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Antimicrobial, insecticidal, and antioxidant activity of essential oil and extracts of *Guarea kunthiana* A. Juss

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The goal of this study was to assess the antimicrobial activity of the essential oil and aqueous, alcoholic, and ethyl acetate extracts of *Guarea kunthiana* A. Juss against ten *Salmonella* serotypes from poultry origin (Enteritidis, Infantis, Typhimurium, Heidelberg, Mbandaka, Give, Saintpaul, Ohio, Gallinarum, and Agona); the insecticidal potential of the oil and extracts against larvae and adults of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) (Panzer, 1797); and also to determine the antioxidant activity of these compounds using the capture method of radical 2.2-diphenyl-2-picrylhydrazyl (DPPH). With respect to the antimicrobial activity of the essential oil of *G. kunthiana*, the most sensitive serotypes were Infantis, Typhimurium, and Give, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 54.6 µg/ml. Regarding the other serotypes tested, the action of the oil was classified as moderate, weak, or inactive. With respect to the extracts, the greatest microbial susceptibility was observed in the activity of the alcoholic extract, with MIC and MBC values of 0.39 mg/ml for the serotype Infantis, and MIC and MBC values of 0.78 mg/ml for the serotype Gallinarum. The results of the insecticidal activity of the essential oil and the extracts were found to be low, with 28% mortality of larvae and 12% of adults, at a concentration of 200 mg/ml. Regarding the extracts, the best results were observed using the alcoholic extract at concentrations of 10%, with 34 and 44% mortality of larvae and adults, respectively. The values of antioxidant activity showed that there were no significant differences between the synthetic antioxidant butylhydroxytoluene (BHT), the essential oil, and the alcoholic extract, revealing that both the essential oil and the alcoholic extract of *G. kunthiana* exhibited high antioxidant capacity.

Key words: Poultry farming, *Salmonella* species, *Alphitobius diaperinus*, mortality.

INTRODUCTION

The intense growth of aviculture sector has caused some problems, such as the emergence of enteric diseases caused mainly by the genus *Salmonella*, which can lead to loss of productivity due to increased mortality and contamination of products of poultry origin (Santos and Turnes, 2005).

Another major problem of the poultry sector is the proliferation of insects and pests, mainly *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) (Panzer, 1797), known in Brazil as "*cascudinho* of the aviaries." Besides

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damaging feed conversion and the weight gain of birds, larval and adult forms are supporters of viable pathogens on the external surface and in their digestive tract. Therefore, they are potential vectors of pathogens that cause diseases, mainly bacteria of the genus *Salmonella* species (Hazeleger et al., 2008).

Due to the constant concern of the population compared to synthetic insecticides, as toxicity to applicators, environmental pollution and the presence of residues in food, researchers have been encouraged to develop studies in order to insert new alternative control practices for pest control, especially the use of insecticides of plant origin (Almeida et al., 2004; Tavares and Vendramim, 2005; Pereira et al., 2008).

Members of Meliaceae family exhibit a wide variety of chemical compounds, including limonoids, triterpenes, steroids, diterpenes, sesquiterpenes and coumarins (Cortez et al., 2000; Garcez et al., 2004; Lago and Roque, 2009). The limonoids are the most abundant compounds, and probably the greatest representatives of the class of terpenes with insecticidal activity (Luo et al., 1999). In addition to these properties, these compounds may also act as antitumor, antifungal, antibacterial, antiviral (Champagne, 1992), antioxidants (Jayaprakasha and Patil, 2007), antileishmanial (Lima, 2006) and antimalarials (Kirandeep et al., 2009).

Studies conducted on the Brazilian native plant *Guarea kunthiana* A. Juss (Meliaceae) reported the insecticidal potential of its hexanic and ethanolic extracts obtained from the leaves (Mesquita et al., 2005; Coelho, 2006; Lima, 2006). However, the antimicrobial and antioxidant potential as well as the insecticidal activity against *A. diaperinus* have not been reported in the literature, thus the present study is the first one on the subject.

The goal of this study was to assess the antimicrobial activity of the essential oil and aqueous, ethyl acetate, and alcoholic extracts of *G. kunthiana* against ten *Salmonella* serotypes in order to determine the insecticidal activity of these compounds against *A. diaperinus* and their antioxidant activity.

