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Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts

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Abstract

The antioxidant activities (AA) of *Sechium edule* extracts were tested by three established in vitro methods, namely reducing power, β-carotene linoleate model and 1,1- diphenyl-2 picrylhydrazyl (DPPH) radical-scavenging. Leaf ethanolic extracts and leaf and seed water extracts showed strong inhibitory activity by β-carotene bleaching (AA values of 90%). Furthermore, these extracts exerted hydrogen-donating ability in the presence of DDPH stable radical (IC_{50} 2 μg/ml). These extracts also showed strong reducing power by the potassium ferricyanide reduction method. Leaf and seed extracts may be exploited as biopreservatives in food applications as well as for health supplements or functional food, to aleviate oxidative stress.

Keywords: *Sechium edule*; Antioxidant capacity

1. Introduction

High levels of free radicals or active oxygen species create oxidative stress, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death (Ames, 1998). Epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant phychemicals (notably flavonoids and other polyphenols) is advantageous for our health (Cao, Sofic, & Prior, 1996; Di Carlo, Mascolo, Izzo, & Capasso, 1999; Pulido, Bravo, & Saura-Calixto, 2000), since they can protect the human body from free radicals and retard the progress of many chronic diseases. Moreover, many biological functions, such as antimutagenicity, anticarcinogenicity and antiaging, among others, originate from this property (Cook & Samman, 1996; Ramos et al., 2003; Yen & Chen, 1995; Yen, Chen, & Peng, 2001). A number of synthetic antioxidant, such as 2-3-*ter*-butyl-4-methoxyphenol (i.e., BHA), 2,6-di-*ter*-butyl-4-methylphenol (i.e., BHT) and *ter*-butylhydroquinone (TBHQ) have been added to foodstuffs but, because of their toxicity, their use has been questioned (Valentaõ et al., 2002). For this reason, the development and isolation of natural antioxidants from plant species, especially edible plants, are in progress.

A by-product of *Sechium edule* (chayote), the bio-catalyzer alpha rho no 11, as it is called, is a health product produced by fermentation of *Carica papaya* Linn, *Penisetum pupureum* and *S. edule*, reported to be useful against neural lipid peroxidation, traumatic epilepsy and aging (Santiago, 1991).

Otherwise, chayote fruit and root, have been important elements in the diet of the people living in many parts of Latin America and other areas of the world. Tender leaves and stems of cultivated *S. edule* are used as daily vegetables (Booth, Bressani, & Johns, 1992). The edible parts of *S. edule* are relatively low in fibre,

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protein and vitamins compared with other vegetables. Nevertheless, they have a high caloric and carbohydrate content, especially in young stems, roots and seeds, and the micro and macronutrient content of the fruit is adequate. The fruit and especially the seeds are rich in several important amino acids.

The diuretic, antihypertensive, cardiovascular and antiinflammatory properties of the leaves, fruits and seeds of chayote have been confirmed by pharmacological studies (Bueno, Moura, & Fonseca, 1970; Losoya, 1980; Salama, Polo, Enrique, Contreras, & Maldonado, 1986; Gordon, Guppy, & Nelson, 2000; Ribeiro et al., 1988).

A protein isolated from seed aqueous extracts of *S. edule*, called Sechiumin, has recently been purified, characterized, cloned and expressed using *Escherichia coli* as the host. It is claimed to possess ribosomal inactivation properties and is a potential chemotherapeutic agent (Wu, Chow, & Lin, 1998). Moreover, antimutagenic activity was found in water extracts of *S. edule*. The heat-stable antimutagenic substances might be phenolic compounds (Yen et al., 2001). In a previous work, we reported the antibacterial activity of ethanolic extracts of *S. edule* (Ordóñez, Gómez, Cudmani, Vattuone, & Isla, 2003). Reports on the chemical constituents of *S. edule* have been difficult to locate (Flick, Brúñete, Aung, Ory, & Saint Angelo, 1978; Ordóñez et al., 2003).

The present work investigates the possible antioxidative effects of *S. edule* (leaves, stems and seeds) to use as a natural preservative in food or functional food.

