Evaluation of the antioxidant activity of aqueous and organic extracts of edible insects to different cooking temperatures and pH conditions

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ABSTRACT

Objective: To evaluate heat treatment and pH on the antioxidant activity of extracts obtained from edible insects endemic to Mexico. Insects known as jumiles, grasshoppers (adults and nymphs), red maguey (*Agave* sp.) wormfrom the maguey penca, white maguey worms from the pineapple, and chicatanas were used.
Design/methodology/approach: Different solvents (water, methanol, ethanol, NaCl, and acetic acid) were used to prepare the extracts. These were characterized by X-ray diffraction, and the effect of temperature and pH on their antioxidant activity was analyzed. The methods used were DPPH and FRAP, and total polyphenols and flavonoids were quantified.
Results: There are presence of antioxidant molecules characterized as quercetin, hesperetin, and DL-α-lipoic acid. The extracts with the highest content of flavonoids and total phenols were from adult grasshoppers in water, chicatanas in 50% ethanol, and maguey worms from the penca in water. However, the antioxidant activity of these extracts was negatively affected by elevated pH and temperature.
Study limitations/implications: The main limitations of the study were the availability of the insects, which are endemic species of Mexico and most are seasonal.
Findings/conclusions: Molecules with antioxidant capacity were identified, and their antioxidant activity decreased when exposed to high pH values.

Keywords: Edible insects, Antioxidants, *Sphenarium purpurascens*, *Atta mexicana*.

INTRODUCTION

Antioxidants are substances that prevent the oxidation of other compounds. The use of antioxidants has become a necessity for food products, which are sensitive to this

type of chemical change. Widely used synthetic antioxidants are effective (*e.g.*, BHA, BHT, TBHQ). Currently, there is a growing consumer demand for the use of natural ingredients in processed foods. Therefore, new sources of natural compounds with antioxidant activity are being sought (Hęś *et al.*, 2019). Some natural antioxidants have a more potent effect than artificial antioxidants (Valenzuela and Pérez, 2016). Naturally occurring antioxidants are mainly found in plants, microorganisms, fungi, and animal tissues (Gallegos *et al.*, 2013).

Polyphenols are organic chemical compounds that occur naturally in plants and constitute one of the most abundant and diverse groups of natural compounds in the plant kingdom. These compounds are classified as secondary metabolites, produced through the phenylpropanoid-derived shikimate pathway and/or the polyketide pathway. There are approximately 80,000 identified polyphenols, with molecular weights reaching up to 30,000 Da. Polyphenols are classified as flavonoids, phenolic acids, lignans, and stilbenes, each defined by unique structural features (Sejbuk *et al.*, 2024). Polyphenols share common phenolic structural features and exhibit structural diversity with several subgroups. Polyphenols are categorized by the number of phenolic rings they contain and by the structural elements that link the rings. The four primary structural groups of polyphenols are flavonoids, phenolic acids, lignans, and stilbenes; these are present in fruits, vegetables, tea, and wine. Caffeic and ferulic acids are prevalent in coffee and grains. Lignans are found in seeds and whole grains, while stilbenes, such as resveratrol, are found in grapes and berries. This wide variety of phenolic structural types forms complex biological functions (Jalouli *et al.*, 2025).

Food processing aims to extend shelf life, improve organoleptic properties, and increase the availability of bioactive compounds present in raw materials. However, some methods can result in the loss of polyphenols. Therefore, the final amount of these compounds depends on the processing technique used, the exposure time, and the characteristics of the ingredients (Marín *et al.*, 2015).

There are various processing strategies; for example, the use of heat is one of the most common. This method includes techniques such as boiling, steaming, frying, baking, dehydration, pasteurization, sterilization, canning, and roasting. The effect of heat on polyphenols varies depending on the specific procedure; in some cases, high temperatures promote their absorption and breakdown of cellular structures, while in others, they can cause degradation through oxidation (Minatel *et al.*, 2017). This study aimed to evaluate the effect of heat treatment and pH on the antioxidant activity of extracts from edible insects endemic to Mexico.

