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# Chemical characterization and bioactivity of organic extracts from *Annona hypoglauca* Mart. on *Aphis craccivora* Koch (Hemiptera: Aphididae)

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**Abstract:** This study aimed to evaluate the bioactivity of ethanolic leaf extracts and hexanic seed extracts from *Annona hypoglauca* Mart. against *Aphis craccivora* Koch, as well as to characterize their chemical composition. The chemical constituents were identified by gas chromatography using a flame ionization detector and a mass spectrometer. Toxicity, repellency, and translaminar action were assessed on nymphs and adults of the cowpea aphid. The ethanolic leaf extract was mainly composed of terpenoids, including β-sitosterol (23.69%), phytol (12.11%), neophytadiene (9.45%), and elemol (9.75%). In contrast, the hexanic seed extract was predominantly composed of fatty acids, such as oleic (50.50%), palmitic (24.90%), and stearic (9.00%) acids. Both extracts exhibited toxicity to *A. craccivora* nymphs, with the hexanic seed extract showing higher potency, requiring lower concentrations to achieve similar effects ($LC_{50}$ = 3.8 mg·mL$^{-1}$; $LC_{90}$ = 59.63 mg·mL$^{-1}$). The $LC_{50}$ values of 14.80 mg·mL$^{-1}$ for the ethanolic leaf extract and 3.80 mg·mL$^{-1}$ for the hexanic seed extract caused significant toxicity in adults of *A. craccivora*, in addition to inducing repellency and translaminar activity.

**Keywords:** Bioinsecticide; insecticidal plants; integrated pest management; plant extracts; bio-inputs.

## Introduction

Pest management remains one of the major challenges in plant production. *Aphis craccivora* Koch (Hemiptera: Aphididae), commonly known as the cowpea aphid, is a key pest affecting cowpea crops in Brazil and worldwide (Silva et al., 2019; Srinivasan et al., 2019). In addition to its high reproductive rate, this hemipteran is one of the main sap-sucking pests of legumes and a vector of viral diseases, causing significant reductions in yield and economic returns.

The control of *A. craccivora*, as with most economically important pests, relies primarily on synthetic insecticides, which contradicts the principles of Integrated Pest Management (IPM). In contrast, the use of plant-derived extracts with insecticidal potential has produced promising results when integrated with other control methods, offering an alternative to the exclusive or rotational use of synthetic insecticides (Jiang et al., 2018).

Extracts from plants of the genus *Annona* contain complex mixtures of secondary metabolites with insecticidal properties widely documented in the literature (Ikbal and Pavela, 2019; Maciel et al., 2019). These extracts have demonstrated efficacy against various phytophagous arthropods, including *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Hidalgo et al., 2018); *Aphis gossypii* (Hemiptera: Aphididae) and *Eriopis conexa* (Coleoptera: Coccinellidae) (Santos et al., 2018); *Sitophilus zeamais* (Coleoptera: Curculionidae), *Zabrotes subfasciatus* and *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) (Ribeiro et al., 2018); *Tetranychus urticae* (Acari: Tetranychidae) (Maciel et al., 2020); and *A. craccivora* (Rabelo and Bleicher, 2014; Bandeira et al., 2017).

From this perspectivr, this study aimed to evaluate the bioactivity of ethanolic leaf extracts and hexanic seed extracts of *Annona hypoglauca* against *A. craccivora* and to characterize the chemical components of these organic extracts.

**Results and Discussion**

A total of 83.69% of the chemical constituents present in the ethanolic extract of *Annona hypoglauca* leaves were identified (Table 1). In characterizing the phytochemical profile of ethanolic and hexanic leaf extracts of *A. hypoglauca*, Santos et al. (2018) reported the presence of saponins, flavonoids, triterpenoids, and tannins, while Rinaldi et al. (2017) identified four alkaloids: actinodafinine, anonaine, isoboldine, and nornuciferine.

Studies on the secondary metabolites predominant in the ethanolic extract of *A. hypoglauca* have associated these compounds with positive insecticidal effects against various pest arthropods (Jiménez-Durán et al., 2021; Sang et al., 2021; Shehawy et al., 2020). For instance, Dolma et al. (2022) detected a high concentration of neophytadiene (9.45%) in *Triadica sebifera* (Euphorbiaceae), which exhibited toxicity against *A. craccivora* through significant inhibition of acetylcholinesterase (AChE) activity and adverse effects on glutathione S-transferase (GST) enzymes. The authors also observed synergistic effects among the chemical constituents compared to their isolated actions.