2. Material and methods

2.1. General

All solvents used were of analytical grade and obtained from Merck and Sigma–Aldrich Canada Ltd.

2.2. Plant material

Phenology. Wild populations of *S. edule* flower from April to December and give fruit from September to January.

The plants were collected during September to March in San Miguel de Tucumán, Tucumán, Argentina.

Voucher specimens were deposited in the Herbarium of the Institute de Estudios Vegetales (IEV), Facultad de Bioquímica, Química y Farmacía, UNT (Tucumán, Argentina). The used parts were leaves, stems and seeds.

2.3. Preparation of S. edule extracts

2.3.1. Preparation methods

Lixiviated (LS), maceration (MS) and alcoholature (AS) were prepared according to the Farmacopea Argentina VII Edition (2003) (Ordóñez et al., 2003).

2.3.2. Lixiviation (fluid extract)

Ground air-dried plant material was lixiviated in an extractor with 80% ethanol for leaves and stems or 90% ethanol for seeds at room temperature for total extraction.

2.3.3. Maceration (tincture)

Ground air-dried plant material was macerated in ethanol (1 g dry tissue/5 ml 96% ethanol) for 7 days with shaking (40 cycles/min) at room temperature.

2.3.4. Alcoholature

Fresh plant material was cut in to small pieces and macerated in ethanol (1 g dry tissue/1 ml 96 ethanol) for 8 days with shaking (40 cycles/min) at room temperature.

2.3.5. Decoctions (DS)

Five grams of dried and powdered plant material were extracted in 100 ml of boiling distilled water for 20 min, then the extract was filtered through Whatman No. 1 filter paper after cooling and the volume made up to 100 ml. Prepared extracts were stored at 4 °C in the dark.

2.4. Determination of total phenolic compounds and flavonoid content in S. edule extracts

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The extract samples (0.5 ml of different dilutions) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent (Sigma–Aldrich) for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents.

Total flavonoids were estimated using the method of Woisky and Salatino (1998). To 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin from a calibration curve.

2.5. Determination of antioxidant activity

2.5.1. β-Carotene-linoleic acid assay

Antioxidant activities of *S. edule* alcoholic and aqueous extracts were determined according to the β-carotene bleaching method. One milliliter of 0.2 mg/ml β-carotene dissolved in chloroform was added to round-bottom flasks (50 ml) containing 0.02 ml of linoleic acid and 0.2 ml of Tween 20. Each mixture was then dosed with 0.2 ml of corresponding plant extract (up to 32 μg of phenolic compounds) or positive (BHT) or negative (water and ethanol) controls. After evaporation to

dryness under vacuum at room temperature, oxygenated distilled water (25 ml) was added. The mixture was shaken for 2 min and then subjected to thermal autooxidation at 50 °C for 60 min. The absorbance of the solution was monitored at 470 nm on a spectrophotometer (Beckman DU-600) by taking measurements at 10 min intervals and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate.

Antioxidant activity (AA) was expressed as percentage AA and calculated by the equation

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

where A_0 is the initial absorbance at 470 nm of the emulsion at time 0; A_t is the absorbance of the tested plant extract at time t (10, 20, 30 and 60 min). A_{00} is absorbance at beginning of the incubation without extract; A_{0t} is absorbance at time t without extract.

2.5.2. DPPH free radical-scavenging activity

The H-donor activity of *S. edule* extracts was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method according to Yamaguchi, Takamura, Matoba, and Terao (1998). Briefly: 1.5 ml of DPPH solution (300 μM in 95% ethanol) were incubated with the samples (2–32 μg of phenolic compounds). The reaction mixture was shaken and incubated during 20 min at room temperature. Then, the absorbance was measured at 515 nm against a blank. The free radical-scavenging activity was determinated by comparison with ethanol or water control. Quercetin, rutin and BHT were used as reference compounds.