MATERIAL AND METHODS

Samples

The samples were obtained from the San Juan exotic meat market in Mexico City. The insects used were: jumiles (*Euschistus taxcoensis*), grasshoppers (*Sphenarium purpurascens*) adults and nymphs (fifth instar), red maguey worms (*Comadia redtenbacheri*), white maguey worms (*Aegiale hesperiaris*), and chicatanas (*Atta mexicana*).

Extracts obtained

The extracts of the insects above were obtained with five different solvents: distilled water, 0.6 M sodium chloride, 50% ethanol, 50% methanol, and 30% acetic acid. The methodology proposed by Mendoza *et al.* (2013) and Calderón *et al.* (2016) was used. Table 1 shows the coding. All extractions were performed by liquefying 5 g of the insect samples and 50 mL of the solvents. The mixtures with distilled water and 0.6 M NaCl were boiled and stirred for 30 min, while the other samples were stirred at 90 rpm for 120 hours at 24 °C. Finally, the extracts were filtered through Whatman No. 1 paper.

Concentration of the different extracts

The ethanol, methanol, and acetic acid extracts were concentrated in a rotary evaporator at 20 rpm for 20 min and 45 min, respectively, at a temperature of 50 °C. All extracts were then frozen with liquid nitrogen and lyophilized (Labconco FreeZone® 2.5, Houston, USA); they were then stored in Mylar bags and kept at room temperature until use.

Quantification of total polyphenols

Quantification was performed using the Folin-Ciocalteu spectrophotometric method proposed by Lin and Tang (2007), with some modifications. A reaction was carried out by mixing 100 μL of extract with 2.8 mL of water and then adding 100 μL of 50% Folin-Ciocalteu reagent for 5 min. Subsequently, 2 mL of 2% sodium carbonate was added, and the mixture was left to stand for 40 min at room temperature, all in the dark. Finally, the absorbance at 750 nm was measured using a spectrophotometer (Genesys 105 UV-VIS Thermo Scientific). Gallic acid was used as a standard for the 0-200 μg/mL standard curve.

Flavonoid quantification

The reaction was carried out using the methodology proposed by López (2010), with the following modifications. The reaction was carried out in 250 μL of extract, 1.25 mL of water, and 75 μL of 5% sodium nitrite. The mixture was then allowed to stand at room temperature for 6 min. 150 μL of 2% aluminum trichloride was then added for 5

Table 1. Coding of edible insects and solvents *.

Edible insects		Solvents	
Jumiles	JU	Water	01
Chicatanas	CH	Sodium chloride 0.6M	02
Grasshoppers in the fifth nymph stage	CC	Ethanol 50%	03
Adult grasshoppers	CG	Methanol 50%	04
Maguey worms from the penca	PE	Acetic acid 30%	05
Pineapple maguey worms	PI		

The extracts were named after the edible insect code. For example, JU01 corresponds to the water extract of jumiles. *Only the following extracts were prepared: PI01, CG01, PE01, CC01, CH02, JU02, CH03, PI04, CC05, PE05, and JU05, according to the preliminary results obtained by the working group.

min, followed by the addition of 500 μL of 1 M sodium hydroxide. The final volume was completed to 2.25 mL with distilled water and incubated for 50 min at room temperature. The reaction was carried out in the dark. The absorbance at 510 nm was then measured in a spectrophotometer (Genesys 105 UV-VIS Thermo Scientific). A standard was routinely used to prepare the calibration curve, ranging from 0 to 250 μg/mL.

Effect of heat treatment and pH on antioxidant activity

The extracts were treated under different pH and heat treatment conditions as shown in Table 2. Subsequently, total polyphenols, flavonoids, and antioxidant activity, FRAPP, and DPPH were quantified.

Ferric Reduction Antioxidant Potential (FRAP)

Antioxidant activity was measured using the FRAP method, following the methodology described by Calderón *et al.* (2016). A reaction was performed with 100 μL of extract and 750 μL of FRAP reagent (López, 2010) and incubated for 5 min at 37 °C. A total of 150 μL was withdrawn and analyzed using a plate reader (Synergy HT BioTek Instruments, Vermont, USA) at 593 nm. A standard curve was created using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) dissolved in 50% methanol in a concentration range of 0-400 μg/mL.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical reducing activity

Kinetics were performed to determine the percentage of DDPH radical reduction in the presence of the extracts and the reference antioxidant (0.0078-0.125 mg/mL of gallic

Table 2. A combination of pH and temperature of the different treatments.