MATERIALS AND METHODS

Collection and identification of plant

The leaves of *G. kunthiana* were collected from September to December, 2013 in a rural property of the western region of the State of Paraná, Brazil (Latitude 24°31' S, Longitude 53°44' W, altitude of 442 m). Drying of the leaves was held in an oven at 35°C for subsequent milling in a knife mill until obtaining the crushed plant material with particle size less than 0.42 mm. An exsiccate specimen was sent to the Herbarium of the State University of Oeste do Paraná (UNOP) for botanical identification, deposited under number 7843 Pandini, J. A.

Obtaining extracts

The preparation of extracts was carried out in accordance with the method proposed by Weber et al. (2014) and Ceyhan et al. (2012), with modifications. For the preparation of the aqueous extract, 10 g of crushed plant material were added to 100 ml of distilled water. This mixture was kept on a rotary shaker at 220 rpm for 24 h. After this period, the solution was filtered using Whatman No. 1 filter paper and centrifuged for 15 min at 5000 rpm. The supernatant was collected, thus obtaining the extract at the final concentration of 100 mg/ml. The extract was stored at 4°C. The organic extracts were prepared according to the same method of the aqueous extract. After collection of the supernatant, the extract was submitted to rota-evaporation. The crude extracts were diluted in 10% dimethylsulfoxide (DMSO) until a final concentration of 400 mg/ml and stored at 4°C.

Phytochemical prospection

The phytochemical tests for detecting the presence of steroids, triterpenoids, tannins, alkaloids, coumarins, saponins, anthocyanidins, and flavonoids were conducted according to the method proposed by Matos (1997).

Obtaining essential oil

The essential oil was obtained in accordance with the method proposed by Weber et al. (2014). To this end, about 60 g of crushed plant material were submitted to extraction by hydrodistillation in 700 ml of distilled water for a period of 3 to 4 h using a Clevenger-type apparatus. After extraction, the essential oil was stored at 4°C in the dark.

Antimicrobial activity

Microorganisms and test conditions

The microorganisms used were 10 *Salmonella* serotypes of greater occurrence in the western region of the State of Paraná, Brazil (Scur et al., 2014). These serotypes were isolated from feces (cloacal swabs), poultry litter (drag swabs), and poultry feed (flours/ingredients of poultry feed) from different poultry houses of the western region of the State of Paraná, Brazil, provided by a veterinary laboratory of Cascavel, Paraná, Brazil. The serotypes were Enteritidis, Infantis, Typhimurium, Heidelberg, Mbandaka, Give, Saintpaul, Orion, Gallinarum, and Agona.

For the test, the microorganisms were recovered from brain heart infusion broth (BHI) and incubated for 24 h at 36 ± 0.1°C. After this period, the microbial strains were standardized in saline solution (0.85%) until they reached the final concentration of 1×10^5 UFC/ml to serve as inoculum.

Determination of the minimum inhibitory concentration (MIC)

Essential oil and plant extracts

The MIC of the essential oil and plant extracts was determined according to the broth microdilution method proposed by Santúrio et al. (2007) and Weber et al. (2014). The essential oil was diluted in methyl alcohol and Mueller-Hinton broth (MHB) at a proportion of 1:10 until it reached the concentration of 7,000 µg/ml. A total of 150 µl of MHB were distributed from the second column in 96-well microtiter plates. The first columns received 300 µl of oil solution of *G. kunthiana* and, thereafter, dilutions of 7,000 to 3.4 µg/ml were performed. Finally, 10 µl of inoculum were added in each well and the plates were incubated for 18 to 24 h at 36 ± 0.1°C. After this time interval, 10 µl of 10% triphenyltetrazolium chloride (TTC) were

added and the plates were again incubated for 3 h at 36 ± 0.1°C. The presence of red coloring was interpreted as negative evidence of inhibitory effect of the essential oil.

Determination of the minimum bactericidal concentration (MBC)

The MBC of the extracts and the essential oil was performed in accordance with the method proposed by Weber et al. (2014). Before the addition of the TTC into all wells, an aliquot of 10 µl was withdrawn and inoculated on MH agar surface. The plates were incubated for 24 h at 36 ± 0.1°C and, after this period, the MBC was defined as the lowest concentration of essential oil able to cause the death of the inoculum.

