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Research

Schistocerca piceifrons piceifrons (Orthoptera: Acrididae) as a Source of Compounds of Biotechnological and Nutritional Interest

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Abstract

The Central American locust, *Schistocerca piceifrons piceifrons* (Walker) is a major agricultural pest in Mexico and Central America. Control measures against this pest have generated much environmental damage and substantial financial costs because chemical insecticides are used. Yet various Orthoptera species also appear to be a potential source of nutrients and a source of bioactive metabolites. Here, we studied the presence of secondary metabolites in the adult stage of *S. p. piceifrons* by applying different colorimetric techniques. Adults were collected from the southern region of Tamaulipas, Mexico, during September–December 2017. These samples were subjected to sequential processes of eviscerating, drying, pulverizing, extracting, and detecting of metabolites. Extractions were carried out in water, 50% ethanol, and absolute ethanol. The presence of phenolic compounds, alkaloids, tannins, saponins, flavonoids, and quantity of antioxidants against the DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2′-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid) radicals were determined and reported. Proximate analysis showed that *S. p. piceifrons* has a high protein content (80.26%), low fat content (6.21%), and fiber content (12.56%) similar to other Orthoptera species. Chitin and chitosan contents of *S. p. piceifrons* were 11.88 and 9.11%, respectively; the recovery percentage of chitosan from chitin was 76.71%. Among the Orthoptera, the protein content of this pest is among the highest while its contents of chitin and chitosan are similar to those of other insect species (e.g., *Bombix mori* Linnaeus [Lepidoptera: Bombycidae]). Our results suggest this pest species is a potential source of bioactive compounds of biotechnological interest for use by pharmaceutical and food industries.

Key words: locust pest, secondary metabolite, bioactive compound, colorimetric technique

Insects in nature fulfill diverse ecological roles as phytophagous, pollinators, predators, parasites, detritivores, and as prey for other animals (Saunders et al. 2016). In addition, they have been used in medicine, nutrition, and industry throughout human history. Many insect species are an important source of proteins, peptides, secondary metabolites, and polymers, such as silk, chitin, and chitosan used by the pharmaceutical and food industries (Milusheva and Rashidova 2017, Teramoto et al. 2019). Since they have numerous metabolic and genetic adaptations to cope with diverse environments, mainly through the production of specialized molecules and enzymes (Dias et al. 2015, Hardersen and Zapponi 2018, Da Costa et al. 2019, Kannan et al. 2019), insects are increasingly considered as an alternative source for discovering compounds of biotechnological interest (Seabrooks and Hu 2017). In this context, insect pests have been analyzed as biological material for their potential as sources of food and bioactive compounds. This represents an alternative to sustainably use of insect pests, and to take advantage of this natural resource that is periodically destroyed by conventional agricultural practices (Torres-Castillo et al. 2015b, Sosa and Fogliano 2017). Among the insect pest groups, the Orthopterans, which include species of locusts and grasshoppers, are considered agricultural pests of global interest due to the crop losses associated with the crops they attack (Hunter 2019). The Central American locust, *Schistocerca piceifrons piceifrons* (Walker), is one of the most important agricultural pests in Mexico and Central America (Díaz-Sánchez et al. 2015). It can feed on approximately 400 plant species, but most commonly on key crops: namely, corn, soybean, bean, sesame, sorghum, peanut, cotton, agave, sugarcane, banana, different fruit trees,

and rice (Díaz-Sánchez et al. 2015). Combatting this pest has harmed the environment immensely and incurred large financial costs due to the repeated use of chemical insecticides, which has prompted the search for benign alternatives, such as the use of natural enemies, entomopathogens, and cultural measures (Barrientos-Lozano et al. 2002). In recent years, using insect pests as food has emerged as a sustainably sound alternative; for example, their collection in the southern region of Tamaulipas can reduce the use of pesticides there (Yen 2009). Some other examples of orthopteran pests being used as food include *Sphenarium purpurascens* Charpentier, *Locusta migratoria* L. and *Schistocerca gregaria* Forskål (Cheseto et al. 2015, Mohamed 2015, Torruco-Uco et al. 2019), which represent important sources of nutrients for local communities. Furthermore, the potential of orthopterans serving as reservoirs of bioactive compounds has been recently highlighted (Torres-Castillo et al. 2018), but their exploration and characterization is first necessary to assess the possible effects they could have in humans. Taking into account the potential use of insects in biotechnology, as a food source, and the need for management alternatives in controlling pest species, this work aimed to assess the compounds of biotechnological and food interest in adults of the Central American locust.

Materials and Methods

Collection and Processing of Biological Material

A sample of 120 adults of *S. p. piceifrons* were collected from various crops in the southern region of Tamaulipas, Mexico (i.e., municipalities of Antiguo Morelos, Mante, and Ocampo). Three groups of 40 adults each were washed with running water to clean them before any processing. Insects were killed by placing them into a freezer for 10 min at –20°C and, before they completely froze, they were eviscerated to remove the intestine and thus reduce contamination associated with remains of undigested plant material (Torres-Castillo et al. 2015b). Each group of insects was dried at 40°C for 72 h in a forced-air oven; this material was then ground in a mortar (Fig. 1) until a fine powder was obtained and passed through a sieve (710-µm sieve 25, U.S.A. Standard test sieve W. S. Tyler), which yielded approximately 35–40 g per insect group. The pulverized material was refrigerated at 4°C until being processed for the extractions. Insect intestines were stored at –20°C for further processing in digestive proteases extraction as indicated below. All further extractions and detections were done in triplicate.

