric pigment concentration in the extract is expressed in cyanidin-3-glucoside.

$$\mathbf{Monomeric\ Anthocyanin\ (mg/100g) = (A \times MW \times DF)/E}$$

A = Absorbance
MW = Molecular weight: 449.2
DF = Dilution Factor
E = Molar extinction coefficient 26,900
Results were expressed as mg/100 g DW.

Fiber

Total soluble and insoluble fiber was determined by the gravimetric enzymatic method proposed by Prosky[14].

Minerals

The samples of crude drug of each species were dried in a heater of forced air at 40°C until reach to the stage of hygroscopic moisture. Later were milled with a Wiley mill series 3379 with a stainless steel container, and a sieve up to 0.50 mm diameter; for each sample was followed this procedure: 0.5g of plant material was put in a porcelain crucible, loosely covered with a lid, and carbonized during 1 h by gentle ignition at 500 C; after cooled, was added 15ml of HCl, 10ml of HNO_3, and 5ml of $HClO_4$; leading up to a final volume of 50ml,

TABLE 4 : Content of soluble, insoluble and total dietary fiber of S. edule

Sample	Soluble dietary fiber	Insoluble dietary fiber	Total dietary fiber
Ses	1.93 ± 0.18	7.21 ± 0.11	9.15 ± 0.21
Sep	2.35 ± 0.36	33.62 ± 3.70	35.97 ± 3.74
Sefc	6.06 ± 0.08	19.41 ± 0.50	25.48 ± 0.49
One-way Analysis of Variance (ANOVA)			
Ses vs Sep	NS	P<0.001	P<0.001
Ses vs Sefc	P<0.001	P<0.001	P<0.001
Sep vs Sefc	P<0.001	P<0.001	P<0.01

Values are mean ± SD (Standard Deviation) of three measurements. NS: No significative

TABLE 5 : Measures of the antioxidant activity of methanol extract of S. edule

Sample	% Inhibición NO	% Inhibición DPPH	% Inhibitión β-carotene
Ses	47.51 ± 1.9	88.87 ± 1.1	63.32 ± 1.5
Sep	55.62 ± 1.3	89.13 ± 1.5	53.15 ± 1.4
Sefc	50.01 ± 1.7	90.42 ± 1.9	33.85 ± 1.3
One-way Analysis of Variance (ANOVA)			
Ses vs Sep	P<0.01	NS	P<0.001
Ses vs Sefc	NS	NS	P<0.001
Sep vs Sefc	P<0.05	NS	P<0.001

Values are mean ± SD (Standard Deviation) of three measurements. NS: No significative

and shaking strongly.

The concentrations of 27 selected minerals were determined using an ICP-OES spectrometer (Varian Vista-PRO™, SL.ICP.04, radial type, solid state detector, 167-785nm, software version v3.1b 394, firmware version 2.15). The wavelength calibration was periodic and automatic (based on Argon and emission lines). Within these minerals zinc, copper and selenium interest for this study.

Vitamins

Were determined vitamins A (ascorbic acid) and C (β-carotene) following the techniques established by AOAC[15].

Scavenging activity against nitric oxide (NO test)

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction[16,17]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be estimated by use of Griess reagent with which nitrite reacts to give a stable product absorbing at 542nm. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite. Sodium nitroprusside solution was prepared immediately before the experiment, dissolving 10 mmol/L sodium nitroprusside in 0.02 mol/l phosphate buffer, pH 7.4, previously bubbled with argon. The samples were diluted in 0.02 mol/l phosphate buffer, pH 7.4, to obtain optimal concentrations. At the beginning of the experiment, a 1mL quantity of the samples (2.5mg/ml) was diluted with 1 ml of sodium nitroprusside solution and incubated at room temperature for 150 min. At the end of the incubation, 2ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylehylenediamine dihydrochloride) was added to each sample, and the absorbance was read at 542 nm (UV-Vis Beckman DK-2[a]). Blank without test extract but equivalent amount of methanol was conducted in an identical manner. The nitrite concentration was calculated by referring to the absorbance of standard solutions of sodium nitrite.

