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Biology-Ecology

Evaluation of antioxidant, antimicrobial, toxicological, and larvicidal activity *of Psychotria fractistipula* L.B. Sm., Klein & Delprete

Avaliação da atividade antioxidante, antimicrobiana, toxicológica e larvicida de *Psychotria fractistipula* L.B. Sm., Klein e Delprete

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ABSTRACT

The objective of this study was to assess the potential antioxidant, antimicrobial, and toxicological properties of crude extracts and fractions obtained from the leaves and stem of *Psychotria fractistipula* L.B. Sm., Klein & Delprete. The content of phenolic compounds varied significantly between samples (783.70–78.22 GAE mg/g), with the highest concentrations being in the ethyl acetate fraction of the leaves and stem (679.39 and 783.70 GAE mg/g, respectively). The latter yielded also the best IC_{50} of the DPPH radical, which amounted to 9.48 and 4.75 µg/mL, respectively; whereas other samples ranged up to 156.64 µg/mL. Similarly, phosphomolybdenum activity varied between 90.17% and 16.00%, with the ethyl acetate fractions of the leaves and stem corresponding to 90.17% and 87.37%, respectively. Antimicrobial activity was elevated in the leaves crude extract (*Staphylococcus aureus*, 62.5 µg/mL), leaves ethyl acetate fraction (*S. aureus*, 31.25 µg/mL; *Enterococcus faecalis*, 62.4 µg/mL), and the stem ethyl acetate fraction (*S. aureus*, 31.25 µg/mL; *Pseudomonas aeruginosa*, 62.5 µg/mL). Hemolytic activity was high in the chloroform fractions of the leaves (1000 µg/mL) and stem (500 µg/mL). Larvicidal activity against *Aedes aegypti* was observed in the hexane fraction of the stem (LC_{50}, 297.44 µg/mL). The ethyl acetate fractions of the stem and leaves were toxic to *Artemia salina*, with LC_{50} values of 277.91 and 933.89 µg/mL, respectively. These results indicate that *P. fractistipula* may constitute an unexplored source of natural antioxidants and antimicrobials with low toxicity.

Keywords: *Psychotria*; Antioxidant; Antimicrobial; Toxicological

RESUMO

O objetivo deste estudo foi avaliar as potenciais propriedades antioxidantes, antimicrobianas e toxicológicas dos extratos brutos e frações obtidos das folhas e caule de *Psychotria fractistipula* L.B. Sm., Klein & Delprete. O conteúdo de compostos fenólicos variou significativamente entre as amostras (783,70-78,22 GAE mg / g), com as concentrações mais altas na fração acetato de etila das folhas e caule (679,39 e 783,70 GAE mg / g, respectivamente). Essas frações também apresentaram a melhor IC_{50} frente ao radical DPPH, que totalizaram 9,48 e 4,75 μg/mL, respectivamente; enquanto outras amostras variaram até 156, 64 μg/mL. Da mesma forma, a atividade do fosfomolibdênio variou entre 90,17% e 16,00%, com as frações de acetato de etila das folhas e caule correspondendo a 90,17% e 87,37%, respectivamente. A atividade antimicrobiana foi elevada no extrato bruto de folhas (*Staphylococcus aureus*, 62,5 μg/mL), na fração de acetato de etila das folhas (*S. aureus*, 31,25 μg/mL; *Enterococcus faecalis*, 62,4 μg/mL) e na fração de acetato de etila do caule (*S aureus*, 31,25 μg/mL; *Pseudomonas aeruginosa*, 62,5 μg/mL). A atividade hemolítica foi alta nas frações de clorofórmio das folhas (1000 μg/mL) e caule (500 μg/mL). Observou-se atividade larvicida contra *Aedes aegypti* na fração hexano do caule (CL_{50}, 297,44 μg/mL). As frações de acetato de etila do caule e das folhas foram tóxicas para *Artemia salina*, com valores de CL_{50} de 277,91 e 933,89 μg/mL, respectivamente. Esses resultados indicam que P. fractistipula pode constituir uma fonte inexplorada de antioxidantes e antimicrobianos naturais com baixa toxicidade.