Preparation of Extracts

The extractions were carried out using 1 g of pulverized insects in each of these solvents: distilled water, 50% ethanol, and absolute ethanol (Abs ethanol) which were applied separately, in a ratio of 1:4 (w/v); each mixture was stirred for 10 min and then allowed to sit for 15 min at 4°C. Samples were clarified by centrifugation at 10,000 × *g* for 8 min at 28–30°C. The supernatant fluid was recovered and used as a source of entomochemicals; these extracts were stored at –20°C until used.

Entomochemicals Detection With Colorimetric Tests

The detection of phenolic compounds and flavonoids was conducted using colorimetric reactions (Torres-Castillo et al. 2018). Specifically, detection of phenolic compounds with $KMnO_4$ was carried out by diluting a 100 µl sample of each of the extracts with an additional 100 µl of distilled water (dH_2O); detection was performed by adding 30 µl of 0.1% $KMnO_4$ (Control Técnico and Representaciones [CTR] Monterrey, Nuevo León, Mexico). The reaction was considered positive when the purple color turned yellowish or greenish. Distilled water, 50% ethanol, and Abs ethanol were used as controls. Detection of phenolic compounds with $FeCl_3$ was carried out by diluting a 100 µl subsample with 100 µl of dH_2O and adding to it 30 µl of 0.5% $FeCl_3$ (CTR). The reaction was deemed positive when dark green, blue to dark blue, or even blackish tones appeared in the mixture. To detect flavonoids, to each 100 µl extract, two to four metallic magnesium chips (CTR) and three drops of 25% HCl solution were added. A positive reaction occurred when the solution's color changed to pink, yellow, or orange.

Tannins Detection

This assay was based on gelatin precipitation in a saline solution. For each extract, 10 tubes were prepared with 2 ml of a solution, as follows: three tubes with a 10% NaCl solution, three tubes with 2% gelatin solution dissolved in 10% NaCl, three tubes with a solution of 2% gelatin in distilled water, with one tube left as the control which consisted of the solvent from the corresponding extraction. Next, 500 µl of an extract was added to each tube; this was allowed to stand for 10 min under observation. The reaction was positive when a whitish precipitation appeared or clots formed in the tubes with gelatin and gelatin with 10% NaCl, but not in the distilled water nor in the tube with just 10% NaCl.

Saponins Detection

In a tube, 300 µl of an extract and 300 µl of boiling water were mixed, then cooled, and vigorously shaken for 30 s. Foaming for more than 10 min was considered a positive reaction for the presence of saponin-like compounds.

Detection of Steroidal Compounds by the Liebermann–Burchard Reaction

For each extract, a 300 µl aliquot was evaporated in a test tube, and then resuspended in 300 µl of chloroform. A reaction solution was also prepared by combining 1 ml of acetic anhydride (CTR) with 1 ml of 100% chloroform and three drops of concentrated sulfuric acid, which were mixed by stirring. To develop the reaction, 50 µl of the reaction solution in the tube was mixed with 300 µl of the extract resuspended in chloroform; this mixture sat for 20–25 min and was considered positive when a color red-violet area appeared.

Alkaloids Detection

Subsamples of 75 µl from each extract were heated to 95°C to which 75 µl of HCl (10%) was added (CTR). The tubes were left at room temperature (28–30°C) to cool and then 20 µl of Wagner reagent was added to each and mixed. The reaction was considered positive when a garnet precipitate formed.

Determination of Total Phenolic Compounds

The total phenolic compound (TPC) content was quantified following Singleton et al. (1999). Gallic acid (Sigma-Aldrich, St. Louis, MO) was used to prepare a standard curve; for this, a solution consisting of 5 mg of gallic acid with 50 ml of distilled water was prepared. The reaction for standards was done using a series of dilutions with distilled water, to obtain a gradient of concentrations of 1–8 µg/ml. A 250 µl aliquot of the sample reactions or each standard was mixed with 250 µl of the Folin-Ciocalteau 1 M reagent (Sigma-Aldrich), followed by the addition of 750 µl of sodium carbonate at 20% (CTR). This mixture was allowed to stand for 2 h in the dark, after which its absorbance at 750 nm was recorded. The concentration of TPC was