Treatment	pH	Temperature/time
Control	Without modification	No treatment
1		64 °C / 30 min
2		72 °C / 15 s
3		95 °C / 30 min
4	4	No treatment
5		64 °C / 30 min
6		72 °C / 15 s
7		95 °C / 30 min
8	6	No treatment
9		64 °C / 30 min
10		72 °C / 15 s
11		95 °C / 30 min
12	8	No treatment
13		64 °C / 30 min
14		72 °C / 15 s
15		95 °C / 30 min

acid). The process was carried out with measurements every 5 min for 30 min at 25 °C, using a plate reader (Synergy HT BioTek Instruments, Vermont, USA) at a wavelength of 517 nm. The reactions were analyzed using the methodology of Mendoza *et al.* (2013), with the following modifications: 5 μL of the sample and 195 μL of a 4×10^{-5} M methanolic DDPH solution were added. For grasshopper samples in 50% ethanol, a concentration of 8×10^{-5} M DPPH was used. In addition, various controls were prepared to verify that the solvents or extracts did not absorb at the wavelength used: 1) Equipment blank (200 μL of water); 2) Solvent blank (5 μL of solvent in which the extract was dissolved and 195 μL of water); 3) Extract blank (5 μL of extract and 195 μL of water); 4) DPPH solvent blank (200 μL of methanol). Finally, plate wells were used to measure the absorbance of the DPPH radical without the presence of an antioxidant.

The DPPH reproduction percentage was calculated using the following equation:

$$\%\,of\ reduction\ DPPH = \frac{\left(A_R - A_{bpR}\right) - \left(A_m - A_{bp} - A_{mp}\right)}{A_R - A_{bp}} \times 100\%$$

Where: A_R=Absorbance of the DPPH radical; A_m=Absorbance of the extract; A_{bpR}=Absorbance of the radical solvent blank − Absorbance of the equipment; A_{bp}=Absorbance of the solvent blank − Absorbance of the equipment; A_{mp}=Absorbance of the extract blank − Absorbance of the equipment.

X-ray diffraction

The analysis was performed in the X-ray laboratory of the Division of Basic Sciences and Engineering at UAM-Iztapalapa.

Statistical analysis

All determinations were performed in triplicate, and the results are presented as the mean and standard deviation. Statistical analyses were performed using XLSTAT software version 2014.5.03 (Addinsoft) using an alpha threshold of 0.05. Results were analyzed using one-way analysis of variance (ANOVA) and the Tukey method for comparison of means between treatments for each of the techniques used. Principal component analysis (PCA) was used for the quantification tests of total polyphenols and flavonoids.

RESULTS AND DISCUSSION

The results indicate that all the samples analyzed may have antioxidant activity; however, it has been reported that the phenolic content in some plants is not related to antioxidant activity, but rather to the action of the polyphenols present (Gutiérrez *et al.*, 2008). For example, some polyphenols are widely distributed in the plant kingdom and are important for antioxidant capacity. The phenolic compounds in legume seeds are particularly flavonoids, phenolic acids, and procyanidins, and the antioxidant capacity of legumes is influenced by their composition of active polysaccharides, proteins, amino acids, vitamins, and microelements (Zhao *et al.*, 2014).

The activity of natural antioxidants depends on the participation of phenolic hydrogen in reaction with radicals, the stability of the antioxidant radical formed during radical reactions, and the chemical substitutions present in the structure. Substitutions in the structure are likely the most significant contribution to the capacity of a natural antioxidant, playing a crucial role in controlling radical reactions and facilitating the formation of resonance-stabilized antioxidant radicals (Figure 1).

The electron-donating capacity of methyl, ethyl, and tertiary-butyl substitutions at the ortho and para positions of hydroxyl groups is enhanced by the antioxidant activity of phenol. Furthermore, hydroxyl group substitutions at these positions improved antioxidant activity (Pokorny *et al.*, 2001).