A total of 92.50% of the chemical constituents in the hexanic seed extract of *A. hypoglauca* were identified (Table 2). Santos et al. (2015), analyzing the fatty acid profile of *A. hypoglauca*, found oleic and linoleic acids as the main components. The insecticidal and acaricidal potential of palmitic and oleic acids against several pest arthropods has also been reported (Romo-Asunción et al., 2016; Liu et al., 2019). Aziz et al. (2018) detected oleic, stearic, palmitic, and linoleic acids in seed oils of *Azadirachta indica*, with linoleic acid representing 34.69% of the total composition, contributing to the oil's toxicity against *A. craccivora*.

A total of 2,536 and 1,263 nymphs were used in bioassays to test the toxicity of ethanolic and hexanic seed extracts of *A. hypoglauca*, respectively. The hexanic extract was more toxic to *A. craccivora* nymphs ($LC_{50}$ = 3.80 mg $mL^{-1}$ and $LC_{90}$ = 59.63 mg $mL^{-1}$) than the ethanolic extract ($LC_{50}$ = 14.80 mg $mL^{-1}$ and $LC_{90}$ = 776.20 mg $mL^{-1}$). The toxicity ratio (RT) analysis showed that the concentrations of the hexanic extract required to cause 50% and 90% mortality were 3.90 and 13.02 times lower, respectively, than those of the ethanolic extract (Table 3).

Previous studies on extracts from *Annona* species have demonstrated that their lethality to insects and mites varies depending on the plant species, the part used for extraction, and the solvent employed—whether ethanolic or hexanic (Giraldo-Rivera and Guerrero-Álvarez, 2021; Gonçalves et al., 2022).

In contact toxicity assays via spraying, the $LC_{50}$ values of 3.80 mg $mL^{-1}$ for the hexanic (apolar) extract and 14.80 mg $mL^{-1}$ for the ethanolic (polar) extract yielded average mortality rates of 48% and 51%, respectively. These rates did not differ statistically from each other but were significantly higher than the control (3%) (Table 4).

The bioactive potential of *A. hypoglauca* extracts has been supported by reports of diverse biological properties, including antimicrobial (Barnabe et al., 2014), antibacterial (Rinaldi et al., 2017), antitumor (Tundis et al., 2017), cytotoxic and anticancer (Sufrredini et al., 2007), anti-caries (Silva et al., 2014), larvicidal (Pimenta et al., 2003), antifungal (Santos et al., 2014), and acetylcholinesterase inhibitory activities (Santos et al., 2015).

The efficacy of ethanolic leaf and hexanic seed extracts of *A. hypoglauca* against nymphs and adults of *A. craccivora* observed in this study aligns with findings from other *Annona* species, such as *A. squamosa* (Dutra et al., 2020; Rabelo and Bleicher, 2014) and *A. montana* (Bandeira et al., 2017).

Chemical compounds including neophytadiene (Dolma et al., 2022) and palmitic acid (Liu et al., 2019), among others identified in *A. hypoglauca* (Tables 1 and 2), support the insecticidal potential of this species against pest arthropods based on its chemical composition.

Young insects (nymphs and larvae) undergo periods of physiological vulnerability during ecdysis. If extract application occurred when sclerotization and lipid deposition in the epicuticle were incomplete, the physical and chemical barriers that normally limit toxin penetration would be reduced, increasing susceptibility to the tested extracts.

In the repellency assays, significant differences were observed between the ethanolic leaf extract and the hexanic seed extract of *A. hypoglauca* compared to their respective controls (Figure 1).

Investigations into the repellent activity of organic extracts from *Annona* species against various pest arthropods have been documented in the literature (Acda, 2014; Ismail and Sleem, 2021). However, no previous studies were found reporting the repellent effects of ethanolic and hexanic extracts of *Annona hypoglauca* on the cowpea aphid, *Aphis craccivora*. In this context, the present results demonstrate the repellent activity of *A. hypoglauca* extracts against *A. craccivora*.

Supporting this finding, Maciel et al. (2020) and Fernandes et al. (2017) observed the repellent action of *Annona* species extracts against *Tetranychus urticae* (Acari: Tetranychidae). Similarly, Blandino et al. (2020) and Rodríguez-Montero et al. (2020) reported repellency of *Annona* species to *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Bemisia tabaci* (Hemiptera: Aleyrodidae), respectively.