The percentage (%) of radical scavenging activity (RSA) was calculated using the following equation:

$$\mathrm{RSA}\% = [(A_0 - A_s)/A_0] \times 100,$$

where A_0 is the absorbance of the control and A_s is the absorbance of the samples at 515 nm. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

2.5.3. Determination of reducing power

The reducing power of extracts was determined according to the method of Yen and Duh (1995). Aqueous extracts (10–1000 μg of phenolic compounds) in 1 ml of distilled water and ethanolic extracts (10–1000 μg of phenolic compounds) in 1 ml of ethanol 95% were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%); the mixture was incubated at 50 °C for 20 min. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid (TCA) were added to the mixture, which was centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. The reducing power was expressed as ASE/mg. ASE means that reducing power of 1 mg sample is equivalent (E) to reducing power of 1 nmol ascorbic acid (AS).

2.5.4. Statistical analysis

For statistical analysis, each extract was considered as one treatment. All measurements were replicated three times. Linear regressions between the content; of total phenolics and data for the antioxidant assays were assessed. All data are expressed as means ± SD.

3. Results and discussion

3.1. General

Four kinds of crude preparations (tincture, fluid extract, alcoholature and decoction) from leaf, seed and stem of *S. edule* were investigated for their antioxidant and antiradical activities. Fresh and dry material from the same plant were compared.

Table 1 shows the results of the extraction using water and ethanol. Because antioxidant activity does not always correlate with the presence of large quantities of polyphenolic compounds, both data need to be examined together. For this, all extracts were analyzed for total phenolic compounds and flavonoid contents *Sechium* leaf extracts obtained with dried material gave the best yields of total phenolic compounds and flavonoid contents (expressed as quercetin equivalents) per ml of crude extract.

3.2. Antioxidant activity

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu, 2001). The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Gao, Igarashi, & Nukina, 2000). Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. The inhibitory capacity of *S. edule* extracts against the coupled oxidation of β-carotene and linoleic acid, was tested. The antioxidant activity of ethanolic extracts exhibited values from 54% to 91%. Otherwise the antioxidant activity with fresh stems and leaves was lower than those of dry plant preparation at the same phenolic compound concentration (Table 1). The highest antioxidant activity was obtained with alcoholic extracts of dry leaves and decoctions of leaves and seeds (AA values between 80% and 91%). Stem extracts showed lower antioxidant activities (AA values between 30% and 65%). Fig. 1A

Table 1
Total phenolic compounds, flavonoid contents and antioxidant activities (AA) of *S. edule* extracts

Extracts	Plant material	Flavonoid content (mg/ml)	Phenolic compounds (mg/ml)	AA (%)
Tincture	Leaf	0.65 ± 0.01	1.16 ± 0.01	85 ± 0.2
	Stem	0.18 ± 0.02	0.25 ± 0.02	65 ± 0.1
	Seed	0.16 ± 0.01	0.42 ± 0.02	76 ± 0.2
Fluid extracts	Leaf	0.75 ± 0.01	1.21 ± 0.02	91 ± 0.1
	Stem	0.20 ± 0.01	0.28 ± 0.01	60 ± 0.3
	Seed	0.15 ± 0.02	0.78 ± 0.01	66 ± 0.2
Alcoholature	Leaf	0.30 ± 0.02	0.50 ± 0.01	58 ± 0.1
	Stem	0.10 ± 0.02	0.33 ± 0.02	54 ± 0.1
	Seed	0.09 ± 0.02	0.23 ± 0.01	72 ± 0.2
Decoction	Leaf	0.20 ± 0.02	0.91 ± 0.02	80 ± 0.2
	Stem	0.07 ± 0.01	0.23 ± 0.02	30 ± 0.2
	Seed	0.02 ± 0.02	0.49 ± 0.03	91 ± 0.1
BHT[a]				98 ± 0.1

The antioxidant activities (AA%) in the *Sechium* extracts were determined according to the β-carotene bleaching method, using 1.28 μg/ml in the incubation mixture at 60 min. BHT was used at the final concentration of 50 μg/ml. Measurements were carried out in triplicate. Means and standard deviations are indicated.

[a] Reference compound.