An amount of 200 mg/ml of gentamicin was used as positive control and methyl alcohol as negative control to test the essential oil, and 10% DMSO was used to test the extracts.

Classification of antimicrobial activity

The MIC and MBC of the essential oil and the extracts were classified according to the criteria proposed by Sartoratto et al. (2004) for the essential oil, and according to Araújo (2010) for the plant extracts. For the essential oil, the activity was classified as high (<100 µg/ml), moderate (between 100 and 500 µg/ml), low (between 500 and 1000 µg/ml), and very low (above 1000 µg/ml). For the extracts, the classification was high (≤12.5 mg/ml), moderate (12.5 to 25 mg/ml), low (50 to 100 mg/mL), and very low (above 100 mg/ml).

Insecticidal activity

The assessment of the insecticidal potential of the essential oil and extracts of *G. kunthiana* was conducted according to the method proposed by Marcomini et al. (2009), with modifications. To this end, larvae and adults of *A. diaperinus* from a commercial poultry house of Maripá, Paraná, Brazil were used.

Application of plant extracts

Aqueous, alcoholic, and ethyl acetate extracts of *G. kunthiana* were diluted in 10% DMSO until they reached concentrations of 10 and 5%. The application of the extracts was performed directly on the insects, arranged in a Petri dish, and 1 ml was sprayed on each plate using a Potter tower calibrated with pressure of 0.70 kgf/cm^2. After application, the insects were transferred to another Petri dish and kept in a BOD camera (26 ± 1°C and 14-h photophase), and fed with about 2 g of poultry feed. Five repetitions with 30 insects each were prepared, with a total of 150 insects per treatment. The control procedure consisted of the application of sterile distilled water (general control) on the insects and also a control procedure with 10% DMSO.

Application of essential oil

The essential oil of *G. kunthiana* was diluted in 10% acetone until it reached concentrations of 100 and 200 mg/ml. The application on the larvae and adults of *A. diaperinus* was carried out following the same procedures of the extracts application. The control procedure consisted of the application of sterile distilled water and acetone at a concentration of 10%, following the same procedure performed in the other treatments. The assessment of insects' mortality was conducted after 10 days, considering dead those insects that did not respond to the touch of a clamp.

Analysis of data

The data were assessed to determine normality and homogeneity using Kolmogorov-Smirnov test and Levene's test (Central Limit Theorem), respectively. From this information, the variance was assessed using the F test and the averages were compared using Tukey's test at 5% significance employing the Statistica® software, version 7.0 (StatSoft Inc, Tulsa, USA).

Antioxidant activity

The antioxidant activity was determined using the method of reducing the free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH) based on the method proposed by Scherer and Godoy (2009), Rufino et al. (2007), and Weber (2014). To this end, aliquots of 0.1 ml of the oil at a concentration of 6,000 µg/ml and aqueous of 100 mg/ml, ethyl acetate and alcoholic of 400 mg/ml extracts were treated with 3.9 ml of DPPH methanolic solution (0.2 mM) and slightly homogenized in a tube agitator. After agitation, the tubes were left to stand for 30 min in the dark. After the reaction time, the absorbance of samples was measured at 515 nm. An aliquot of 0.1 ml of the control solution (methyl alcohol, acetone, and water) was used for the negative control and the synthetic antioxidant butylhydroxytoluene (BHT) was used for the positive control under the same conditions of the negative control. Methyl alcohol was used as blank for the calibration of the spectrophotometer. The ability of free radical sequestration was expressed by the equation:

$$I\% = [(Abs_0 - Abs_1) / Abs_0] \times 100,$$

where Abs_0 is the absorbance of the control and Abs_1 is the absorbance of the sample. The IC_{50} (amount of antioxidant substance required to reduce by 50% the initial DPPH concentration) was calculated on the basis of the equation of the line obtained from the calibration curve. The tests were carried out in triplicate.

Analysis of data

The data obtained by calculations of DPPH radical sequestration capacity and the IC_{50} were assessed using Tukey's test, at 5% significance, employing the Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

Phytochemical prospecting

The phytochemical profile of extracts of *G. kunthiana* revealed the occurrence of triterpenoids in the aqueous and ethyl acetate extracts, and tannins and flavonoids in the aqueous and alcoholic extracts. The presence of alkaloids, coumarins, saponins, anthocyanins, and anthocyanidins was not observed (Table 1).