Palavras-chave: *Psychotria*; Antioxidante; Antimicrobiano; Toxicológico

1 INTRODUCTION

The genus *Psychotria* belongs to the Rubiaceae family and contains approximately 2000 species, including trees and shrubs typical of the tropical and subtropical regions (DAVIS *et al.*, 2009). This genus is notable for the presence of alkaloids, mainly indole alkaloids. Its best known representative is *Psychotria viridis*, which is rich in the indolic alkaloid N,N-dimethyltryptamine (SOARES *et al*., 2017). The alkaloids bahienoside A and bahienoside B have been identified in *Psychotria bahiensis* (PAUL *et al*., 2003), psychollatine has been found in *Psychotria umbrellata* (KERBER *et al*., 2008), strictosidinic acid in *Psychotria myriantha* (FARIAS *et al*., 2012), and psychotrine and psychopentamine in *Psychotria rosata* (TAKAYAMA *et al*., 2004).

Some species of *Psychotria* have been demonstrated to exert various pharmacological effects, including antifungal (*P. carthagenensis* and *P. prunifolia)*, antibacterial (*P. carthagenensis* and *P. micralabastra*), anti-inflammatory (*P. ipecacuanha* and *P. octosulcata*), cytotoxic and antitumor (*P. prunifolia*), as well as anxiolytic and anticonvulsant (*P. umbrellata*). Moreover, *Psychotria rigida* and

Psychotria bracteocardia are used routinely to kill mice (MARIYAMMAL; KAVIMANI, 2013; SOUZA *et al.,* 2013).

Psychotria fractistipula L.B. Sm., Klein & Delprete is native and endemic to Brazil. It grows in Paraná and Santa Catarina states, and its phytogeographical domain corresponds to the Atlantic Forest (TAYLOR *et al*., 2015). Phytochemical screening by thin-layer chromatography indicated the presence of alkaloids, flavonoids, tannins, sterols, and terpenes in this species (OLIVEIRA *et al*., 2014).

The objective of the present study was to evaluate the *in vitro* antioxidant, antimicrobial, toxicological, and larvicidal activities of crude extracts and fractions obtained from the leaves and stem of *P. fractistipula*.

2 MATERIAL AND METHODS

2.1 Plant material

The leaves and stem of *P. fractistipula* were collected in Capão of Ciflomaat, Parana Federal University of Curitiba (25°26’54" “S; 49°14’27”" W), between March and April 2013. The samples were identified and deposited at the Botanical Garden of Curitiba (MBM) Herbarium under serial number 389153.

This study was authorized by the Genetic Heritage Management Council (CGEN), a legislative and deliberative body under the Ministry of the Environment of Brazil, under the number 02001.001165/2013-47.

2.2 Extraction of *P. fractistipula*

The crude ethanolic extract was prepared with 96°GL ethanol in a Soxhlet extractor under continuous reflux for 6 h at 50°C. Fractions were obtained through liquid-liquid partitioning in a modified Soxhlet extractor. Solvents of analytical grade were used in increasing order of polarity as follows: hexane, chloroform, and ethyl acetate (CARVALHO *et al*., 2006; SOUZA *et al*., 2014).

2.3 Determination of total phenolic content (TPC)

The Folin-Ciocalteu methodology was used to assay TPC (SINGLETON, 1999). Extracts and fractions diluted in methanol were tested at concentrations between 80 and 320 μg/mL. TPC was calculated using a calibration curve for gallic acid. The results were expressed as gallic acid equivalents (GAE) of dry plants.

2.4 Antioxidant activity evaluation using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH was used according to Mensor *et al*. (2001) to measure free radical scavenging activity. The concentrations of extracts and fractions ranged from 5 to 450 μg/mL. The standards used were vitamin C and rutin. Absorbance was measured at 518 nm. Three replicates per treatment were applied and activity was expressed as the concentration (in μg/mL) required for 50% DPPH inhibition (IC_{50}). The percentage inhibition of DPPH was evaluated using the following formula:

$$\text{DPPH scavenging effect (\%)} = 100 \times [(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})] \quad (1)$$

where A_{control} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

2.5 Antioxidant activity assessment using phosphomolybdenum

The phosphomolybdenum assay was performed according to Prieto *et al*. (1999). The absorbance of the solution was measured at 695 nm. The percentage of antioxidant activity (AA%) relative to ascorbic acid was evaluated using the following formula:

$$\text{AA\% relative to ascorbic acid} = [(A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}}) - (A_{\text{blank}})] \times 100 \quad (2)$$

where A_{sample} is the absorbance of the test compound, A_{blank} is the absorbance of the blank, and A_{control} is the absorbance of ascorbic acid.

2.6 Antimicrobial activity

Antimicrobial activity was tested against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853.