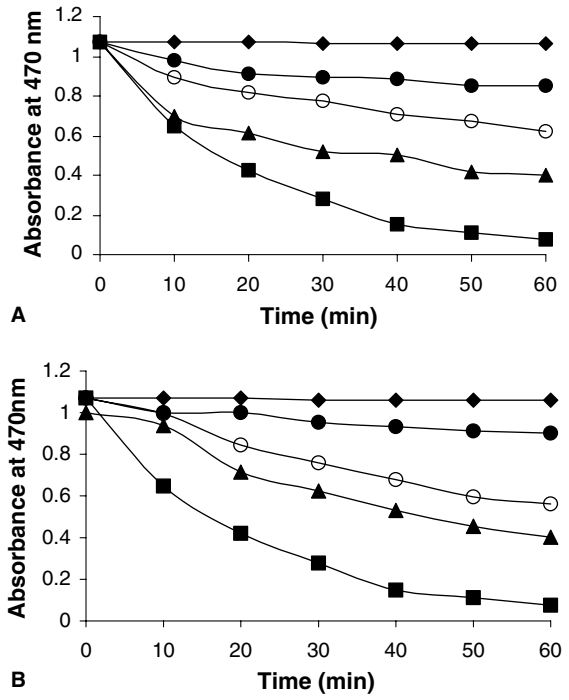

Fig. 1. Antioxidant activity of (A) leaf decoction, (B) leaf fluid extract of *Sechium edule* in the β-carotene-linoleic acid system at different incubation times. Each sample was used for the assay at the final concentration of (-●-) 1.28 μg/ml, (-O-) 0.64 μg/ml and (-▲-) 0.32 μg/ml of phenolic compounds. BHT was used at the final concentration of 50 μg/ml (-■-). Measurements were carried out in triplicate.

and B show the AA of ethanolic and aqueous extracts using different phenolic compound concentrations and compared with the activities of a known antioxidant (BHT) at different incubation times. The content of total phenolic compounds of each analyzed plant extract showed positive correlation with AA ($R^2 = 0.90$). This suggests that the content of phenolic compounds can play a major role in the antioxidant activity of *S. edule*.

The different values of AA obtained with each extract can be ascribed to their different chemical compositions (Ordóñez et al., 2003). Thus, the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content. Otherwise, synergism of polyphenolic compounds, with one another, and/or other components present in an extract, may contribute to the overall observed antioxidant activity (Shahidi, Wanasundara, & Amarowicz, 1994).

The above findings show the presence of natural antioxidant phenolic compounds in *S. edule* with better performance that BHT, known as a very efficient synthetic antioxidant agent and widely used in food technology (Potterat, 1997).

3.3. DPPH radical scavenging activity of *S. edule* extracts

DPPH assay evaluates the ability of antioxidants to scavenge free radicals. Hydrogen-donating ability is an index of the primary antioxidants. These antioxidants donate hydrogen to free radicals, leading to non-toxic species and therefore to inhibition of the propagation phase of lipid oxidation (Lugasi, Dworschák, Blázovics, & Kéry, 1998). According to Hochstein and Atallah (1998), the antimutagenic activity of antioxidants is due to their ability to scavenge free radicals or induce antioxidative enzymes. The %RSA values of the crude extracts of *S. edule* were examined and compared with one another. All *S. edule* extracts inhibited DPPH absorption with values up to 86% (32 μg/ml). These percentages can be considered as a full absorption inhibition of DPPH because, after completing the reaction, the final solution always possesses some yellowish colour (Miliauskas, Venskutonis, & Van Beek, 2004). The %RSA was phenolic compound concentration-dependent (Fig. 2A and B). The content of total pheno-