Antimicrobial activity

Considering the MIC and MBC values, it was possible to confirm that the essential oil exhibited high activity

Table 1. Classes of secondary metabolites present in the aqueous, alcoholic, and ethyl acetate extracts of *G. kunthiana*.

Classes of metabolite	Extracts		
	Aqueous	Alcoholic	Ethyl acetate
Pyrogallic tannins	+	+	-
Alkaloids	-	-	-
Coumarins	-	-	-
Saponins	-	-	-
Anthocyanins	-	-	-
Flavonoids	+	+	-
Triterpenoids	+	+	+
Steroids	-	-	-

+: Presence of the compound; -: Absence of the compound.

against *Salmonella* Infantis, *Salmonella* Typhimurium, and *Salmonella* Give, with MIC and MBC values of 54.6 µg/ml. Regarding *Salmonella* Saintpaul and *Salmonella* Agona, the values were moderate, with MIC and MBC of 218.7 µg/ml for the serotype Saintpaul, and MIC and MBC of 437 µg/ml for serotype Agona. For the serotypes Ohio and Gallinarum, the values showed low activity, with MIC and MBC of 875 µg/ml. With respect to *Salmonella* Heidelberg, the MIC and MBC values of 1750 µg/ml were considered very low. The essential oil had no activity against *Salmonella* Enteritidis and *Salmonella* Mbandaka (Table 2).

With respect to the classification of antimicrobial activity of extracts, it was possible to confirm that the alcoholic extract exhibited activity against the serotypes Infant and Gallinarum, with MIC and MBC values of 0.39 and 0.78 mg/ml, respectively. On the other hand, the ethyl acetate extract showed low antimicrobial activity against the serotypes tested, with MIC and MBC values ranging from 100 to 200 mg/ml. Lastly, the aqueous extract did not exhibit antimicrobial activity (Table 3).

Insecticidal activity

The 10% alcoholic extract exhibited the highest percentage of larval mortality (34.0%), whereas the ethyl acetate extract exhibited 21.3 and 26.0% at concentrations of 5 and 10%, respectively. On the other hand, the water extract exhibited the lowest mortality values, with 12.5 and 14.6% at concentrations of 5 and 10%, respectively, therefore showing low activity (Table 4).

With respect to the essential oil, it was observed that the concentration of 200 mg/ml exhibited higher mortality percentage (28.6%), followed by the concentration of 100 mg/ml, which exhibited lower mortality value (14.0%) (Table 5).

With respect to adult mortality values resulting from the application of the essential oil, it was observed that the mortality values were low, with 10.0% for the concentration of 100 mg/ml and 12.0% for the concentration of 200 mg/ml, since the two concentrations did not differ statistically (Table 5).

Antioxidant activity

Considering the results of the antioxidant activity, it should be noted that the IC_{50} values are inversely related to the DPPH sequestration percentage, since the higher the sequestration rate, the lower the IC_{50} value. It is observed that there were no significant differences between the positive control (BHT), the essential oil, and the alcoholic extract, this way demonstrating that they exhibited good antioxidant activity. Comparing the aqueous extract with the synthetic antioxidant, it was possible to observe that there were significant differences, thus demonstrating a moderate antioxidant capacity. On the other hand, the ethyl acetate extract exhibited low capacity of radical sequestration (Table 6).

The results of phytochemical screening were in line with data from the literature. Lago and Roque (2009) assessed the compounds present in the ethanolic extract of *Guarea macrophylla* and observed the presence of triterpenoids. Pereira et al. (2012) assessed the compounds present in the methanolic extract and observed the presence of flavonoids. Hernes and Hedges (2004) found tannins and triterpenoids in extractions with ethyl acetate solvents and water in *Guarea rubriflora* and *Guarea trichiliodes*.

Flavonoids are phenolic compounds which have broad biological and therapeutic functions and distributed in the leaves, seeds, flowers and roots of plants, and their biological activity depends on its chemical structure and relative orientation of groups in the molecule (Agati et al., 2012). Tannins are compounds responsible for the astringency of many fruits and other plant products. Having the ability to complex with proteins, this factor is responsible for the action in the control of insects, fungi and bacteria (Mello and Santos, 2002).