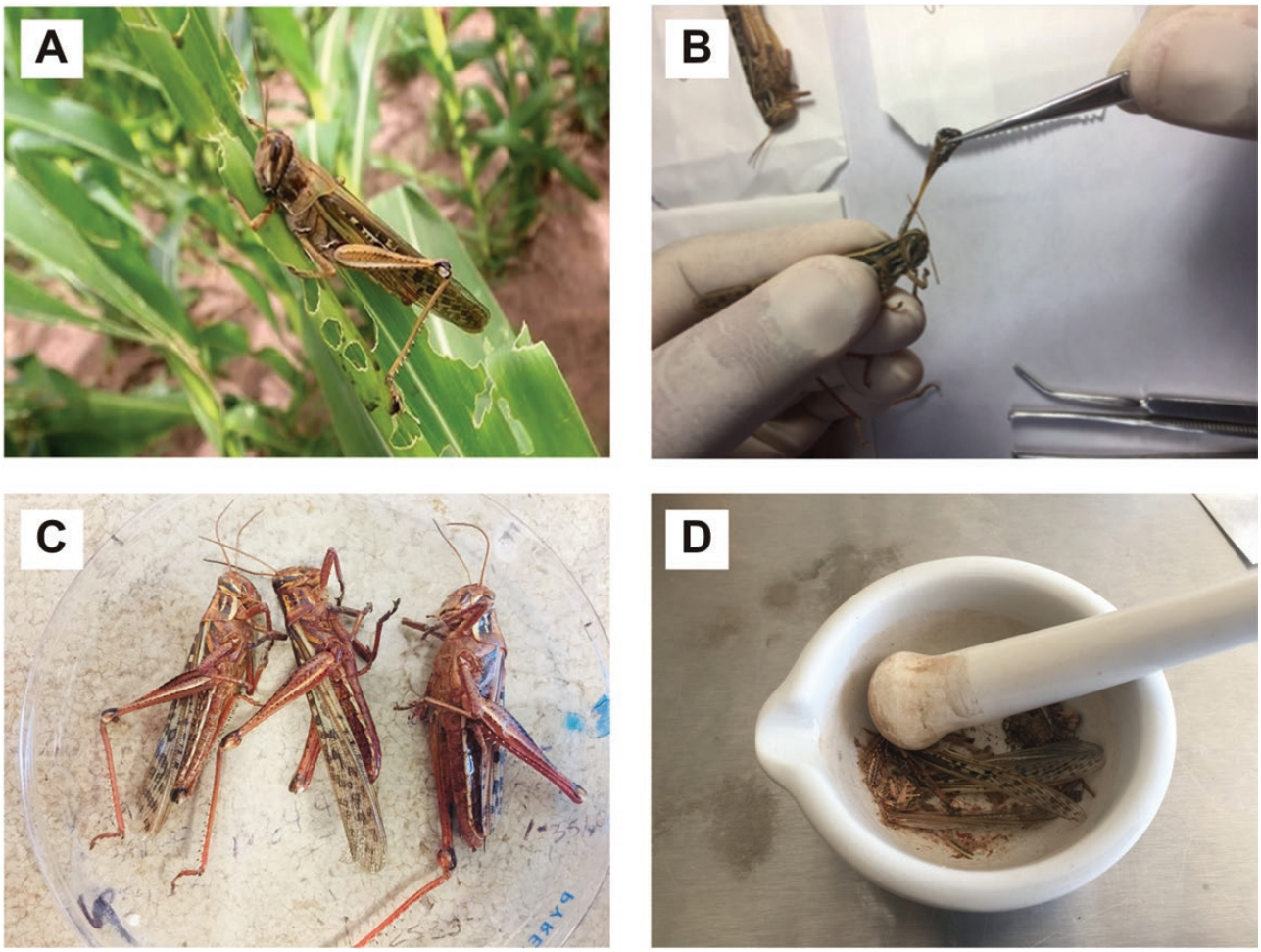

Fig. 1. (A) Adults of *S. p. piceifrons* feeding on foliage in the field; (B) extraction of intestines; (C) dehydrated adult bodies; (D) grinding in mortar to obtain fine powder.

expressed as millimolar of gallic acid per gram of dry weight (mM GAE/g DW) according to the standard curve.

Inhibition of the DPPH Radical

The in vitro free radical-scavenging capacity against DPPH· (2, 2-diphenyl-1-picrylhydrazyl) was evaluated according to the protocol of Brand-Williams et al. (1995). The DPPH solution (Sigma-Aldrich) was prepared at 0.002% by dissolving the compound in methanol (CTR), and this solution kept in the dark and adjusted to 0.7 of absorbance at 515 nm (working solution). Then it was used for the reactions with standards and samples, by mixing 25 µl of samples or standards with a 975 µl working solution, which was allowed to react for 30 min in the dark at room temperature (28–30°C). After this incubation, absorbance of each solution sample was recorded and compared with respect to the absorbance of the working solution. Free radical-scavenging capacity was determined through a comparison with a standard curve prepared for Trolox (Sigma-Aldrich), with concentrations ranging from 100 to 1,200 µM Trolox, and expressed as millimolar equivalents of Trolox by g of dry weight (mM TE/g DW).