In the no-choice repellency test, a significant interaction was observed between *A. hypoglauca* extract treatments and evaluation time in influencing the repellency behavior of *A. craccivora*. Both the ethanolic leaf extract and hexanic seed extract, at their respective median lethal concentrations, were repellent to the cowpea aphid compared with the control across all evaluation periods. After 60 minutes, the ethanolic (polar) leaf extract showed the highest repellency among the treatments (Table 5).

**Table 1.** Chemical composition of the foliar ethanolic extract of *Annona hypoglauca.*

| RT * (min) | Concentration (%) | Chemical Compound |
|---|---|---|
| 8.498 | 0.58 | 3-hydroxyoxolan-2-one |
| 9.302 | 1.40 | 3-hydroxy-2-methyl-gamma-butyrolactone |
| 14.144 | 0.36 | Allyl carbamate |
| 14.492 | 3.97 | β-Elemene |
| 15.284 | 1.49 | Caryophyllene |
| 18.462 | 9.75 | Elemol |
| 19.283 | 3.07 | Caryophyllene oxide |
| 19.938 | 0.55 | Humulene epoxide II |
| 20.975 | 3.53 | β-Eudesmol |
| 24.825 | 9.45 | Neophytadiene |
| 30.107 | 12.11 | Phytol |
| 27.323 | 0.67 | Decanoic acid |
| 27.963 | 3.10 | Palmitic acid |
| 31.146 | 4.23 | *E,E,Z*-1,3,12-Nonadecatrieno-5,14-diol |
| 31.643 | 0.97 | Ethyl stearate |
| 37.221 | 1.17 | Octadecanal |
| 47.642 | 3.60 | Stigmasterol |
| 48.861 | 23.69 | β-Sitosterol |
| Identified | 83.69 | |

* Retention time (RT)

**Table 2.** Chemical composition of the seed hexanic extract of *Annona hypoglauca.*

| Fatty Acid | Concentration (%) | Chemical Compound |
|---|---|---|
| C14:0 | 0.10 | Myristic acid |
| C16:0 | 24.90 | Palmitic acid |
| C18:0 | 9.00 | Stearic acid |
| C18:1 | 50.50 | Oleic acid (ω-9) |
| C18:2 | 3.30 | Linoleic acid (ω-6) |
| C18:3 | 0.40 | α-Linolenic acid (ω-3) |
| C20:0 | 1.80 | Arachidic acid |
| C20:1 | 2.10 | Gadoleic acid |
| C22:0 | 0.30 | Behenic acid |
| C24:0 | 0.10 | Lignoceric acid |
| Identified | 92.50 | - |

It is noteworthy that the lethal efficacy of plant extracts may not necessarily correlate with feeding deterrence. This dissociation suggests that repellency may result from how compounds act on insect physiology and behavior at different developmental stages, as well as from the synergistic effects of secondary metabolites present in the extracts.

Even under no-choice conditions, the tested extracts maintained strong repellency, indicating potential practical value for pest management. Soares et al. (2021) emphasized that, for vector insects, repellency can prevent feeding and/or colonization, thereby reducing the transmission of plant viruses. These authors also reported that the ethanolic extract of *A. muricata* exhibited oviposition inhibition activity.

Sayed et al. (2022), when evaluating the repellency of *Mentha piperita*, *Mentha longifolia*, *Salvia officinalis*, and *S. rosmarinus* against *Aphis punicae* (Hemiptera: Aphididae), found that all species caused significant repellency. Among the compounds identified, β-elemene, caryophyllene oxide, and caryophyllene—also detected in the ethanolic extract of *A. hypoglauca* (Table 1)—were highlighted by the authors as potential contributors to the observed repellency in *A. craccivora*. These sesquiterpenes have documented insecticidal activity; for instance, caryophyllene, a secondary metabolite found in rosemary and clove, exhibits proven toxicity against several insect species (Plata-Rueda et al., 2018; Santos, 2018).