Results were expressed as percentage (%) of nitrite production with respect to blank.

$$\% \text{ Nitrite Production} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

A = Absorbance

DPPH free radical-scavenging assay

This method relates the sample capacity to inhibit the action of free radicals generated by DPPH, in a highly polar environment, in the absence of an oxidable lipid. The hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple-colored MeOH solution of DPPH. This spectrophotometric assay uses stable radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) as a reagent[18]. Various concentrations of the extract (2.5mg/ml) in MeOH (50µl) were added to 5 ml of a 0.004% MeOH solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517nm (UV-Vis Beckman DK-2[a]). Blank contain all reagents except the test compound. The synthetic antioxidant buthylated hydroxy toluene (BHT) was included in experiments as a positive control (1.6µg/ml).

The percentage (%) of DPPH scavenging was calculated using the following equation:

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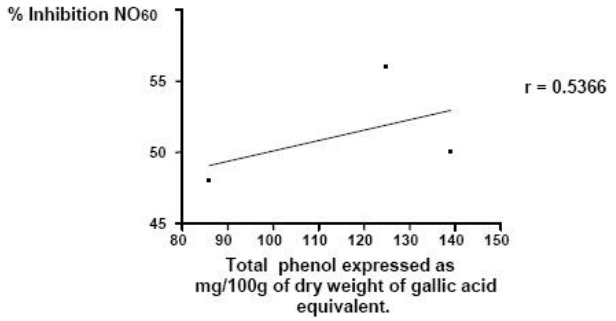

Figure 1 : Correlation between total phenols content and NO assay. Values are mean of three determinations ± SD

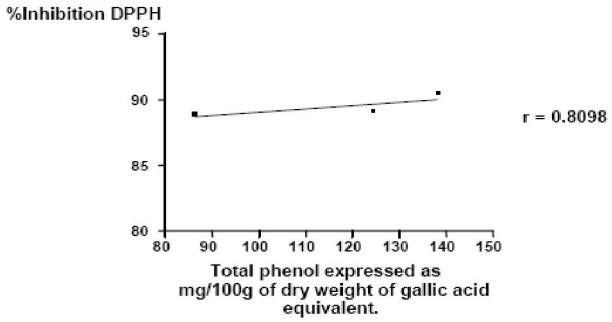

Figure 2 : Correlation between total phenolics content and DPPH assay. Values are mean of three determinations ± SD

% DPPH Scavenging = $(A_{blank} - A_{sample}/A_{blank})\times100$

A = Absorbance

β-carotene-linoleic acid assay

This technique involves measuring β-carotene bleaching at 470nm resulting from β-carotene oxidation by linoleic acid degradation products. Tween is used for dispersion of linoleic acid and β-carotene in[19].

β-carotene (0.2mg) were dissolved in 0.2ml of chloroform and were added to round-bottom flasks (50ml) containing 0.02ml of linoleic acid a and 0.2ml of Tween 20. Chloroform was completely evaporated using a vacuum evaporator. Then 50ml distilled water was added with a vigorous shaking (40 min). This reaction mixture (3ml) was dispersed to test tubes that contain a 200µl portion of the sample extracts (5mg/ml). The same procedure was repeated with synthetic antioxidant BHT (100µg/ml) as positive control and methanol as negative control. The mixture was shaken for 2 min and then the tubes were placed at 50 C in a water bath, and the absorbance at 470nm was taken at zero time (t = 0). Measurement of absorbance was continued until the color of β-carotene disappeared in the control tubes (t = 60 min) at an interval of 15 minutes. A mixture prepared as above without β-carotene served as blank. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β-carotene and was expressed as percentage (%) and calculated for the following equation:

% Bleaching β-carotene = $100\times[1 - (A_0 - A_t / A_{00} - A_{0t})]$

A_0 = Initial Absorbance at 470nm of the test sample at time 0

A_t = Absorbance of the tested plant extract at time t

A_{00} = Absorbance at beginning of the incubation without extract

A_{0t} = Absorbance at time t without extract

Statistical analysis

Results are expressed as mean ± standard deviations. Statistical differences were tested by One-way Analysis of Variance (ANOVA) or Student's test .A probability of 0.05 or less indicated significant difference[20].