The extracts were evaluated by D-DX and compared with different antioxidant compound structures. It was observed that all the insects contained ascorbic acid, gallic acid, and quercetin dihydrate, of which gallic acid (GA) represents the primary class of phenolic acids. In contrast, quercetin dihydrate (QDH) is the main representative of the flavanol group of flavonoids. Gallic acid is available in various parts of plants (fruits, seeds, leaves, and wood). Fruits, such as blackberries, blueberries, strawberries, walnuts, grapes, plums, and mangoes. QDH is a bioflavonoid found in fruits and vegetables. Vegetables include onions, kale, broccoli, and peppers. Fruits include apples, tomatoes, blueberries, cherries, red grapes, raspberries, and cranberries (Bibila Mayaya Bisseyou *et al.* 2024).

On the other hand, jumiles and chicatanas do not contain ferulic acid, which has generated worldwide interest due to its antioxidant capacity, meaning they can potentially protect DNA and lipids from oxidation through reactive oxygen species; its activity has been linked to anti-inflammatory, anti-aging, anticancer, antidiabetic, antihypertensive, and neuroprotective effects. Ferulic acid is found in many plants, including vegetables, coffee, nuts, and cereals (Han & Mackie, 2023). Another antioxidant compound absent in chicatanas is hesperitin, a flavone present in citrus fruits, and is also present in its glycosidic form as hesperidin (Tanwar *et al.*, 2020). Flavanones are distinguished among flavonoids by the absence of the C2-C3 double bond, forming a non-planar C-ring (Amisha and Arati, 2023). On the other hand, hesperetin is a promising potential anticancer compound for inhibiting the aromatase enzyme, which acts as an important therapeutic agent for breast cancer in postmenopausal women (Iqbal *et al.*, 2018).

Lactic acid is not synthesized in grasshoppers at the nymph stage, compared to the adult stage and other insects. Molecules such as halite are also observed, but this was only observed in samples extracted with NaCl and distilled water. Finally, the compounds meso-tartaric acid and β-D-mannose were found in maguey worms extracted with water

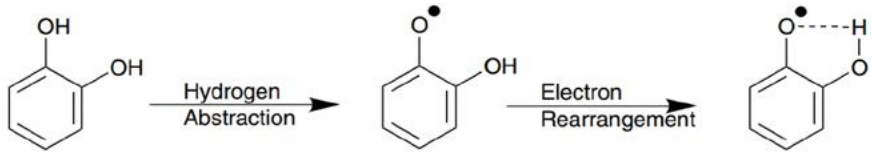

Figure 1. Baum and Perun proposed intramolecular hydrogen bonding of ortho-substituted phenols. Taken from Pokorny *et al.* (2001).

(PE01); these compounds are highly soluble in water. On the other hand, several authors have mentioned some antioxidant compounds present in edible insects such as: gallic acid and quercetin in *Polyrhachis vicina*, *Acheta domesticus*, *Holotrichia parallela*, *Alphitobius diaperinus*, *Tenebrio molitor* (Zhang *et al.*, 2022; Nino *et al.*, 2021; Liu *et al.*, 2012; Gumul *et al.* 2023), additionally quercetin is reported in grasshoppers such as *Dissoteira carolina*, caterpillars: *Rondotia menciana* and *Bombyx mori* worms (Hopkins and Ahmad, 1991; Hirayama *et al.*, 2013; Kurioka and Yamazaki. 2002). Gumul *et al.* (2023) reported levels of 0.45, 7.23, and 0.54 mg/100g of ferulic acid for *A. diaperinus*, *A. domesticus*, and *T. molitor*, respectively. Fu *et al.* (2021) reported the presence of hesperitin in the moth *Antheraea pernyi*.

Based on the quantification of total polyphenols and flavonoids, a Principal Component Analysis (PCA) was performed to select the extracts with the highest concentration of potential antioxidant molecules (Figure 2). The PCA accounted for 100% of the sample variability. The samples with the highest concentrations of total phenols and flavonoids were found in penca maguey worms in water (PE01), adult grasshoppers in water (CG01), and chicatanas in 50% ethanol (CH03).

Table 4 shows the reducing capacity using the FRAP technique. The PE01 extract from treatment 15 showed a significant increase compared to the control (176.43±2.082 and 56.81±0.267, $P<0.05$, respectively). Phenolic compounds had greater reducing capacity because they formed a blue complex with tripyridyltriazine at acidic pH, reducing

Table 3. Antioxidant compounds in edible insect extracts by D-RX.