Acda (2014) studied the phytochemical profile of seed extracts from *A. squamosa* and *A. muricata* and their activity against *Coptotermes gestroi* (Isoptera: Rhinotermitidae), identifying oleic, linoleic, stearic, and palmitic acids, as well as *E,E,Z*-1,3,12-nonadecatriene-5,14-diol. The author suggested that these compounds act synergistically to produce a repellent effect on subterranean termites. Since these substances were also identified in the leaf extract of *A. hypoglauca* (Table 1), it is plausible that they contribute to or enhance the repellency observed against *A. craccivora*. Further systematic studies with polar, intermediate, and apolar extracts from *Annona* species are recommended to better elucidate these effects.

**Table 3.** 50 % ($LC_{50}$) and 90 % ($LC_{90}$) lethal concentrations (mg/mL$^{-1}$) of the foliar ethanolic extract and seed hexanic extract of *Annona hypoglauca* on *Aphis craccivora* nymphs.

| Extract | No. of aphids | Slope (± EP)[a] | $LC_{50}$ (CI 95 %) | $LC_{90}$ (CI 95 %) | $\chi^{2}$ [b] | p-value | RT $LC_{50}$[c] | RT $LC_{90}$[c] |
|---|---|---|---|---|---|---|---|---|
| Ethanolic extracts of leaves | 2536 | 0.74±0.16 | 14.80 (12.0; 18.7) | 776.20 (402.00; 1933.00) | 52.12 | 0.999 | 3.90 | 13.02 |
| Hexanic extracts of seeds | 1265 | 1.07±0.19 | 3.80 (3.08; 4.88) | 59.63 (33.77; 138.29) | 26.25 | 0.999 | | |

[a] Standard error; [b] Chi-square; [c] Toxicity ratio between the extracts at the same concentration (x) as the lethal concentration: RT = (higher LCx value - Extract 1)/(lower LCx - Extract 2).

**Table 4.** Mortality (mean ± SD) of adults of *A. craccivora* under an $LC_{50}$, applied via spraying using the foliar ethanolic extract and seed hexanic extract of *Annona hypoglauca.*

| Treatment | Mortality (%) * |
|---|---|
| Seed hexanic extract - $LC_{50}$ of 3.80 mg/mL$^{-1}$ | 48.00 ± 2.49 a |
| Foliar ethanolic extract - $LC_{50}$ of 14.80 mg/mL$^{-1}$ | 51,00 ± 3.79 a |
| Control | 3.00 ± 2.13 b |
| CV (%) | 26.91 |

* Values followed by the same letter do not differ by Tukey's test (p 0.05). CV: Coefficient of variation.

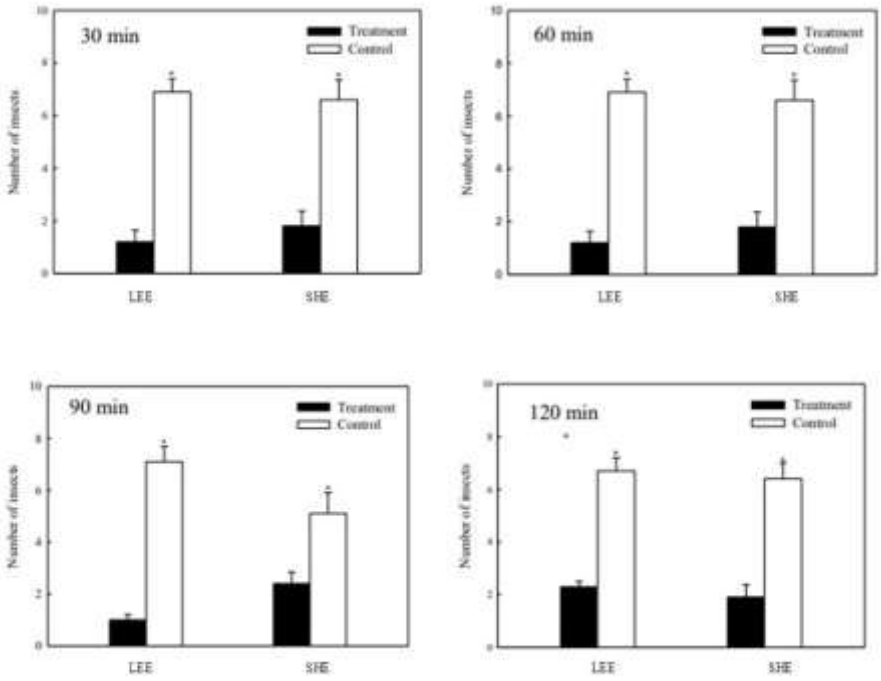


**Figure 1.** Number of adult aphids (mean ± SD*) recorded on leaf discs of cowpea beans treated with the foliar ethanolic extract (LEE) and seed hexane extract (SHE) of *Annona hypoglauca* (choice test) (25 ± 2 ºC, RH 60 ± 10 % and 12-hour photophase). Means in the columns differed by the t-test (P ≤ 0.05).