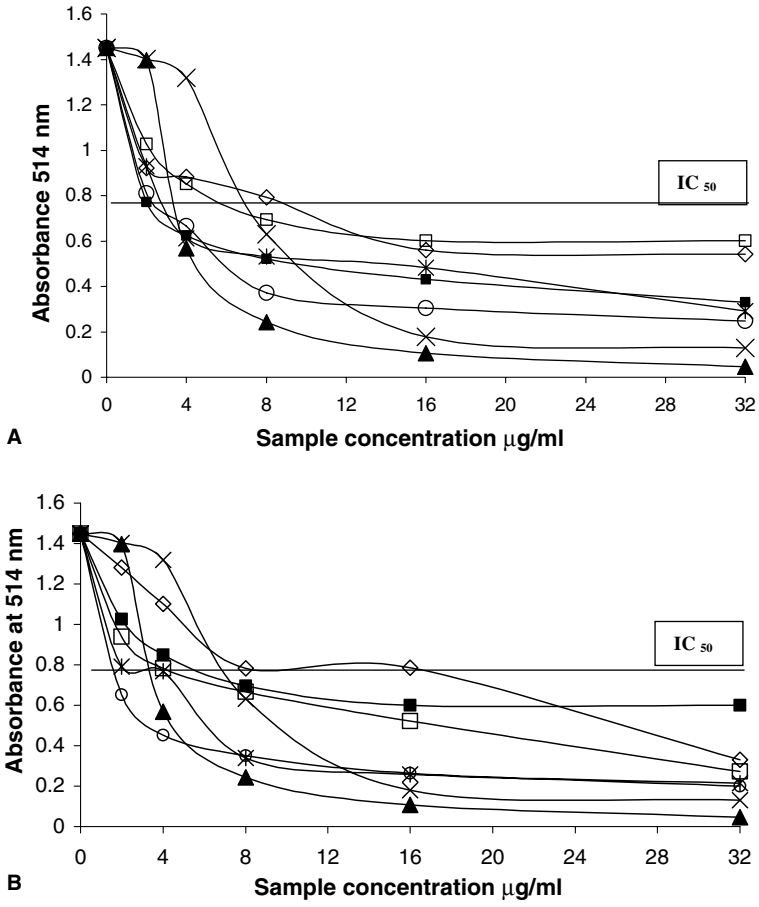

Fig. 2. DPPH radical scavenging activity of *Sechium edule* extracts. (A) leaf extracts (B) seed extracts. *Sechium* extracts and the reference samples (rutin, quercetin and BHT) were used for the assay at the final concentration between 2 and 32 μg/ml. Measurements were carried out in triplicate. (-■-) BHT, (-▲-) quercetin, (-x-) rutin, (-*-) fluid extract, (-O-) tincture, (-□-) alcoholature, (-◇-) decoction.

lic compounds of each analyzed plant extract showed positive correlation, with RSA ($R^2 = 0.80$). The IC_{50} values of leaf and seed ethanolic extracts were lower than those of decoctions (2–4 μg/ml and 8–32 μg/ml, respectively). At a dosage of 32 μg of phenolic compounds, the seed decoction afforded 86.6% RSA on the stable DPPH free radical, followed by leaf fluid extract, tincture and alcoholature with 82%, 78% and 75%, respectively (Table 2). Otherwise, if the rate of DPPH radical-scavenging is considered, leaf fluid extracts were the most effective with 80% of absorption inhibition in 5 min, while seed decoction reaches that level of inhibition in 15 min. Between positive controls, the highest antioxidant activity was observed for flavonoids (quercetin and rutin), followed by BHT. The above findings show the presence of natural antioxidant phenolic compounds in *S. edule*.

3.4. Reducing power

The reducing power property indicates that these compounds are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen & Chen, 1995). Fig. 3 shows the reducing power of *S. edule* extracts. Thus, tincture and decoction of leaves were good electron and hydrogen donors and could terminate the radical chain reaction, converting free radicals to more stable products.

In this study, ethanolic extracts of dried leaves and water extracts of seeds were found to possess higher radical-scavenging, reducing power and antioxidant activities. The bioactive components of *S. edule* extracts can act as primary and secondary antioxidants, scavenging free radicals, and can therefore inhibit the lipid peroxidation. The qualitative (Ordóñez et al., 2003) and quantitative analysis of phenolic compounds in active, extracts showed, the presence of flavonoids (Table 1) principally flavonol, that could be responsible for the effects, but further experiments are necessary to verify relation between chemical composition and antioxidant activity.