Name of antioxidant compound	Chemistry Formula	Edible insect extract										
		JU02	JU05	CC01	CC05	PI04	PI04	PE01	PE05	CH02	CH03	CG01
Cinnamic Acid	$C_9H_80_3$	X	X	X	X	X		X	X	X		X
Gallic acid hydrate	$C_7H_6O_5 \bullet H_20$	X					X			X		.
Quercetin dihydrate	$C_{15}H_{14}O_7 \bullet 2H_20$	X	X		X	X	X	X	X	X	X	X
Rutin	$C_{27}H_{30}O_{16}$	X		X	X					X		X
Hesperetin	$C_{28}H_{34}O_{15}$	X	X		X	X	X	X	X			X
B-Carotene	$C_{40}H_{56}$	X	X	X		X		X			X	X
Folic acid	$C_{19}H_{19}N_7O_6$	X	X			X	X	X	X	X	X	X
Ascorbic acid	$C_6H_8O_6$	X	X		X	X	X	X	X	X	X	X
Glutathione	$C_{10}H_{17}N_3O_6S$	X		X		X	X	X	X	X	X	X
dl-a-Lipoic acid	$C_6H_{14}O_2S_2$	X	X	X	X	X	X		X	X	X	X
Halite	NaCI	X		X	X					X		X
Ferulic acid	$C_{10}H_{10}O_4$			X	X	X	X	X	X			X
acid o-coumaric acid	$C_9H_8O_3$			X				X			X	X
Vitamin B12	$C_{63}H_{88}CoN_{14}O_{14}P \bullet 16H_20$			X								
Rhodium, platinum	(Rh, Pt)					X	X					
Meso Tartaric acid	$C_4H_6O_6$							X				
13-D-Mannose	$C_6H_{12}O_6$							X				X

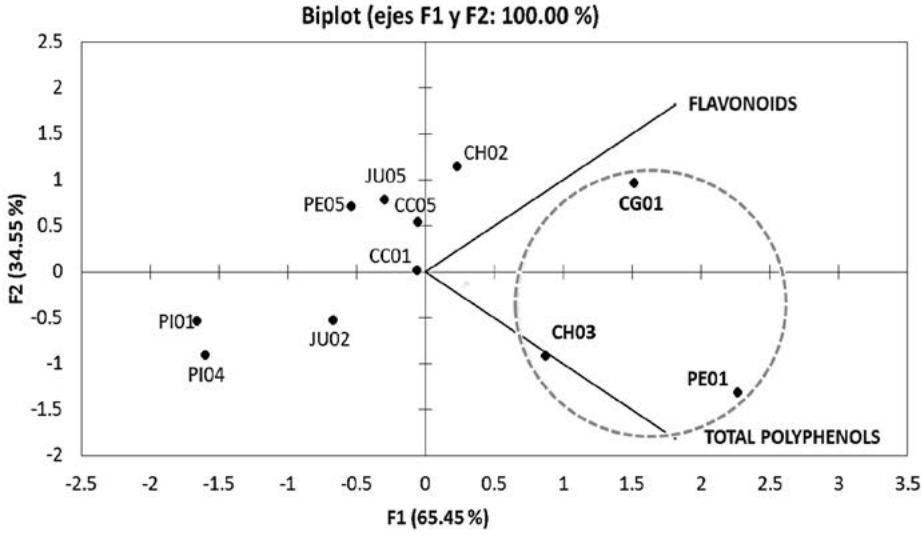

Figure 2. Principal component analysis of insect extracts.
Variables analyzed: total phenols and flavonoids. Extracts with the highest concentrations were grouped into clusters marked with circles.

Table 4. Antioxidant activity and quantification of antioxidant molecules after treatment with pH and temperature of the PE01 extract.