A significant interaction was also observed between extract treatments and evaluation time regarding *A. craccivora* mortality, with evidence of translaminar action of the extracts compared with the control 48 hours after application. Aphid mortality increased progressively over time (Table 6).

Previous studies have reported translaminar effects of *Annona* extracts on various pest arthropods, consistent with the findings for *A. hypoglauca* on *A. craccivora*. Soares et al. (2021) observed that the ethanolic seed extract of *A. mucosa* exhibited translaminar action, promoting 31.1% mortality in *Bemisia tabaci*. Brito et al. (2020) evaluated extracts from different structures of *A. montana*, *A. muricata*, and *A. sylvatica* against *Tuta absoluta*, and found that leaf and seed extracts of *A. muricata* caused pronounced toxicity and growth inhibition in larvae through residual contact and translaminar activity.

**Table 5.** Number of adult aphids (mean ± SD) recorded on leaf discs of cowpea beans treated with the foliar ethanolic extract and seed hexanic extract of *Annona hypoglauca* (no-choice test) (25 ± 2 ºC, RH 60 ± 10% and 12-hour photophase)*.

| Treatment | Time (minutes) | | | |
|---|---|---|---|---|
| | 30 | 60 | 90 | 120 |
| Control | 9.90 ± 0.10 a | 9.70 ± 0.21 a | 10.00 ± 0.00 a | 9.80 ± 0.13 a |
| Hexanic extracts of seeds ($LC_{50}$ of 3.80 mg/mL$^{-1}$) | 6.60 ± 0.62 b | 6.60 ± 0.82 b | 7.00 ± 0.39 b | 6.00 ± 0.30 b |
| Ethanolic extracts of leaves ($LC_{50}$ of 14.80 mg/mL$^{-1}$) | 6.40 ± 0.54 b | 5.00 ± 0.70 c | 6.80 ±0.53 b | 5.90 ± 0.38 b |
| CV (%) | 19.65 | | | |

* Values followed by the same letter, in the column, do not differ by Tukey's test (p 0.05). CV: Coefficient of variation

**Table 6.** Mortality (%) (mean ± SD) of *Aphis craccivora* nymphs after application of the foliar ethanolic extract and seed hexanic extract of *Annona hypoglauca* (translaminar effect) on cowpea leaves (25 ± 2 ºC, RH 60 ± 10% and 12-hour photophase).

| Treatment | Times | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| Hexanic extracts of seeds ($LC_{50}$ of 3.80 mg/mL$^{-1}$) | 0.00 ± 0.00 a | 9.35 ± 3.47 a | 21.55 ± 3.76 a |
| Ethanolic extracts of leaves ($LC_{50}$ of 14.80 mg/mL$^{-1}$) | 0.00 ± 0.00 a | 8.50 ± 2.32 a | 18.04 ± 4.50 a |
| Control | 0.00 ± 0.00 a | 0.44 ± 0.33 b | 0.96 ± 0.48 b |
| CV (%) | 58.62 | | |

* Values followed by the same letter, in the column, do not differ by the Tukey test (p 0.05). CV: Coefficient of variation.

Translaminar action refers to the ability of certain insecticides to penetrate the leaf lamina and reach insects located in areas not directly contacted by the spray solution. This depth effect provides an additional advantage among pesticide modes of action. Therefore, if such properties are also present in plant extracts, they may extend their potential as alternatives to conventional chemical control.

**Material and Methods**

***Rearing of Aphis carccivora***

Nymphs and adult females of *A. craccivora* were collected from a cultivated cowpea (*Vigna unguiculata*) field at the Agrarian Sciences Center, Federal University of São Francisco Valley (UNIVASF). The aphids were reared on cowpea plants ('BRS Aracê') grown in pots and maintained in 2.00 × 2.00 m cages covered with an anti-aphid screen.