Inhibition of Radical ABTS·$^{+}$

Free radical-scavenging capacity against ABTS·$^{+}$ was investigated by using a solution of ABTS (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) (Sigma-Aldrich) at 7 mM (77.6 mg dissolved in 20 ml of distilled water). This solution was mixed with 13.2 mg of potassium persulfate (2.45 mM) (Sigma-Aldrich) (Re et al. 1999), and allowed to react in the dark for 12 to 16 h at room temperature (28–30°C). The ensuing working solution was diluted with Abs ethanol to obtain an initial absorbance of 0.7 (± 0.02) at 732 nm; this was used to evaluate the extracts and standards. For the reaction, 1 ml of the working solution was mixed with 10 µl of a standard or sample, and reacted for 5 min; its absorbance value was then recorded in relation to the work solution. Concentrations were determined using a standard curve of Trolox (range of 100–1,200 µM), and expressed as mM TE/g DW.

Extraction and Detection of Digestive Proteases

Extraction of intestinal proteases was done by grinding 1 g of each intestine sample in a cold porcelain mortar, and mixing it with acidified distilled water (pH 3) in a ratio of 1:4 (w/v). This mixture was stirred and allowed to stand for 10 min at 4°C, then centrifuged at 10,000 × *g* for 10 min. The supernatant was recovered for use as a source of digestive enzymes, and stored at –20°C.

Detection of Intestine Proteolytic Activity of *S. p. piceifrons* and Effect of Chemical Inhibitors

Detection of intestine proteolytic activity (IPA) was conducted following Torres-Castillo et al. (2015a), and trypsin-like activity was

related to the release of *p*-nitroanilide from the substrate *N*-benzoyl-d,l-arginine-4-nitroanilide hydrochloride (BApNa, Sigma-Aldrich). The unit of proteolytic activity was defined as an increase in 0.01 absorbance units at 405 nm for 15 min at 37°C. The blank reaction was prepared by mixing together 135 µl of 0.01 M Tris-HCl (pH 8), 15 µl of 0.01 M BApNa substrate dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich), and 300 µl of 30% acetic acid solution (CTR). The control reaction of bovine trypsin was prepared with 127.5 µl of 0.01 M Tris-HCl (pH 8) and 7.5 µl of trypsin enzyme (50 µg/ml) (Sigma-Aldrich), to which 150 µl of substrate was added, followed by incubation at 37°C for 15 min, after which the reaction was stopped by adding 300 µl of 30% acetic acid solution. The IPA reaction of *S. p. piceifrons* was prepared in the same way as that for the trypsin control reaction, but using only 5 µl of extract; absorbance of the proteolytic activity reaction was used to calculate units of proteolytic activity (UPA) in this way:

$$\mathrm{UPA} = (\mathrm{AUE} - \mathrm{AUB}) / (0.01 * V_e)$$

where AUE indicates the final absorbance units of enzyme activity, AUB is the absorbance of the blank reaction, and V_e is the volume of enzyme (ml).

To confirm the mechanistic type of serine proteinase activity, specifically trypsin, a chemical inhibition was carried out using phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich) at 5 mM dissolved in DMSO, of which 10 µl was added to the IPA reaction (compensated by removing the equivalent volume of reaction buffer). The dependence of IPA on Ca^{+2} as a cofactor of digestive proteases was determined in another series of reactions, where Na ethylenediaminetetraacetic acid (EDTA) (25 mM) was used as an inhibitor, from which 10 µl was added to the IPA reaction solution (as before, this added volume was compensated by withdrawing an equivalent volume of reaction buffer). Once the IPA reactions ended, in the presence of PMSF and EDTA, absorbance was recorded and compared to that of the IPA reaction alone, to determine the remaining activity and percentage inhibition.

Determining the Content of Chitin and Chitosan

From the locust powder, 30 g samples were washed with 5% commercial dishwashing detergent solution and stirred for 15 min at 90°C. Next, they were rinsed by first passing the sample through a sieve (150 µm sieve 100, U.S.A. Standard test sieve W. S. Tyler) and then pouring running water on it to remove the detergent and any residues. The pellet was deproteinized by washing it twice with 500 mM NaOH (CTR); these washes were carried out by continuous stirring of the sample in NaOH solution for 1 h at 90°C, followed by decanting it to let the liquid discard and recovery of the solid sample. After each process, samples were rinsed to remove soluble residues, as described above. The solid material was washed with 1 M NaOH at 90°C for 1 h, by using continuous stirring and decanting as above, and then rinsed until the rinsed liquid attained pH 8; this was performed by placing the sample in the sieve described previously and pouring running water on the sample. The solid material was dried for 48 h at 60°C in an oven. The resulting material was chitin, and its weight was recorded. The obtained chitin was processed via thermoalkaline deacetylation, by immersing the solid, dry material in 70% NaOH in a ratio of 1:3 (w/v) for 2 h at 120°C under 1 kg of pressure, followed by a 12-h resting period. The ensuing material was washed with water to a pH 8, then dried at 60°C for 48 h, at which point its recorded dry weight represented the chitosan content. Chitin content was estimated relative to percentage weight of material recovered here to the initial weight of grounded insects; for percentage of chitosan recovered, this was calculated here as the residue weight obtained from the thermoalkaline deacetylation process with respect to the initial weight of the processed chitin.