Treatment	Antioxidant activity		Antioxidant molecules, quantification	
	FRAP[1]	DPPH (%)	Polifenols total[2]	Flavonoids[3]
Control	56.81 ± 0.27^{C}	58.15 ± 3.72^{BC}	13.46 ± 0.62^{F}	41.92 ± 5.20^{ABC}
1	18.78 ± 0.29^{G}	28.55 ± 0.98^{F}	24.75 ± 1.32^{BC}	$35.25 \pm 9.43^{A,BC}$
2	27.9 ± 1.63^{F}	52.43 ± 7.21^{BCD}	20.92 ± 0.80^{D}	26.95 ± 4.74^{BC}
3	62.99 ± 1.75^{B}	108.37 ± 2.19^{A}	27.17 ± 0.52^{AB}	53.58 ± 14.14^{AB}
4	15.95 ± 0.09^{GI}	37.36 ± 4.92^{DEF}	13.71 ± 0.44^{F}	34.83 ± 1.77^{ABC}
5	17.21 ± 0.24^{G}	35.97 ± 2.95^{DEF}	26.25 ± 0.66^{AB}	59.14 ± 11.09^{A}
6	59.25 ± 1.57^{BC}	58.92 ± 9.18^{B}	20.92 ± 0.80^{D}	20.25 ± 4.74^{B}
7	61.34 ± 1.98^{B}	108.06 ± 2.12^{A}	26.75 ± 1.56^{AB}	25.25 ± 7.07^{C}
8	12.63 ± 0.35^{HI}	51.35 ± 7.36^{BCD}	12.71 ± 0.61^{F}	40.67 ± 4.12^{ABC}
9	17.64 ± 0.53^{G}	34.97 ± 3.94^{EF}	27.17 ± 1.61^{AB}	34.42 ± 1.18^{ABC}
10	40.20 ± 1.41^{D}	47.48 ± 3.48^{BCDE}	17.42 ± 2.04^{E}	25.25 ± 2.33^{C}
11	33.80 ± 0.72^{E}	96.00 ± 4.59^{A}	24.92 ± 0.88^{ABC}	61.92 ± 11.79^{A}
12	8.78 ± 0.55^{J}	43.47 ± 0.97^{CDEF}	13.75 ± 0.55^{F}	46.64 ± 8.09^{ABC}
13	$9.65 \pm 0.213^{I,J}$	43.39 ± 0.33^{CDEF}	27.92 ± 1.01^{A}	46.92 ± 2.35^{ABC}
14	29.71 ± 2.095^{F}	49.18 ± 2.45^{BCD}	14.92 ± 0.63^{EF}	22.48 ± 1.93^{C}
15	176.43 ± 2.082^{A}	49.65 ± 5.35^{BCD}	22.58 ± 0.38^{CD}	32.47 ± 5.09^{BC}

[1] mg TROLOX equivalent/g extract, 2 mg gallic acid/g extract, 3 mg rutin/g/g extract. Superscript letters represent significant differences between rows within the same column ($p<0.05$).

Fe^{3+} to Fe^{2+} (Benzie and Strain, 1996). Treatments 3 and 7 also had a greater increase than the control (62.99±1.751, 61.34±1.976 and 56.81±0.267, P<0.05, respectively); in these treatments there was a reduction in the DPPH radical, the highest value was 108.37±2.19% for PE01 (treatment 3), and 108.06±2.12% for PE01 (treatment 7), This was due to the total phenol content (27.17±0.52 and 26.75±1.56, respectively), but the extract from treatment 7 had a decrease in flavonoid content compared to the control;. In contrast, treatment three did not have a significant difference (25.25±7.07, 41.92±5.20, 53.58±14.14, P>0.05, respectively). The important difference was in treatment 3, where the thermal effect favored the persistence of flavonoids.

Table 5 shows the antioxidant activity values for the extract of adult grasshoppers in water (CG01), treatment 5 (pH 4, 64 °C/30 min), had the highest antioxidant activity in ferric reduction (FRAP) with 23.24±1,050 mg TROLOX equivalent/g of extract (p<0.05) *vs*. Control and other treatments. There are no references on the antioxidant power with ferric reduction (FRAP). In the case of crickets (Orthoptera), there is a report by Gumul *et al*. (2023). In *Acheta domesticus*, the value was 71.15±0.5 mM Fe/kg, lower than that reported by Kurdi *et al*. (2021). *Gryllus bimaculatus* showed 300±100 mM Fe/kg; this value was because the crickets were previously defatted. DPPH showed higher levels in the Control group and Treatment 4 (pH 4, without heat treatment), 47.7±1.35 and 54.75±6.34 mg of gallic acid/g of extract, respectively (P<0.05). The increase in

Table 5. Antioxidant activity and quantification of antioxidant molecules after pH and temperature treatment of the CG01 extract.