***Collection of botanical material***

Leaves and seeds of *Annona hypoglauca* Mart. were collected in March 2018 from a flooded forest (igapó) area in the municipality of Mucajaí, Roraima State, Northern Amazon, Brazil (2°25′48″ N; 60°54′00″ W). A voucher specimen was deposited in the indexed herbarium of the Federal University of Roraima (UFRR 9045). Access to the genetic material was registered in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen), in accordance with Brazilian legislation (registration no. AE6C7D9).

***Preparation of plant powders***

After collection, the plant materials (leaves and fruits) were transported to the Phytotechnology Laboratory, Agrarian Sciences Center, Federal University of Roraima. The leaves and seeds of *A. hypoglauca* were selected, weighed, washed under running water, and dried in a forced-air oven at 40 °C to prevent denaturation of secondary metabolites. The materials remained in the oven until constant mass was achieved, after which they were ground to a fine powder (20–40 mesh). The resulting leaf and seed powders were stored in hermetically sealed glass containers until extraction.

***Preparation of organic extracts***

The ethanolic leaf extract was obtained following the methodology described by Carvalho et al. (2017), and the hexane seed extract according to the procedure described by Melo Filho et al. (2018).

***Chemical composition***

The chemical constituents of the hexane seed extract were identified by gas chromatography coupled to mass spectrometry (GC–MS) using two preparatory steps: (1) microwave hydrolysis and (2) methylation of free fatty acids, as

described by Sande et al. (2018) and Melo Filho et al. (2018). The chemical components of the ethanolic leaf extract were determined by gas chromatography with a flame ionization detector (GC–FID), following Carvalho et al. (2013).

***Formulation of emulsions***

For the bioassays, liquid emulsions of ethanolic leaf and hexane seed extracts of *A. hypoglauca* were prepared. The formulations consisted of reverse osmosis water, Tween 80®, and the corresponding botanical extracts. To minimize experimental error, a stock emulsion (standard) was also prepared by mixing 10 g of extract, 50 mL of reverse osmosis water, and 30 µL of Tween 80®. This stock solution facilitated accurate aliquot extraction and preparation of working concentrations used in the bioassays against *A. craccivora*.

***Toxicity bioassays on nymphs***

To assess the toxicity of *A. hypoglauca* extracts on *A. craccivora* nymphs and estimate the lethal concentrations at 50% ($LC_{50}$) and 90% ($LC_{90}$), preliminary tests were conducted with varying concentrations of ethanolic leaf and hexane seed extracts. These tests determined the upper (≈100%) and lower (≈0%) mortality limits, following the method of Bliss (1934). In bioassay 1, the concentrations tested for the ethanolic leaf extract were: water and Tween 80® (0.06%), 1.00, 1.98, 3.91, 7.74, 15.32, 30.30, and 59.95 mg $mL^{-1}$. In bioassay 2, for the hexane seed extract, the concentrations were: water and Tween 80® (0.06%), 0.46, 0.79, 1.37, 2.36, 4.07, 7.02, and 12.10 mg $mL^{-1}$.

Cowpea plants ('BRS Aracê') were grown in 500 g pots, and three adult aphids from the stock colony were placed per leaf (experimental unit) and confined in mini cages (Ø 4 × 2 cm) for 48 h to allow colony establishment and nymph production. After this period, adults were discarded, and only nymphs (2–3 days old) were retained on the leaves. Treatments were applied by spraying with a double-action professional airbrush (STEULA CB66-05®, 0.5 mm nozzle) connected to a pneumatic pump at 5 psi. Preliminary tests determined that a 30 µL aliquot of each working solution provided optimal coverage for spraying.

Nymph mortality was evaluated 24 h after application. The experiment followed a completely randomized design with eight treatments and ten replicates, each plot represented by a leaf with an average of 20 nymphs. The insects were not manipulated to avoid damage to their mouthparts. Lethal concentrations were estimated by Probit analysis using R Studio (R Studio Team, 2020).

***Toxicity bioassays on adults***

Toxicity tests on adult *A. craccivora* followed the same methodology and application procedures used to determine $LC_{50}$ and $LC_{90}$. Ten adult aphids (8–10 days old) were confined in mini cages attached to cowpea leaves. The treatments were: a) Control (water and Tween 80® - 0.06%); b) $LC_{50}$ of ethanolic leaf extract plus Tween 80® - 0.06%; and c) $LC_{50}$ of hexanic seed extract plus Tween 80® - 0.06%. Treatments were applied by spraying, and mortality was assessed 24 h after application. The experimental design was completely randomized with three treatments and ten replicates, each consisting of one leaf with ten adult aphids. Data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at a 5% significance level.