Regarding the results of antimicrobial activity, it was observed that there was variation of antimicrobial susceptibility among the serotypes tested. This variation was also reported by Carramiñana et al. (2004) and Scur et al. (2013), who explained the differences in some serotypes tested against different antimicrobials. The authors attributed this variation to the origin of the serotypes and the selective pressures that they may suffer as a result of the use of different antimicrobials, leading to the selection of resistant serotypes.

The mechanism of antimicrobial action of essential oils involve different actions, such as the cytoplasmic membrane disruption, interruption of proton motive force and the flow of electrons, active transport, and cellular content coagulation (Burt, 2004). One of the most important aspects is hydrophobicity of the chemical components, which allows a partition of lipids of the bacterial

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oil of *G. kunthiana* against the microorganisms tested.

	MIC/MBC [a]		
Microorganism	**Essential oil[b]**	**Methanol[c]**	**Gentamicin[d]**
S. Enteritidis	Na/Na	Na/Na	0.78/0.78*
S. Infantis	54.6/54.6	Na/Na	0.78/0.78*
S. Typhimurium	54.6/54.6	Na/Na	0.78/0.78*
S. Heildelberg	1750/1750	Na/Na	0.78/0.78*
S. Mbandaka	Na/Na	Na/Na	0.78/0.78*
S. Give	54.6/54.6	Na/Na	0.78/0.78*
S. Saintpaul	218.7/218.7	Na/Na	0.78/0.78*
S. Ohio	875/875	Na/Na	0.78/0.78*
S. Gallinarum	875/875	Na/Na	0.78/0.78*
S. Agona	437/437	Na/Na	0.78/0.78

[a]Minimum inhibitory concentration (MIC)/Minimum bactericidal concentration (MBC); [b]Tested at a concentration of 7,000-3.4 µg/ml; [c]Tested at a concentration of 7,000 µg/ml; [d]Tested at a concentration of 100-0.78 mg/ml; Na = No activity; Nt = Not tested* mg/ml.

Table 3. Minimum inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) of different extracts of *G. kunthiana* against the *Salmonella* serotypes tested.

	MIC/MBC (mg/ml)[a]				
Microorganism	**Aqueous[b]**	**Ethyl acetate[c]**	**Alcoholic[d]**	**DMSO[e] 10%**	**Gentamicin[f] (200 mg/ml)**
S. Enteritidis	Na/Na	200/200	200/200	Na/Na	0.78/0.78
S. Infantis	Na/Na	200/200	0.39/0.39	Na/Na	0.78/0.78
S. Typhimurium	Na/Na	200/200	200/200	Na/Na	0.78/0.78
S. Heildelberg	Na/Na	200/200	200/200	Na/Na	0.78/0.78
S. Mbandaka	Na/Na	200/200	200/200	Na/Na	0.78/0.78
S. Give	Na/Na	100/200	100/200	Na/Na	0.78/0.78
S. Saintpaul	Na/Na	200/200	200/200	Na/Na	0.78/0.78
S. Ohio	Na/Na	100/200	Na/ Na	Na/Na	0.78/0.78
S. Gallinarum	Na/Na	200/200	0.78/0.78	Na/Na	0.78/0.78
S. Agona	Na/Na	200/200	200/200	Na/Na	0.78/0.78

[a]Minimum inhibitory concentration (MIC)/Minimum bactericidal concentration (MBC) mg/ml; [b]Tested at a concentration of 50-0.04 mg/ml; [c]Tested at a concentration of 200 to 0.09 mg/ml; [d]Tested at a concentration of 200 to 0.09 mg/ml; [e]Tested at a concentration of 10%; [f]Tested at a concentration of 100-0.78; Na = No activity.

cell membrane and the mitochondria, entailing a possible leakage of cellular content (Burt, 2004; Holley and Patel, 2005).

The antimicrobial potential of the alcoholic extract can be explained by the presence of some secondary metabolites, such as tannins, triterpenoids, and flavonoids (Table 1). The antimicrobial action of flavonoids is probably related to the capacity to complex extracellular and soluble proteins, as well as the structures of the bacterial cell wall (Sato et al., 1996). Cushnie and Lamb (2011) suggest that the antibacterial activity of flavonoids can be attributed to damages in the cytoplasmic membrane (perforation and/or reduction of membrane fluidity), inhibition of the synthesis of nucleic acids (caused by inhibition of topoisomerase), and inhibition of the energetic metabolism (caused by inhibition of NADH-cytochrome C reductase).