Treatment	Antioxidant Activity		Antioxidant molecules, Quantification	
	FRAP[1]	DPPH (%)	Polifenols total[3]	Flavonoids[4]
Control	15.50 ± 0.660^{CD}	47.7 ± 1.35^{DE}	7.46 ± 0.14^{ABCD}	63.31 ± 9.66^{DE}
1	15.28 ± 0.496^{CD}	24.20 ± 3.10^{AB}	7.50 ± 0.87^{ABCD}	80.53 ± 8.67^{EF}
2	16.16 ± 0.496^{D}	17.16 ± 7.13^{AB}	6.83 ± 0.38^{ABC}	30.25 ± 4.71^{A}
3	19.32 ± 0.717^{E}	28.07 ± 1.35^{ABC}	8.92 ± 0.38^{CDE}	91.92 ± 2.35^{F}
4	13.36 ± 0.402^{BC}	54.75 ± 6.34^{E}	7.42 ± 0.56^{ABCD}	65.25 ± 4.41^{DE}
5	23.24 ± 1.050^{F}	20.57 ± 3.09^{AB}	7.44 ± 0.44^{ABCD}	51.08 ± 3.54^{BCD}
6	18.66 ± 0.538^{E}	20.30 ± 0.77^{AB}	6.00 ± 0.43^{AB}	71.77 ± 7.28^{DEF}
7	19.11 ± 0.580^{E}	27.84 ± 3.04^{ABC}	13.08 ± 1.18^{F}	63.59 ± 4.72^{DE}
8	15.79 ± 0.255^{D}	20.16 ± 5.21^{AB}	5.29 ± 1.58^{A}	58.59 ± 1.18^{CD}
9	15.46 ± 0.317^{CD}	12.93 ± 1.93^{A}	6.00 ± 0.45^{AB}	55.67 ± 3.54^{BCD}
10	16.04 ± 0.797^{D}	18.39 ± 4.63^{AB}	8.08 ± 1.38^{BCD}	41.92 ± 7.07^{ABC}
11	17.38 ± 1.244^{DE}	24.52 ± 3.28^{AB}	11.42 ± 0.80^{EF}	58.59 ± 16.50^{CD}
12	15.51 ± 0.433^{CD}	30.39 ± 3.54^{BC}	7.50 ± 0.69^{ABCD}	66.92 ± 5.89^{DE}
13	11.22 ± 0.616^{B}	32.29 ± 3.09^{BCD}	5.75 ± 0.78^{AB}	61.64 ± 6.79^{CDE}
14	13.33 ± 1.281^{BC}	40.34 ± 13.30^{CDE}	8.42 ± 1.63^{BCD}	35.25 ± 4.71^{AB}
15	6.85 ± 0.840^{A}	19.07 ± 6.36^{AB}	10.08 ± 1.01^{DE}	28.29 ± 2.35^{A}

[1] mg equivalente de TROLOX/g de extracto, 2 mg de ácido gálico/g de extracto, 3 mg de rutina/g de extracto. Las leras como superíndice representan diferencia significativa entre los renglones de la misma columna (p<0.05).

temperature and pH decreased the antioxidant activity. On the contrary, Kurdi *et al.* (2021) observed a temperature of 135 °C and 15 min increased DPPH activity in *Gryllus bimaculatus* from 0.07±0.08 to 45.51±2.64%. For the quantification of antioxidant molecules, there were differences ($P<0.05$) between the Control group *vs.* Treatment 7, having a higher content of total polyphenols, while Treatments 1, 3, and 6 were for the flavonoid content.