Crude extracts and fractions were prepared in 10% ethanol and 2% dimethyl sulfoxide (DMSO) and filtered through a 0.22-μm Millipore membrane. Bacterial suspensions were prepared in saline solution at a concentration of 10 x 10^8 CFU/mL, corresponding to a 0.5 McFarland tube.

The minimum inhibitory concentration (MIC) was determined according to the Clinical and Laboratory Standards Institute (2008). The diluents (ethanol and DMSO) were used as negative controls, whereas vancomycin was used as positive control. Formation of a red color in the microplate wells indicated absence of bacterial growth inhibition. MIC values were classified as indicative of good inhibitory potential (< 100 μg/mL), moderate inhibitory activity (100–500 μg/mL), weak inhibitory activity (500–1000 μg/mL), and absence of inhibitory activity (> 1000 μg/mL) (AYRES *et al.,* 2008).

2.7 In vitro hemolytic activity

Crude extracts and fractions were prepared in a solution made of 10% methanol and 90% phosphate-buffered saline (PBS), adjusted to pH 7.4. This same solution was used as negative control, whereas 0.1% Triton X-100 was used as positive control. The protocol was adapted from Banerjee *et al.* (2008). Extracts and fractions were used at concentrations of 1000, 500, 200, and 100 μg/mL. Briefly, 5 mL defibrillated sheep's blood was homogenized, poured into a glass test tube, and centrifuged for 5 min at 3000 rpm. The supernatant was decanted and the viscous pellet was washed three times with 5 mL chilled (4°C) PBS solution (pH 7.4). The washed cells were suspended in chilled PBS to obtain 2% erythrocytes. Then, 200 μL of the erythrocyte suspension was placed in a microcentrifuge tube in the presence of 200 μL of sample or controls. The tubes were homogenized and incubated for 3 h in an oven at 37°C. They were then centrifuged in an Eppendorf® Minispin Plus centrifuge at 3000 rpm for 5 min. Finally, 200 μL of the supernatant was placed into 96 well plates and microquant absorbance at 540 nm was measured. The experiment was performed in triplicate. The percentage of hemolysis was calculated using the following formula:

$$\% \text{ hemolysis} = [(A_{sample} - A_{blank})] / A_{control}] \times 100 \quad (3)$$

where A_{sample} is the absorbance of the test compound, A_{blank} is the absorbance of the blank, and $A_{control}$ is the absorbance of ascorbic acid.

2.8 Toxicity against *A. aegypti*

Crude extracts and fractions were diluted in 0.5% DMSO and then dissolved in dechlorinated water to obtain the desired concentration. An aqueous 0.5% DMSO solution was used as negative control, whereas the insecticide Temophos was used as positive control. The protocol was adapted from the World Health Organization (2005). Eggs of *A. aegypti* (originally from the Rockefeller University, New York City, NY, USA, and made available by the Oswaldo Cruz Foundation - Fiocruz) were placed in dechlorinated water and incubated in B.O.D at a controlled temperature of 27 ± 2°C and relative humidity of 80 ± 5%. The larvae diet consisted of fish feed (Aldon basic, MEP 200 complex). After hatching, 10 larvae in the 3rd stage were placed in contact with the extracts and fractions at concentrations of 1000, 100, and 10 µg/mL, as well as the negative and positive control for 24 h, after which live and dead larvae were counted. Three replicates were used for each treatment, totaling 30 larvae per sample dose. The positive control was added at a concentration of 0.06 mg/mL, as recommended by the World Health Organization (1981).

2.9 Brine shrimp lethality assay

The extracts and fractions were diluted in 1% DMSO and then dissolved in artificial seawater to the desired concentration. Artificial seawater with 1% DMSO was used as negative control and quinidine sulfate was used as positive control. The assay was performed according to Meyer *et al*. (1982), with some modifications. The cysts of *Artemia salina* L. were placed in artificial seawater (38 g marine salts dissolved in 1000 mL purified water) and incubated in B.O.D. at a controlled temperature of 30 ± 2°C for 48 h. After hatching, 10 nauplii were placed in contact with the extracts and fractions at concentrations of 1000, 500, 100, and 10 μg/mL, as well as the negative and positive controls for 24 h, after which live and dead nauplii were counted. Each treatment was run in triplicate, totaling 30 nauplii per sample dose. The positive control was used at concentrations of 10, 20, 30, 40, and 50 μg/mL.