The antimicrobial action of tannins may be related to the fact that these compounds are able to complex macromolecules such as polysaccharides and proteins. This way, tannins may cause denaturation and, consequently, they may change the proteins of the bacterial cell membrane. This action occurs with proteins due to non-specific interactions, such as hydrogen bridges, hydrophobic effects, and through covalent bonds (Simões et al., 2007).

Triterpenes are very frequent in plants and they have many biological activities, mainly antimicrobial and insecticidal action (Chung et al., 2011; Garcez et al., 2013). Their mechanism of action in the bacterial cell is

Table 4. Mortality percentage of cascudinho larvae (*A. diaperinus*) ten days after being submitted to direct application of the extracts and the essential oil of *G. kunthiana*, under laboratory conditions (26 ± 1ºC, 14-h photophase).

Treatment (extracts)	Mortality (%)
Control (water)	3.3 ± 1.49^{e}
DMSO - 10%	4.0± 1.24^{e}
Aqueous extract - 5%	12.5 ± 1.59^{d}
Aqueous extract - 10 %	14.6 ± 1.69^{cd}
Acetate extract - 5 %	21.3 ± 1.33^{bc}
Acetate extract - 10 %	26.0 ± 2.21^{b}
Alcoholic extract - 5 %	24.6 ± 2.26^{b}
Alcoholic extract - 10 %	34 ± 1.63^{a}
Treatment (essential oil)	**Mortality (%)**
Control (water)	2.6 ± 1.49^{c}
Acetone - 10%	3.3 ± 1.24^{c}
Oil - 100 mg/ml	14 ± 1.24^{b}
Oil - 200 mg/ml	28.6 ±1.69^{a}

Averages followed by the same letter in the column do not differ from each other according to Tukey's test ($p<0.05$).

Table 5. Mortality percentage of cascudinho adults (*A. diaperinus*) ten days after being submitted to direct application of the plant extracts and the essential oil of *G. kunthiana*, under laboratory conditions (26 ± 1ºC, 14-h photophase).

Treatment (plant extracts)	Mortality (%)
Control (water)	2.0 ± 1.33^{c}
DMSO - 10%	2.0 ± 0.81^{c}
Aqueous extract - 5%	16.6 ± 2.35^{b}
Aqueous extract - 10%	19.3 ± 2.44^{b}
Acetate extract - 5%	23.3 ± 1.82^{b}
Acetate extract - 10%	28.0 ± 2.26^{b}
Alcoholic extract - 5%	26.6 ± 3.49^{b}
Alcoholic extract - 10%	44.6 ± 4.42^{a}
Treatment (essential oil)	**Mortality (%)**
Control (water)	1.3 ± 1.33^{b}
Acetone - 10%	2.0 ± 0.81^{b}
Oil - 100 mg/ml	10 ± 1.49^{a}
Oil - 200 mg/ml	12 ± 1.33^{a}

Averages followed by the same letter in the column do not differ from each other according to Tukey's test ($p<0.05$).

related to the disruption of lipophilic compounds (Bagamboula et al., 2004).

Even though the presence of steroids, tannins, flavonoids, and triterpenoids in the aqueous extract was detected (Table 1), it did not exhibit antimicrobial activity. This fact can be explained by the assumption that these compounds are present in very small quantities or due to the amount of extract applied, which may not have been enough to promote inhibitory action (Degáspari et al., 2005). This finding can also be reported for the ethyl acetate extract, which had triterpenoids in its composition (Table 1) and exhibited low antimicrobial activity.

With respect to the effect on adults of *A. diaperinus*, the 10% alcoholic extract exhibited the greatest mortality value (44.6%), followed by ethyl acetate extract (23.3 and 28.0%) at concentrations of 5 and 10%, respectively, and lastly, the aqueous extract exhibited the lowest mortality values at the same concentrations (16.6 and 19.3%) (Table 5). It was possible to observe that there were differences in the activity of the different solvents used, which confirms the issue of affinity difference of solvents with respect to the different plant compounds. According to Ferri (1996), extreme polar extracts (aqueous) exhibit less activity when compared with extracts of intermediate polarity (ethanolic).