Proximate Analysis

This was carried out through a service provided by the Laboratory of Animal Nutrition and Bromatology of the Faculty of Veterinary Medicine and Zootechnics at the Universidad Autónoma de Tamaulipas, Mexico. Moisture and dry matter contents were determined by dehydration of three samples—10 g each of adult locusts freshly collected—that had been eviscerated and subjected to dehydration on previously weighed dishes. They were dehydrated at 60°C in a forced-air oven for 48 h and their contents calculated by difference in weights; expressed as gram per 100 g of dry weight.

Crude protein determination relied on the Kjeldahl method (A.O.A.C. 1975). Here, the value of the protein is obtained by multiplying the sample's nitrogen content by 6.25; this factor assumes proteins consist of 16% nitrogen (100/16). The crude protein contains true protein, as well as non-protein nitrogen compounds.

Fat contents were determined with the Soxhlet protocol (A.O.A.C. 1975). For this, 3 g of a given sample (free of moisture) was weighed and deposited in an asbestos thimble. Then ethyl ether (Sigma-Aldrich) was added to the flask, up to a little more than half its volume, which was then connected to the extractor and condenser. The extraction process lasted 4 h. The difference in weight corresponded to fat content.

Ash content was measured using 1 g subsample deposited in a clean and previously weighed crucible. This was heated to 600°C in a muffle furnace for 2 h. Subsequently, it was extracted and the crucible placed in a desiccator for 30 min. Upon its removal, it was weighed to determine the ash content (=difference between initial and final weight).

Fiber content was determined by the successive acidic and alkaline boiling of the insect samples; the obtained residue after boiling was considered to be crude fiber. For this analysis, 2 g of each residual sample from the prior fat determination analysis was weighed. A sample was placed in a Berzelius glass, to which 200 ml of 1.25% sulfuric acid was added and the vessel then connected to a raw fiber digester and boiled for 320 min, under constant boiling; next, 200 ml of 3.75% NaOH was added and it was left to boil for another 30 min. The solution was removed and its residue washed with hot distilled water on a pre-weighed filter paper, which was subsequently dried at 50°C for 24 h. Percentage of fiber was calculated as difference in a sample's weight.

Statistical Analysis

All experiments were performed in triplicate. In the case of spectrophotometric determinations and the extraction of chitin and chitosan, the data obtained were analyzed by one-way analysis of variance (ANOVA) ($P < 0.05$), the fixed factor being tested was the type of solvent (i.e., three levels); next, a Tukey pairwise comparison of means test was performed by using the statistical package PAST, 3.16 (Hammer et al. 2001).

Results

Using different extracts revealed a particular detection pattern for the entomochemicals (Table 1). In the case of water extraction, positive reactions were observed for five metabolites, but for the extracts with 50% ethanol and Abs ethanol a positive reaction was obtained

for four metabolites (i.e., one fewer in each case). Furthermore, phenolic compounds determined using both reagents ($KMnO_4$ and $FeCl_3$) occurred in both the water and 50% ethanol solvent-based extractions, but not Abs ethanol, for which phenolic compounds reactive with $KMnO_4$ were not detected. This represents a differential phenolic profile pattern depending on the solvent used and the polarity of the diverse phenolic compounds. For the flavonoids, a subgroup of phenolic compounds, detections were positive only with the Abs ethanol but not with the more polar solvents. Detection of tannins, another group of phenolic compounds, gave a positive reaction when water and Abs ethanol extracts were used. This differential pattern in the diversity of phenolic compounds was evident among the solvents. Saponins and steroidal compounds only showed a positive reaction in one solvent, that for saponins was 50% ethanol but it was water for steroidal compounds. Finally, for the alkaloids detection, a positive reaction was observed for these in all three solvents used, indicating a wide range of solubility of these compounds. In sum, each solvent exhibited a differential extraction behavior.

The TPC content varied significantly among the three extraction solvents used ($F = 216.2$; df = 2, 24; $P < 0.001$), being higher for water whose value was slightly superior to the content obtained from 50% ethanol extract, and more than three times that obtained via Abs ethanol (Table 2). Abs ethanol extracted the lowest amount of TPC, only 0.047 mGAE/g DW. The level of scavenging activity against DPPH· differed significantly among the solvents ($F = 3590$; df = 2, 24; $P < 0.001$), this being higher when water was used for the extraction (3.905 mM TE/g DW) than either the 50% ethanol or Abs ethanol as extracts. The Abs ethanol extract provided the lowest activity, with only 0.29 mM TE/g DW, or 13 times less than that obtained from water (Table 2). Regarding the activity levels against the radical ABTS·$^+$ ($F = 907.7$; df = 2, 24; $P < 0.001$), the water extract yielded the highest value, followed by the level obtained with 50% ethanol, at approximately 1 unit lower, and finally the Abs ethanol extract, at more than seven times less than that of the water extract (Table 2). These differences in water and 50% ethanol solvents-derived activity against ABTS·$^+$ vis-à-vis the Abs ethanol were significant. In sum, each solvent had a characteristic extraction profile, among which water seems to be a good solvent to extract compounds with activity against free radicals. By contrast, Abs ethanol was a weak solvent, with little potential to extract compounds with activity against both radicals and TPC (Table 2).