2.10 Statistical analysis

Statistical analysis was performed for antioxidant and hemolytic activities. Results are presented as means ± standard deviation (SD) from three replicates of each experiment. A p value < 0.05 was used to denote significant differences among means determined by analysis of variance (ANOVA). Results were compared using one-way and multivariate ANOVA followed by Duncan' s multiple range tests. The probit method (FINNEY, 1971) was used to determine the lethal concentration (LC_{50} and LC_{90}) as well as the corresponding 95% confidence intervals and chi square values for the assays with *A. aegypti* and *A. salina*. Calculations were performed in IBM SPSS Statistics version 20.0.

3 RESULTS AND DISCUSSION

3.1 Percentage yield and TPC

Hexane, chloroform, and ethyl acetate fractions were obtained from 25 g of crude leaf extract and 66 g of crude stem extract using a modified Soxhlet extractor (Table 1). Ethyl acetate from the stem presented the highest yield (47.23%), followed by hexane (31.81%) and ethyl acetate (30.53%) of the leaves. These high yields can be explained by the polarity of the chemical compounds present in the samples.

The highest TPC content was obtained in the ethyl acetate fractions of the stem (783.70 GAE mg/g) and leaves (679.39 GAE mg/g), followed by crude leaves and stem extracts (Table 1). These results corroborate existing evidence stating that the yield of phenolic compounds is related to the polarity of the solvent and, hence, extraction is improved in highly polar solvents (LOPEZ *et al*., 2011).

The most common phenolic compounds in plants are simple phenols, phenolics, flavonoids, tannins, lignins, and coumarins (SOARES, 2002). In the *Psychotria* genus, some of these compounds have been identified as quercetin, 3-O-glycosides of quercetin, quercetin 3-O-D-rhamnoside, quercetin 3-O-rutinoside, kaempferol, kaempferol 7-O-glucopyranoside, kaempferol 3-O-rutinoside, coumarin, umbelliferone, psoralene, and scopoletin (BENEVIDES *et al*., 2005; DA ROSA *et al*., 2010; LU *et al*., 2014; MORENO *et al*., 2014).

Numerous activities have been linked to phenolic compounds, including antibacterial, antiviral, antiparasitic, anti-inflammatory, antioxidant, antitumor, vasodilator, and hepatoprotective (BALASUNDRAM *et al*., 2006; DORMAS *et al*., 2008).

3.2 Antioxidant activity by the DPPH radical scavenging assay

The DPPH assay evaluated the ability of extracts and fractions to reduce the free radical DPPH by 50%. The ethyl acetate fraction of the stem presented the lowest IC_{50} (4.75 µg/mL ,(which was not significantly different from the IC_{50} of the standard (4.92 µg/mL). Similar results were found for the crude extract of the stem (8.58 µg/mL) and the ethyl acetate fraction of the leaves (9.48 µg/mL) (Table 1).

Other species of *Psychotria* (*P. carthagenensis, P. leiocarpa, P. capillacea, and P. deflexa*) were also reported to exhibit antioxidant activity by the DPPH method (FORMAGIO *et al*., 2014); however, their values were lower than in *P. fractistipula.* The high TPC in this species indicates abundant phenolic compounds, which can sequester or neutralize free radicals due to their reducing action and chemical conformation (DEWICK, 2002; SOUSA *et al*., 2007; DORMAS *et al*., 2008).

3.3 Antioxidant activity of phosphomolybdenum

This method has the ability to evaluate the activity of lipophilic and hydrophilic components (PRIETO *et al*., 1999). Relative to the standards, activity was particularly high in the ethyl acetate fraction of leaves (90.17%) and stems (87.37%) (Table 1). Antioxidant activity in the phosphomolybdenum assay is thought to be related to the presence of flavonoids and phenolic compounds (DEWICK, 2002; SOUSA *et al*., 2007; DORMAS *et al*., 2008).

Table 1 – Yield, total phenolic contents (TPC), DPPH radical scavenging activity, and phosphomolybdenum activity of *P. fractistipula* leaves and steam extracts and fractions

Extracts and fractions	**Methods**			
	Yield of extracts and fractions (g)	TPC (GAE mg/g)	DPPH IC_{50} (µg/mL)	Phosphomolybdenum assay relative to ascorbic acid (%)
			Leaves	
Crude extract	25.00 ± 0.06	529.74 ± 0.11^{c}	25.62 ± 0.85^{d}	39.75 ± 1.69^{c}

Continued...