RESULTS AND DISCUSSION

Reactive oxygen species participate in the etiology and physiology of human disorders such as neurodegenerative conditions, inflammation, viral infections, digestive disorders and gastric ulcer. In order to understand the protective effect of natural compounds, studies of antioxidant components must be conducted, followed by an assessment of their antioxidant function. In the present study we evaluated bioactive components such as total phenols, flavonoids, anthocyanins, some minerals, vitamins and fibers, and their antioxidant activity was assessed *in vitro*.

Flavonoids and most polyphenols contribute to the reduction of oxidant agents in the organism, due to their free radical scavenging capacity. Scavenging occurs by the general reaction of losing a hydrogen atom and becoming a low-reactivity radical, or by forming chelates with iron ions, which are well known catalysts of Fenton´s reaction which leads to the formation of hydroxyl radicals and the consequent inactivation of new reactive species[21].

The three analyzed samples showed significantly different contents ($P<0.001$) of total phenols, expressed as mg/100g DW of gallic acid equivalent, with the value found in *Sep* being higher than in *Ses* (124.83 ± 1.23;

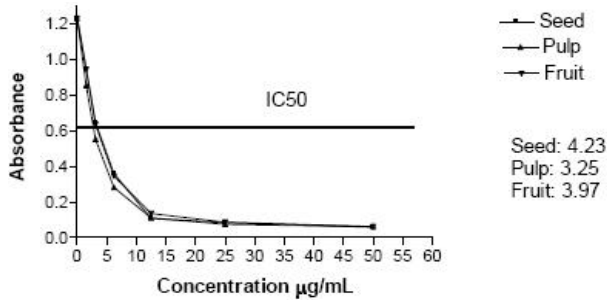

Figure 3 : DPPH radical scavenging activity of S. edule extracts. The samples were used for the assay at the final concentration between 0.25 and 5 mg ml^{-1}

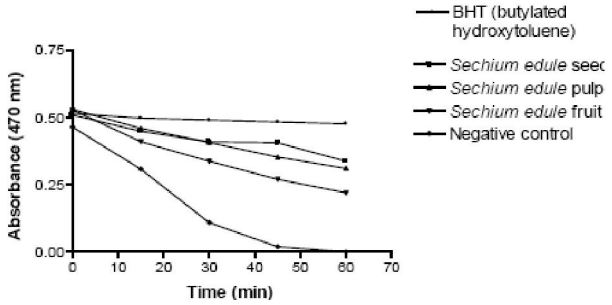

Figure 4 : Kinetic reaction antioxidant activity of methanolic extract using β-carotene-linoleate model system

86.36 ± 1.55, respectively) and coincident with the higher content of flavonoids, expressed as mg/100g DW of catechin equivalent, in *Sep* (85.83 ± 2.13) as compared to *Ses (*62.38 ± 2.60), see TABLE 1. These values are comparable to those reported by Ordoñez et al. (2006)[5].

Anthocyanines have high potential for use as natural colorants due to their favorable colors. At present, they are becoming increasingly important as antioxidants[22,23] and these have been reported to help reduce the risk of coronary heart disease and prevent several chronic diseases[24].

In this study, the content of anthocyanins was significantly different among the three analyzed samples (P<0.001), with a higher percentage in *Sep* (21.35 %) as compared to the *Ses* (TABLE 1).

The analysis of the whole fruit showed a total value of the studied bioactive substances that was lower than the sum of the concentrations obtained in pulp and seed. This might be explained by the fact that the concentrations of these compounds depend on their microenvironment, since they interact with each other, which may lead to synergic or inhibitory effects[25].

Zinc and copper are important antioxidant oligoelements, since they are part of various antioxidant enzymatic systems, such as superoxide dismutase, which constitute the first line of defense against free radicals[26]. Selenium is an essential component of the glutathione antioxidant enzymatic system, and as such it plays a major role in the cell antioxidant defense system[27].

The content of these micronutrients is shown in TABLE 2. As can be seen, the zinc content is higher in *Ses* than in *Sep* (86.17 ± 2.34ppm and 37.47 ± 0.92ppm, respectively). The lower Zn content in *Sep* might be explained by its high humidity content (91.17%). The Zn intake of 8-11mg/day suggested by the Recommended Dietary Allowance (RDA) would be met by ingestion of 250g of *Sep*; and the RDA suggested value for Cu (0.9 mg/day) would be met with ingestion of only 50g of *Sep*. The selenium values obtained for *Sep* and *Seef* are in agreement with the typical values for vegetables, lower to 0.1ppm[28].