Table 1. Colorimetric detection profiles for each solvent

Reaction	Water	Ethanol 50%	Abs ethanol
Phenolic compounds/$KMnO_4$	+	+	–
Phenolic compounds/$FeCl_3$	+	+	+
Flavonoids	–	–	+
Tannins	+	–	+
Saponins	–	+	–
Steroidal compounds	+	–	–
Alkaloids	+	+	+

(+), the presence (positive reaction); (–), the absence (negative reaction). All tests were performed in triplicate.

Table 2. Contents of TPC and free radical-scavenging capacity against DPPH· and ABTS·$^+$ extracted with different solvents

Parameter	Water	Ethanol 50%	Abs ethanol
TPC (mGAE/g DW)	$0.154^a \pm 0.009$	$0.131^b \pm 0.015$	$0.047^c \pm 0.009$
DPPH (mM TE/g DW)	$3.905^a \pm 0.031$	$3.626^b \pm 0.165$	$0.290^c \pm 0.044$
ABTS$^+$ (mM TE/g DW)	$3.779^a \pm 0.130$	$2.923^b \pm 0.234$	$0.492^c \pm 0.121$

Each solvent represents a different polarity condition during extraction. Different letters between columns and rows indicate statistically significant differences, $P < 0.05$ according to Tukey tests.

In the case of proteolytic activity detection, the IPA detected according to the pH and prototype substrate used was related to a trypsin-like activity (at approximately 26,000 UPA/ml). This confirmed that mechanistic type was present in *S. p. piceifrons*, as further corroborated by the specific inhibition of serine proteases by the PMSF inhibitor, which reduced total activity recorded in the control reaction by as much as 70%. Additionally, the presence of a divalent cation as a cofactor that increases total IPA was confirmed, since the addition of Na EDTA reduced such hydrolytic activity by approximately 40% (Fig. 2). Concerning the hydrolysis of BApNa and inhibition by PMSF, the IPA of *S. p. piceifrons* was similar to that of bovine trypsin used as the control.

Chitin and chitosan contents determination showed the yield of *S. p. piceifrons* for chitin was 11.88 g/100 g of dry weight, and for chitosan it was 9.11 g/100 g of dry weight. Of the obtained chitin, the recovery percentage of chitosan was 76.71%. Those values were then compared to the same contents reported for other insect species in previous studies (Table 3). This investigation showed that *S. p. piceifrons* nutrimental contents are similar to those found in other insects.

Proximate Analysis

The proximate composition of *S. p. piceifrons* adults was as follows: 65.84% moisture content and 34.15% dry matter, with protein, fat, ash, and fiber contents of 80.26, 6.21, 3.35, and 12.56 g per 100 g of dry weight, respectively. Table 4 compares the proximate composition of *S. p. piceifrons* with those of other Orthoptera species.

Discussion

Interest in using insects as food and a source of materials by industry has increased, prompting explorations to obtain compounds of pharmaceutical and nutritional interest, which is of special importance when trying to promote management strategies of pests that allow for their rational exploitation (Paul et al. 2016, Sosa and Fogliano 2017). This study showed that *S. p. piceifrons* is a potential source from which to obtain metabolites of industrial interest, namely phenolic compounds, alkaloids, tannins, flavonoids, steroids, and antioxidants, in addition to chitin and chitosan. Beyond this, the pest is also a worthy candidate for human feeding enterprises due to its nutritional contents from our proximate analysis. Although *S. p. piceifrons* was similar in content to various Orthoptera species previously studied (Table 4), it had the highest protein (80.26 g/100 g dry matter) and one of the lowest fat (6.21 g/100 g dry matter). These findings imply this locust and its material could be used to develop food products with important nutritional benefits.

That *S. p. piceifrons* harbored secondary metabolites or entomochemicals with free radical-scavenging activity supports this insect's potential use as a raw material for industry, medicine, and biotechnology. Entomochemicals have been reported in several insect species, and usually include low-molecular-weight compounds corresponding to diverse chemical groups (Goodey et al. 2015, Erb and Robert 2016, Torres-Castillo et al. 2018). In the case of *S. p. piceifrons*, entomochemicals were detected in the extracts using three solvents, which reflects the diversity of compounds extracted under different polarities. Furthermore, the presence of proteases and polysaccharides highlights this insect as a plausible source of

material for industrial processes (e.g., leather manufacturing, laundry uses, and painting industry) or food interests (e.g., dairy industry, as a meat tenderizer, in dietary supplements). This lets us view this insect as a natural resource with potential uses instead of a pest only, one whose destruction is sought by conventional control strategies.