Table 1 – Conclusion

Extracts and fractions	Methods			
	Yield of extracts and fractions (g)	TPC (GAE mg/g)	DPPH IC_{50} (µg/mL)	Phosphomolybdenum assay relative to ascorbic acid (%)
			Leaves	
Hexane	8.61 ± 0.07	-	40.52 ± 0.33^{f}	31.38 ± 3.74^{e}
Chloroform	0.73 ± 0.09	78.22 ± 0.01^{e}	156.64 ± 2.79^{g}	25.15 ± 3.29^{f}
Ethyl acetate	8.2 ± 0.06	679.39 ± 0.07^{b}	9.48 ± 0.16^{c}	90.17 ± 0.65^{b}
			Stem	
Crude extract	66.00 ± 0.08	503.89 ± 0.01^{c}	8.58 ± 0.75^{bc}	35.08 ± 1.27^{d}
Hexane	10.38 ± 0.04	-	-	16.00 ± 1.30^{g}
Chloroform	5.25 ± 0.04	286.23 ± 0.02^{d}	33.98 ±1.85^{e}	32.02 ± 0.60^{de}
Ethyl acetate	32.15 ± 0.06	783.70 ± 0.0^{4}a	4.75 ± 0.19^{a}	87.37 ± 0.07^{b}
Ascorbic acid	N/A	N/A	4.92 ± 0.06	100^{a}

Values correspond to the mean ± SD of three separate experiments ($p < 0.05$); (-), no effect of antioxidant activity at the tested concentrations; N/A, not applicable; TPC, total phenolic content; DPPH, 2,2-diphenyl-1-picrylhydrazyl

3.4 Antimicrobial activity

The antimicrobial activity of extracts and fractions from the leaves and stem of *P. fractistipula* was evaluated against a range of pathogenic microorganisms. The antimicrobial activity of the genus *Psychotria* has already been demonstrated for *P. reevessi* Wall (GIANG *et al*., 2007), *P. gardneri, P. micralabastra, and P. stenophylla* (YANG *et al*., 2016). Here, the MIC of plant extracts and fractions against the different bacteria ranged from 31.25 to 1000 µg/mL (Table 2).

The ethyl acetate fraction of the leaves showed potent inhibitory activity against *S. aureus* (31.25 µg/mL) and *E. faecalis* (62.5 µg/mL). Analogous values were obtained for the ethyl acetate fraction of the stem against *S. aureus* (31.25 µg/mL) and *P. aeruginosa* (62.5 μg/mL). Significant activity against *S. aureus* (62.5 μg/mL) was shown also by the crude leaves extract.

The activity demonstrated by the crude extract and the ethyl acetate fraction is likely the result of phenolic compounds (DAGLIA, 2011). Flavonoids may act by interfering with the energy metabolism of the bacterium or by altering its cytoplasmic membrane (FOWLER *et al*., 2011; HENDRA *et al*., 2011; KUREK *et al*., 2011); whereas flavonols and tannins are mostly responsible for suppressing microbial virulence, inhibiting biofilm formation, reducing host binding, and neutralizing bacterial toxins (DAGLIA, 2011).

Table 2 – Antimicrobial activity of extracts and fractions of leaves and stem of *P. fractistipula*

Extracts and fractions	**MIC (μg/mL)** **Microorganisms**				
	S. aureus	*E. faecalis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
			Leaves		
Crude extract	62.5	250	500	500	500
Hexane	250	1000	1000	-	-
Chloroform	500	500	-	-	-
Ethyl acetate	31.25	62.5	125	250	250
			Stem		
Crude extract	500	125	500	1000	500
Hexane	1000	500	-	-	-
Chloroform	125	500	1000	1000	-
Ethyl acetate	31.25	250	62.5	250	250

(-) no effect on growth inhibition with the concentrations tested. MIC, minimum inhibitory concentration

The moderate activity of the stem hexane fraction against *S. aureus* and *E. faecalis* (Table 2) may be linked to steroids and triterpenes. Some studies have shown that triterpene may be responsible for antimicrobial activity (LIMA *et al*., 2011); however, its mechanism of action is not yet fully understood and is only known to rupture the plasma membrane upon binding to it (SALEEM *et al*., 2010).

-βSitosterol and campesterol, which have proven antimicrobial activity (LOPES *et al*., 2000), have been reported in this species of *Psychotria* (OLIVEIRA *et al*., 2020). These compounds are capable of inhibiting the growth of *S. aureus* and *E. faecalis* (TOMOKOU *et al*., 2011; DOGAN *et al*., 2017).