Table 6 shows the antioxidant activity values for the chicatanas extract with 50% ethanol (CH03), the highest FRAP activity was in Treatments 5 (pH 4, 64 °C/30 min), and 8 (pH six and without heat treatment) of 15.37±0.02 and 14.93±0.42 mg TROLOX equivalent/g of extract, respectively ($P<0.05$), in the literature no references were found on the antioxidant power for chicatanas, Campos *et al.* (2014) reported DPPH% activity with a concentration of 50 μg/mL of *Melipona orbignyila* propolis (75±3.5%), being higher than the insect of the Hymenoptera order (71.78±1.67%). Regarding antioxidant molecules, information was only found in total polyphenols from methanolic extracts of beetles *Pharochilus punctiger* and *Titoceres jaspideus* (7.81±0.21 and 6.44±0.30 mg gallic acid/g, respectively) and flavonoids (6.88±0.20 and 5.34±0.27 mg quercetin/g, respectively); these values were lower than in another study (Kibet *et al.*, 2024). On the other hand, increases in pH and temperature decreased FRAP activity; meanwhile, DPPH activity at 95 °C increased compared to pH 4.

Table 6. Antioxidant activity and quantification of antioxidant molecules after pH and temperature treatment of the CH03 extract.

Treatment	Antioxidant Activity		Antioxidant molecules, Quantification	
	FRAP[1]	DPPH (%)	Polifenols total[2]	Flavonoids[3]
Control	13.22 ± 0.35^{CDE}	59.33 ± 9.13^{ABC}	9.13 ± 0.19^{AB}	113.46 ± 8.24^{G}
1	13.77 ± 0.34^{EFG}	62.62 ± 2.38^{ABC}	9.50 ± 0.41^{AB}	44.57 ± 4.74^{CDEF}
2	14.74 ± 0.01^{FG}	54.67 ± 7.08^{ABC}	22.59 ± 0.85^{D}	46.79 ± 8.66^{CDEF}
3	14.80 ± 0.52^{FG}	59.85 ± 2.56^{ABC}	21.54 ± 2.09^{D}	54.30 ± 5.89^{F}
4	13.51 ± 0.22^{DEFG}	70.91 ± 3.91^{C}	9.23 ± 0.20^{AB}	34.02 ± 5.55^{BCD}
5	15.37 ± 0.02^{G}	61.84 ± 6.23^{ABC}	9.50 ± 0.44^{AB}	37.07 ± 3.47^{BCDE}
6	11.37 ± 1.84^{BCD}	69.53 ± 6.44^{BC}	21.25 ± 1.47^{D}	39.30 ± 1.18^{BCDEF}
7	12.64 ± 0.13^{CDEF}	71.78 ± 1.67^{C}	21.88 ± 2.05^{D}	49.29 ± 3.54^{DEF}
8	14.93 ± 0.42^{G}	56.91 ± 8.72^{ABC}	10.17 ± 0.62^{AB}	54.29 ± 6.51^{F}
9	13.11 ± 1.04^{CDEFG}	54.67 ± 4.32^{ABC}	10.71 ± 0.53^{B}	46.24 ± 8.22^{CDEF}
10	13.74 ± 0.75^{EFG}	53.80 ± 9.89^{ABC}	22.42 ± 1.58^{D}	46.79 ± 7.27^{CDEF}
11	13.83 ± 1.65^{EFG}	52.51 ± 2.57^{ABC}	22.79 ± 2.19^{D}	52.63 ± 5.89^{EF}
12	11.14 ± 0.34^{BC}	46.89 ± 3.93^{A}	7.25 ± 0.44^{AB}	26.24 ± 4.59^{AB}
13	12.42 ± 0.43^{CDE}	50.35 ± 8.64^{AB}	6.92 ± 0.50^{A}	32.63 ± 3.54^{ABCD}
14	9.81 ± 0.24^{AB}	57.26 ± 6.77^{ABC}	15.00 ± 0.69^{C}	16.80 ± 2.35^{A}
15	8.80 ± 0.53^{A}	57.69 ± 11.37^{ABC}	15.05 ± 0.80^{C}	30.13 ± 0.00^{ABC}

[1]mg TROLOX equivalent/g extract, 2 mg gallic acid/g extract, 3 mg rutin/g/g extract. Superscript letters represent a significant difference between rows in the same column ($p<0.05$).

CONCLUSION

Molecules with antioxidant capacity were identified and quantified in insects endemic to Mexico, particularly grasshoppers, maguey worms, and chicatanas. However, it was observed that their antioxidant activity is susceptible to changes in pH and temperature.

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