Phenolic compounds and free radical-scavenging capacity are usually associated with biological activities, including combatting oxidative stress, reduced damage to cell membranes, antidiabetic effects, and antimicrobial properties (Martins et al. 2016, Shahidi and Yeo 2018). Therefore, the presence of these compounds in an insect enhances not only its nutritional potential, but also its possible role as a source of nutraceuticals. The contents of TPC and antiradical activities show that *S. p. piceifrons* is a key source of these compounds and that its behavior, in terms of the extraction process, is similar to that found for the orthopteran *Pterophylla beltrani* Bolívar & Bolívar. For *P. beltrani*, its profiles of antioxidants and phenolic compounds also depended on the solvent used, in that case water and alcoholic extracts (Torres-Castillo et al. 2018). This is consistent with our results for *S. p. piceifrons*, in that the type of solvent used to extract its contents had a marked influence with respect to TPC yields or antiradical activity, with the Abs ethanol being the solvent providing the lowest levels and water the best solvent to obtain high yields. This is likely associated with the polarity and molecular weight of entomochemicals and with the polarity of the solvents used, as indicated in previous works (Ngo et al. 2017, Kim et al. 2018). Hence, our findings could be helpful during the optimization of extraction processes, or for specific application of entomochemicals due to their solubility and polarity behavior; this would be most relevant to the idea of using *S. p. piceifrons* as raw material for industrial processes, where this chemical behavior could be useful to recover high-quality and high-activity enriched concentrates (Castro-López et al. 2016).

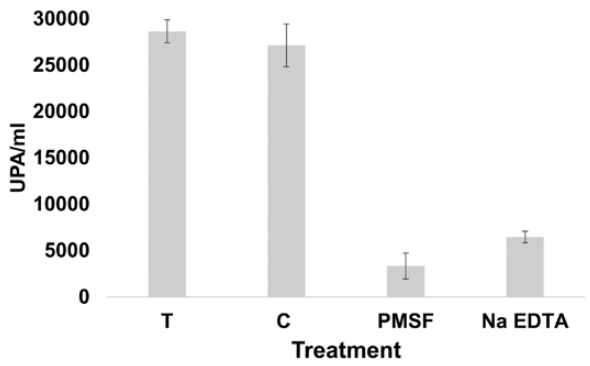

Fig. 2. Units of proteolytic activity (UPA) per ml of crude extract present in *S. p. piceifrons* compared with trypsin and challenged with PMSF and Na EDTA inhibitors. T, trypsin activity used for comparison. C, control of proteolytic activity from *S. p. piceifrons*. Mean for each of the three reactions and SD are shown.

The phenolic contents and free radical-scavenging activity of *S. p. piceifrons* were similar to those observed in plant sources used for feeding; for example, extracts with free radical-scavenging capacity from cranberry (*Vaccinium macrocarpon* L.) contained 194.4 and 144.8 µM Trolox/g DW against ABTS·$^+$ and DPPH, respectively (Oszmiański et al. 2015). This fruit is considered a potent source of antioxidants, yet its ABTS·$^+$ content is lower than what we found in *S. p. piceifrons*, while their DPPH levels are similar; hence, we propose that *S. p. piceifrons* is a good source of these activities and could compete with food products derived from cranberry. In the case of strawberry (*Fragaria ananassa* Duch.), it has 6.05 mGAE/ml, 4.25 mg TEAC/ml against ABTS, and 6.63 mg TEAC/ml against DPPH·$^+$ (Arend et al. 2017), albeit reported values are considerable and these detections were done directly on natural juice. Nonetheless, those contents of strawberry are lower than those extracted by water for *S. p. piceifrons*; a main reason for strawberries' consumption are its touted antioxidant properties and, here, we have shown that an insect pest has higher levels of antioxidant activities and phenolic compounds for consumption. In the case of chili fruits' flesh

Table 3. *Schistocerca piceifrons piceifrons* performance of chitin and chitosan vs other insect species

Species	Chitin	Chitosan	Reference
Schistocerca piceifrons piceifrons Walker	11.88 ± 0.12	9.11 ± 0.64	This work
Bombyx mori L.	2.5–4.2	73–96	Milusheva and Rashidova (2017)
Holotrichia parallela Motsch.	15	NR	Liu et al. (2012)
Apis mellifera L.	19 – 36	16–30	Lootsik et al. (2016)
Calliptamus barbarus Costa	20 ± 0.7	74–75	Kaya et al. (2015a)
Oedaleus decorus Germar	16.5 ± 0.7	75–76	Kaya et al. (2015a)
Pterophylla beltrani Bolívar & Bolívar	11.8 ± 1.05	58.8 ± 2.3	Torres-Castillo et al. (2015b)
Zophobas morio Fabricius	5.22	70.88	Soon et al. (2018)

Contents expressed in g/100 g of dry matter. NR, non reported.

Table 4. Proximate composition of *S. p. piceifrons* vs other orthopteran species

Insect	Protein	Fat	Ash	Fiber	Reference
Schistocerca piceifrons piceifrons Walker	80.26	6.21	3.35	12.56	This work
Zonocerus variegatus L.	17.2	1.18	1.05	1.14	Ademolu et al. (2017)
Sphenarium purpurascens Charp.	65.2	10.8	2.95	9.41	Ramos-Elorduy Blázquez et al. (2012)
Taeniopoda auricornis Walk.	63	10.2	3.97	8.34	Ramos-Elorduy Blázquez et al. (2012)
Schistocerca gregaria Forsk.	76	12.97	2.53	3.33	Zielińska et al. (2015)
Locusta migratoria L.	42.16	18.9	5.72	14.21	Mohamed (2015)
Teleogryllus emma Ohmachi & Matsuura	55.65	25.14	8.17	10.37	Ghosh et al. (2017)
Gryllus bimaculatus De Geer	58.32	11.88	9.69	9.53	Ghosh et al. (2017)

Data expressed as g/100 g of dry matter.