A comparison between gram-positive and gram-negative bacteria revealed greater activity against the former, confirming earlier studies on the effect of triterpenes and flavonoids in these microorganisms (TALEB-CONTINI *et al*., 2003; TIWARI *et al*., 2009; SOUZA *et al*., 2014).

3.5 In vitro hemolytic activity

Hemolytic activity was defined as the amount of extract that successfully hemolyzed 50% of erythrocytes in comparison with the control (EC_{50}) (JUNIOR *et al*., 2010). Of the extracts and fractions of *P. fractistipula* tested for hemolytic activity, only the chloroform extract of the leaves and stem exhibited significant activity, with 50% of erythrocytes being hemolyzed at 1000 and 500 μg/mL, respectively. Hemolysis occurs due to damage to the erythrocyte cell membrane, which may involve the transport of specific ions, modification of the lipid layer structure, or toxic effects that prevent cell volume control (LIMA; SOTO-BLANCO, 2010). The *Psychotria* genus is known to contain indole alkaloids (SOARES *et al*., 2017), which are responsible for hemolytic and other cytotoxic activities (JAGETIA *et al*., 2005; KATAJIMA, 2007; LIMA; SOTO-BLANCO, 2010).

3.6 Toxicity against *A. aegypti*

Among the extracts and fractions tested against 3rd-stage larvae of *A. aegypti*, a promising result was obtained in the hexane fraction of the stem (Table 3). The toxicity of this fraction may be ascribed to the presence of apolar compounds, such as steroids and terpenes, which have demonstrated larvicidal activity, although its mechanism of action has not been completely elucidated (SHAALAN *et al*., 2005; GHOSH *et al*., 2012). This larvicidal potential can be associated also with the presence of -βsitosterol, which has proven action against *A. aegypti* (RAHUMAN *et al*., 2008).

3.7 Brine shrimp lethality assay

Toxic activity against *A. salina* was observed in the ethyl acetate fractions of the leaves and stem (Table 3), whose LC_{50} was < 1000 µg/mL (MEYER *et al*., 1982). The cytotoxicity of these fractions is likely related to the presence of phenolic compounds, as has been proven for flavonoids (MOREIRA *et al*., 2003), tannins (YAMASAKI *et al*., 2002), and catechin (CHOBOT *et al*., 2009). Moreover, studies have demonstrated a correlation between cytotoxicity in *A. salina* and antiviral, antiparasitic, and antitumor activities (MCLAUGHLIN *et al*., 1988; SIQUEIRA *et al*., 2001).

Table 3 – Activity against *A. aegypti* and *A. salina*

Extracts and fractions	***A. aegypti***				***A. salina***			
	LC_{50} (µg/mL) (LCL–UCL)	LC_{90} (µg/mL) (LCL–UCL)	χ^2	df	LC_{50} (µg/mL) (LCL–UCL)	LC_{90} (µg/mL) (LCL–UCL)	χ^2	df
Leaves								
Raw extract	> 1000	> 1000	-	-	> 1000	> 1000		
Hexane	> 1000	> 1000	-	-	> 1000	> 1000		
Chloroform	> 1000	> 1000	-	-	> 1000	> 1000		
Ethyl acetate	> 1000	> 1000	-	-	277.9 (43.3–777.6)	> 1000	30	2
Stem								
Raw extract	> 1000	> 1000	-	-	> 1000	> 1000		
Hexane	297.44 (70.26–868.68)	> 1000	1,4	1	> 1000	> 1000		
Chloroform	> 1000	> 1000	-	-	> 1000	> 1000		
Ethyl acetate	> 1000	> 1000	-	-	933.9 (764.3–1392.4)	> 1000	3,3	2

No mortality was observed in the controls. LC_{50}, lethal concentration that kills 50% of the exposed organisms; LC_{90}, lethal concentration that kills 90% of the exposed organisms; UCL, 95% upper confidence limit; LCL, 95% lower confidence limit; χ^2, chi square test; df, degrees of freedom; (-), no activity against the tested concentrations.

4 CONCLUSIONS

This is the first study to reveal considerable antimicrobial and antioxidant potential by the leaves and stem extracts and fractions of *P. fractistipula*. The hemolytic, toxicological, and larvicidal activities demonstrated a safety margin, which indicates that *P. fractistipula* can serve as a new, natural source of antimicrobials and antioxidants. While this is a preliminary study and the exact chemical composition of the extracts from this plant remains to be determined, our results offer nevertheless a promising basis for the development of medicinally useful drugs.

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