***Food repellency bioassay***

Two repellency tests of *A. hypoglauca* extracts on *A. craccivora* were conducted. In test 1 (choice test), two bioassays were performed, one for each type of *A. hypoglauca* extract. In this case, aphids were allowed to choose between two treatments (plant extract and control). Ten adult *A. craccivora* (8–10 days old) from the rearing stock were released at the center of an arena consisting of a Petri dish (Ø 7 cm × 5 cm high) containing four leaf discs (Ø 4 cm), properly labeled and arranged equidistantly. Of the four discs used, two were treated with $LC_{50}$ extract solutions plus Tween 80® (0.06%) (ethanolic and hexanic) of *A. hypoglauca*, and the other two were treated with water and Tween 80® (0.06%) as control. Before testing, aphids were deprived of food for two hours. Evaluations were carried out at 30, 60, 90, and 120 minutes, recording the number of aphids present on each leaf disc (treated and untreated). The experimental design was completely randomized in a split-plot arrangement. Each treatment had ten replicates, each represented by an arena with ten adult aphids. Data were analyzed by ANOVA, and means were compared using Student's *t*-test at a 5% significance level.

In test 2 (no-choice test), two bioassays were performed, one for each type of *A. hypoglauca* extract. The methodology was similar to that used in the choice test, except that in this case, the leaf discs (Ø 4 cm) treated with the plant extract and the control were offered individually in separate Petri dishes, so the aphids had no choice between treatments. The experimental design was completely randomized in a split-plot arrangement over time. For each treatment, ten replicates were used, each consisting of one arena (Petri dish) with ten adult aphids (8–10 days old). Evaluations were conducted at 30, 60, 90, and 120 minutes, recording the number of aphids on each leaf disc. Data were analyzed by ANOVA, and means were compared using Tukey's test at a 5% significance level.

***Translaminar action bioassay***

To evaluate the translaminar effect of *A. hypoglauca* extracts on *A. craccivora*, the same methodology described for determining $LC_{50}$ and $LC_{90}$ was used, except that in this case, treatments were applied to the opposite side of the leaf where the insects were confined. The experimental design was completely randomized with three treatments and fifteen replicates, each plot consisting of one leaf with an average of 20 nymphs (2–3 days old) of *A. craccivora*. After the treatments dried, the nymphs were placed on the leaf surface and protected by mini cages. Handling was minimized to

avoid damaging the insects' mouthparts. Mortality was assessed 72 hours after treatment application. The results were subjected to analysis of variance, and means were compared using Tukey's test at a 5% significance level.

## Conclusion

The chemical composition of the ethanolic extract from *Annona hypoglauca* leaves was predominantly composed of terpenoids—β-sitosterol, phytol, neophytadiene, elemol, and *E,E,Z*-1,3,12-nonadecatriene-5,14-diol—whereas the hexanic extract from the seeds was mainly characterized by fatty acids, including oleic, palmitic, stearic, linoleic, and gadoleic acids. The hexanic seed extract exhibited greater toxicity to *Aphis craccivora* nymphs than the ethanolic leaf extract. Both nymphs and adults of *A. craccivora* were equally affected by the ethanolic leaf and hexanic seed extracts.
At their respective median lethal concentrations, both extracts demonstrated repellency to *A. craccivora* adults in choice and no-choice assays. Additionally, at these concentrations, the extracts exhibited translaminar activity against *A. craccivora* nymphs 48 hours after application.

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**Statement of Author Contributions**
Moisés Felix de Carvalho Neto: experimental design, data collection, scientific writing, and statistical analysis; Antonio Cesar Silva Lima: supervision and revision of scientific writing; Rita de Cássia Rodrigues Gonçalves-Gervásio: co-supervision, review of scientific writing, and statistical analysis; Antonio Alves de Melo Filho: co-supervision, review of scientific writing, and chemical characterization; Edigênia Cavalcante da Cruz Araújo: review of scientific writing and chemical characterization; Gabriel Lopes Bezerra: support in conducting experiments; Camila Alves de Carvalho Melo: support in conducting experiments; Maria da Conceição Campêlo Ferreira: support in collecting and obtaining plant extracts; Caroline Bogo Rota: review of the English language; Diones Lima de Souza: support in conducting experiments.

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