(*Capsicum*), concentrations obtained varied from 119.97 to 438.76 mGAE/g DW and are higher than those we found in *S. p. piceifrons*. But for ABTS·$^{+}$ activity, the range of chilies is between 17.17 and 97.40 µM of TE/g DW while DPPH· ranges from 2.24 to 3.09 µM TE/g DW (Sora et al. 2015), all values lower than those found in *S. p. piceifrons*. Despite phenolics being responsible for antioxidant and antiradical activities, other compounds like alkaloids, sugars, peptides, and proteins also have the potential to scavenge free radicals; this may apply to *S. p. piceifrons*, which had lower TPC contents and higher ABTS·$^{+}$ and DPPH· levels, a pattern opposite to that for chili. The comparisons made above support the potential of this insect as a source of bioactive compounds, one associated mainly with activities against free radicals and related to the chemical diversity of phenolic.

Enzymes used by the biotechnology industry, either their genes or directly through their extraction from diverse sources, has promoted the exploration of novel sources to obtain candidates with properties for industrial applications (Choi et al. 2015). Among the enzymes with applications in the laundry, food, and pharmaceutical industries, the proteases stand out most (Sundus et al. 2016). It has been suggested recently that some insects can be important and diverse suppliers of proteases, either endogenously, or as obtained from the microorganisms associated with host-insects' digestive tract, these findings support the potential of insects as a source of enzymes for application in the food industry (Mika et al. 2015). In this context, the detection of IPA from *S. p. piceifrons* is indicative of its potential regarding the intestinal proteolytic enzymes, which are considered suitable candidates for product development, such is the case of active ingredients now used by the laundry and detergent industries (Akbar and Sharma 2017). Our detection of trypsin-like enzymes in *S. p. piceifrons* agrees with those reported for other orthopterans, such as *Teleogryllus commodus* (Walker), *Gryllus bimaculatus* (De Geer), and *P. beltrani* (Torres-Castillo et al. 2015b, Woodring and Weidlich 2016), which suggest this catalytic mechanism is common in this insect order. Although the above reports used different units to quantify IPA levels, it is nonetheless evident that values detected in *S. p. piceifrons* could be equivalent, underscoring the potential of this insect as a source of proteolytic enzymes for diverse applications.

Chitin and chitosan are polymers with wide existing applications in the medical, agricultural, food, textile, and biotechnology industries, and they are preferably extracted from arthropods (crustaceans and insects) as well as from some fungal species (Cheung et al. 2015, Kaya et al. 2015b, Hamed et al. 2016). In the case of chitosan, this biopolymer has been envisioned for use in manufacturing nanoparticles and generating bioplastics and as an elicitor of plant defenses in crops (Ramkissoon et al. 2016, Dang et al. 2017, Kumaraswamy et al. 2018). However, the functional properties of this biopolymer are influenced by its organismal source and by the extraction process to obtain it; therefore, exploring sources that guarantee the uniformity, costs, and versatility of the materials obtained has been suggested (Cheung et al. 2015, Kaya et al. 2015a). Regarding the potential of *S. p. piceifrons*, obtaining chitin and chitosan from it is feasible because the registered yields are in line with other reported sources (Liu et al. 2012, Kaya et al. 2015a, Torres-Castillo et al. 2015b, Soon et al. 2018). These results thus strengthen the argument for using orthopterans as viable sources of chitin and chitosan (Kaya et al. 2015a). Moreover, polymers obtained from these sources do not contain accumulations of carbonates or other minerals, as in the chitin from crustaceans (Luo et al. 2019), which further hints at the promising use of chitin and chitosan from *S. p. piceifrons* with respect to their purity. Yet more research is still needed to determine the ashes deacetylation degree, and molecular weight, among other covarying factors.

In terms of its nutritional potential, our proximate results suggest this insect has one of the highest known protein content of orthopterans to date, being similar to that of *S. gregaria*. But for its fat content, *S. p. piceifrons* has one of the lowest values, only above *Zonocerus variegatus*; whereas for its contents of ash and fiber, the pest's values were intermediate with respect to other orthopteran. Our study indicates this insect may hold promising applications for diets for humans or domesticated animals, since its food-related contents are comparable to reports on other species of Orthoptera (a group with the greatest potential for the food industry) (Paul et al. 2016), and that it could be used for developing healthier food alternatives in general due to its high protein content, fiber presence, and lower fat content. Even if so, further studies are necessary to assess the digestibility and quality of this insect's proteins and fat contents, which would allow to support its use as a reliable source of nutrients for human consumption in the future. We conclude that, overall, *S. p. piceifrons* is a good candidate for extracting entomochemicals, enzymes, nutrients, and polymers. Contents of some entomochemicals and enzymes are similar to those found in other insects and plants already being used as food. Since our work revealed some biochemical characteristics of entomochemicals and compounds with free radical-scavenging capacities, this should spur and invite further procedures for their extraction, deeper characterization, and future applied uses.

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