Selected active extracts of *S. edule* (Jacq.) Swartz may be an alternative to more toxic synthetic antioxidants as additives in food, pharmaceutical and cosmetic preparations.

Table 2
DPPH free radical-scavenging activities of *Sechium edule* extracts

DPPH inhibition(%)				
Extracts	Incubation time (min)			
	5	10	15	20
Leaf				
Fluid extract	74.7 ± 0.1	75.2 ± 0.1	72.6 ± 0.1	81.5 ± 0.1
Tincture	45.4 ± 0.1	47.3 ± 0.1	62.7 ± 0.1	77.9 ± 0.1
Alcoholature	45.9 ± 0.2	67.8 ± 0.1	65.4 ± 0.1	65.4 ± 0.1
Decoction	58.7 ± 0.2	61.8 ± 0.2	68.0 ± 0.2	71.4 ± 0.1
Stem				
Fluid extract	42.0 ± 0.2	45.8 ± 0.2	57.2 ± 0.2	68.6 ± 0.2
Tincture	41.3 ± 0.2	45.4 ± 0.2	59.2 ± 0.2	67.9 ± 0.2
Alcoholature	46.8 ± 0.2	56.9 ± 0.2	64.0 ± 0.2	57.2 ± 0.2
Decoction	56.9 ± 0.2	68.9 ± 0.3	61.3 ± 0.3	52.7 ± 0.3
Seed				
Fluid extract	57.8 ± 0.1	61.3 ± 0.2	63.9 ± 0.2	70.2 ± 0.2
Tincture	63.1 ± 0.1	68.0 ± 0.1	68.2 ± 0.1	74.4 ± 0.1
Alcoholature	52.2 ± 0.2	63.5 ± 0.3	70.2 ± 0.1	72.3 ± 0.1
Decoction	48.6 ± 0.2	63.9 ± 0.1	80.1 ± 0.1	86.6 ± 0.2
Quercetin[a]	79.3 ± 0.2	90.9 ± 0.1	94.3 ± 0.1	96.3 ± 0.3
Rutin[a]	90.1 ± 0.1	90.6 ± 0.1	91.1 ± 0.1	93.8 ± 0.1
BHT[a]	57.0 ± 0.3	68.8 ± 0.2	69.2 ± 0.2	78.4 ± 0.3

DPPH free radical-scavenging activities of *Sechium edule* extracts. Each extract sample was used for the assay at the final concentration of 32 μg/ml. The reference samples (quercetin, rutin and BHT) were used at the final concentration of 40 μg/ml. Measurements were carried out in triplicate. Means and standard deviations are indicated.

[a] Reference compounds.

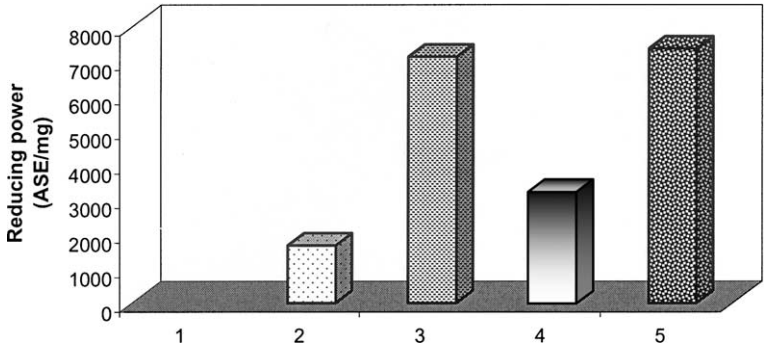

Fig. 3. Reducing power of *Sechium edule* leaf extracts at 700 nm: 1, control negative; 2, fluid extracts; 3, tincture; 4, alcoholature; 5, decoction. (Activity presented, ASE/mg reducing power of 1 mg sample, is equivalent [E] to reducing power of 1 nmol ascorbic acid [AS].)

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