Ascorbic acid or vitamin C is one of the main hydrosoluble antioxidants and it is therefore capable of detoxifying the reactive species present in the plasma and tissues. Vitamin C enhances the activity of vitamin E in cells by regenerating α-tocopherol from its oxidized derivative[29]. It has also been suggested that vitamin C preserves glutathione in vivo[30]. As a result, vitamin C can contribute to optimizing the antioxidant capacity of tissues, which probably constitutes its most important feature at physiological level. TABLE 3 shows the vitamin content in the *S. edule* fruit. The levels in *Sep* are good, considering that kiwi, one of the vitamin C richest fruits, contains 500.00mg/Kg. These content cover a large percentage of the daily requirement of vitamin C because the recommended intake values vary between 39 and 75mg/day, according to age, sex and physiological state.

Some authors have established a correlation between dietary fiber content and antioxidant activity, which has been confirmed by J.A. Larrauri et al.[31] in mango and lemon skin fiber. Considering that the recommended intake of total fiber is 20-30g per day, with 30% of soluble fiber and 70% of insoluble fiber, 100g of *Sep* may provide the daily requirement of fiber, although not the necessary percentage of soluble dietary fiber. The physiological benefits of high content of insoluble dietary fiber are related to the increase of intestinal motility and consequent reduction of colon cancer risk

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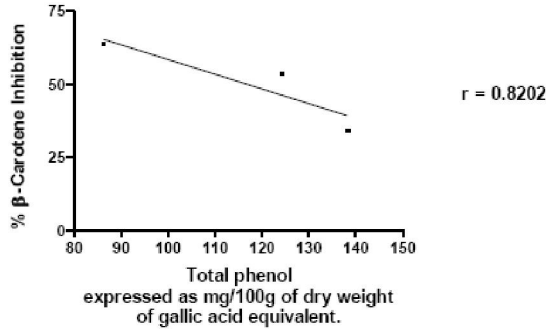

Figure 5 : Correlation between total phenolics content and β carotene assay. Values are mean of three determinations ± SD

(TABLE 4).

Antioxidant activity is a complex process that involves several different mechanisms. For this reason, it was assessed by three different methods (TABLE 5).

The methanol extracts studied presents moderate activity of NO scavenging. *Sep* exhibited the highest inhibition percentage (55.62 ± 1.3). In this case, a moderate correlation with total phenols was obtained (r = 0.54) (Figure 1).

DPPH radical scavenging activity measures the capacity to absorb reactive species by antioxidants and constitutes one of the most widely used methods due to its simplicity and efficiency. In the three studied samples, the inhibition varied between 88.87 % and 90.42 % (TABLE 5), which can be considered as high radical scavenging capacity. The values obtained by DPPH are in correlation with the total phenols in the sample (r = 0.81) (Figure 2).

The IC_{50} values were obtained from the three samples. *Sep* presents the highest inhibition of the DPPH radical (IC50 = 3.25μg /ml) followed by the *Seef* and the *Ses*, with 3.97μg/ml and 4.23μg/ml, respectively (Figure 3).

The β-carotene bleaching assay determines the inhibition effect on lipid oxidation. Figure 4 shows the kinetic reaction of the three extracts compared with the activity of BHT, a well known antioxidant, at different incubation times. The *Ses* extract exhibited the highest activity.

The highest % bleaching β-carotene was obtained by the *Ses* 63.32 ± 1.5, with 53.15 ± 1.4 and for *Sep* (P< 0.001) (TABLE 5). A good correlation was also observed between the values obtained by this method and total phenol values (r = 0.82) (Figure 5).

CONCLUSION

The present study provides information that *Sechium edule* can be a source of natural antioxidants and that consumption of its fruit may play a role in the prevention of disorders related with free radicals generation. The presence of polyphenols compounds and micronutrients such as vitamin A and C, selenium, zinc and copper also play a role in protection by means of various mechanisms. Besides, these findings demonstrated that *Se* has good correlation between DPPH values and total phenols indicate that these may be responsible for antioxidant activity in vitro.

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