It is observed that for both larvae and adults the alcoholic extract exhibited the highest efficiency. Compounds such as tannins, present in the composition of this extract (Table 1), may explain the activity found, since they act against pests. They have the ability to bind with digestive proteins of insects acting as digestive reducers, significantly reducing the growth and survival of insects by turning off digestive enzymes and creating a tannin-protein complex of difficult digestion (Mello and Filho, 2002; Cavalcante et al., 2006).

Studies on the use of extracts of Meliaceae family against larvae of *A. diaperinus* are scarce on the literature, specifically G. *kunthiana* are not found. Similar results were reported for Zorzetti et al. (2012), evaluating the action of ethanol and aqueous extracts of some plants of Meliaceae family against the beetle *Hypothenemus hampei* results obtained in 44% mortality for the ethanol extract and 12% for the aqueous extract of *Azadirachta indica*, both in the concentration of 10%. Cosme et al. (2007), assessing the mortality of the larvae of coleopteran *Cycloneda sanguinea* with compound azadirachtina isolated from *A. indica*, had an index of 83.3 and 65.3% mortality at a concentration of 100 mg/ml for the first and second larval instar, respectively.

The essential oil exhibited greater activity against the larvae than against adults. The insecticidal action of essential oils can occur in different ways and may cause mortality, deformities at different stages of development, repellency, and deterrence. Through the contact, essential oils can interact with the tegument of insects, besides acting in digestive and neurological enzymes (Isman, 2006; Knaak and Fiuza, 2010).

The high antioxidant capacity of the alcoholic extract can be associated with the presence of phenolic compounds detected in the phytochemical prospection, such as flavonoids and tannins (Table 1). The antioxidant potential of phenolic compounds is due mainly to their reducing properties and chemical structure. These

Table 6. Index of 2.2-diphenyl-2-picrylhydrazyl (DPPH) (% of sequestration) and IC_{50} of the essential oil and the different extracts tested.

Test solution	DPPH sequestration (%)	IC_{50}
Positive control (BHT)	95.85±0.04[a]	9.27 ± 0.08[a]
Essential oil	91.52±0.09[a]	17.54 ± 0.18[a]
Alcoholic	92.60± 0.86[a]	15.33 ± 1.62[a]
Aqueous	76.54± 2.00[b]	45.30 ± 3.75[b]
Ethyl acetate	6.61 ±1.04[c]	176.84 ± 1.96[c]

The values correspond to the average and standard deviation of the triplicates. Values followed by the same letter in the column do not differ from each other according to Tukey's test ($p<0.05$).

characteristics are responsible for playing an important role in the neutralization or sequestration of free radicals and chelation of transition metals, acting both in the initiation step and in the propagation of the oxidative process (Chun et al., 2005; Souza et al., 2007).

Flavonoids can act as reducing agents, sequestrators of free radicals, metal chelators, or deactivators of singlet oxygen (Melo and Guerra, 2002; Canterle, 2005). Various tannins act as sequestrators of free radicals, which intercept the active oxygen forming stable radicals (Mello and Santos, 2007).

According to the results obtained, the essential oil and the extracts of *G. kunthiana* tested exhibited antimicrobial, insecticidal, and antioxidant activity. This study is the first report in the literature on antimicrobial activity and insecticidal potential against *A. diaperinus*, and antioxidant activity of the essential oil and the aqueous, alcoholic, and ethyl acetate extracts of *G. kunthiana*. At the same time, this study can serve as the basis for conducting further research on plants that have unknown biological potential. It is important to stress the importance of further studies in order to determine the action of the compounds present in the essential oil and extracts tested in isolation and in synergism, and even toxicity tests, which can contribute to the use of these products in the poultry sector.

Conclusion

The essential oil of *G. kunthiana* exhibited greater antimicrobial activity against the serotypes Infantis, Typhimurium, and Give. With respect to the extracts, the highest antimicrobial susceptibility was observed in the alcoholic extract against the serotypes Infantis and Gallinarum. The insecticidal activity values were considered low for the test with the essential oil and the plant extracts. The results of the antioxidant activity revealed that the essential oil and the alcoholic extract did not exhibit significant differences when compared with the synthetic antioxidant. The importance of further studies were highlighted to determine the action of the compounds present in the essential oil and extracts tested alone and in synergism and even toxicity tests, which may contribute to the application of these products in the poultry sector.

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Conflicts of interest

The authors declare have not declare any conflicts of interest.

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