Article

CHEMICAL AND DEREPLICATION STUDIES OF *Palicourea tomentosa* (Aubl.) Borhidi AND THEIR ANTIMICROBIAL AND ANTICHOLINESTERASE ACTIVITIES

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Received: 11/01/2023; accepted: 02/20/2024; published online: 05/03/2024

Palicourea tomentosa (Aubl.) Borhidi (Rubiaceae, Palicoureeae), earlier classified as *Psychotria poeppigiana* Müll. Arg., is used in folk medicine for a variety of diseases. Biological studies on the plant describes their vasorelaxant, antiplasmodial, antitumoural and anticholinesterase activities. To expand the knowledge on the chemical and biological potential of this species, in this work, phytochemical and dereplication studies, as well as antimicrobial and anticholinesterase evaluation of *Palicourea tomentosa* were carried out. Phytochemical investigation deals with the isolation of lutein (**1**), a mixture of dehydrovomifoliol (**2**) and megastigma-4,7-dien-3-one (**3**), loliolide (**4**), 5,13-epoxy-9-hydroxy-megastigma-7-en,3-one (**5**), vomifoliol (**6**), asperuloside (**7**), 3-*O*-*p*-coumaroylquinic acid (**8**), and a mixture of the pyrrolizidinoindoline alkaloids hodgkinsine (**9**), chimonanthine (**10**) and psychotriasine (**11**). The ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS/MS) based on molecular networking dereplication of dichloromethane fraction led to putative identification of other two megastigmanes, one terpene lactone and three carotenoids, besides seven triterpenes and four indole alkaloids. The dichloromethane, ethyl acetate and hydromethanol fractions of *P. tomentosa* were able to inhibit *Candida albicans* with minimum inhibitory concentrations (MICs) of 125, 250 and 125 μg mL^{-1}, respectively. The methanol crude extract, dichloromethane and alkaloid fractions inhibited selectively the butyrilcholinesterase (BuChE) at percentages of 63.09, 60.39 and 56.22%, respectively.

Keywords: *Palicourea tomentosa*; pyrrolizidinoindoline alkaloids; megastigmanes; antimicrobial activity; anticholinesterase activity.

INTRODUCTION

The genus *Palicourea* Aubl. (Rubiaceae, Palicoureeae) has been considered closely related to the pantropical *Psychotria* L., being both classified in the tribe Psychotrieae.[1] More recently, molecular data showed that Psychotrieae comprises two distinct groups, being one of them *Psychotria* and other constituted by various genera, including *Palicourea*.[1] From the separation between these genera, morphological and molecular studies have confirmed that most of the Neotropical *Psychotria* subg. *Heteropsychotria* Steyerm. belong to the *Palicourea* genus.[2] In its new circumscription, *Palicourea* was classified within the tribe Palicoureeae and comprises around 800 species, occurring from the Bahamas, Greater Antilles and southern Mexico to northern Argentina.[1]

Chemical studies on *Palicourea* genus showed the presence of alkaloids belonging to different classes as main specialized metabolites. Based on phytochemical data available for *Palicourea* species, Berger[3] pointed strictosidine and related monoterpene-indole glucosides as the predominant classes of the genus. Other alkaloid classes include pyrrolidinoindolines, β-carbolines and tryptamine analogues. Beside alkaloids, triterpenes and coumarins,[4,5] chlorogenic acids, polyphenols, flavonoids[6] and iridoids[7,8] were also found in *Palicourea* species.

Our previous work on *Palicourea* genus led to isolation of monoterpene indole alkaloids,[9] and demonstrated the anti-inflammatory and anti-hyperalgesic potential of the methanol extract and the isolated alkaloid croceaine A of *Palicourea crocea*.[10] From the biological study of Palicourea tomentosa we showed that its methanolic crude extract significantly inhibited the acetylcholinesterase (AChE) activity in the frontal cortex, as well as inhibited pain and inflammatory parameters in a carrageenan model.[11] The ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS/MS) analysis of *P. tomentosa* methanolic crude extract permitted to identify putatively two alkaloids (calycanthine and hodgkinsine), one coumarin (scopoletin), one iridoid (asperuloside) and two terpene derivatives (vomifoliol and loliolide).[11]

Palicourea tomentosa (Aubl.) Borhidi (Rubiaceae, Palicoureeae),[12] earlier classified as *Psychotria poeppigiana* Müll. Arg.,[13] is a shrub used in folk medicine for a variety of diseases, as pain, gastrointestinal disorders, stomachaches, fever and for treatment of dyspnea.[14,15] Other studies on the plant, with the earliest classification as *Psychotria poeppigiana*, describes their vasorelaxing effects,[16] antitumoural and anticholinesterase activity.[17] Phenolic compounds, aromatic acids, terpenes, steroids, coumarins, iridoids and alkaloids were identified by gas chromatography-mass spectrometry (GC-MS) and/or UHPLC-HRMS/MS analysis in extracts of the plant.[16,17] Besides that, *in vitro* antiplasmodial activity against chloroquine-resistant *Plasmodium falciparum* was reported for *Psychotria poeppigiana* acid-base extract. The high performance liquid chromatography coupled to diode array detector and electrospray ionization high-resolution mass spectrometry (HPLCDAD-ESI-HRMS) analyses of bioactive extract led to putative identification of pyrrolidinoindoline alkaloids, indicating its association to the high antiplasmodial activity (GI > 90%) observed.[18]

Despite the related studies on chemical composition of this plant, there is only one report[16] on the isolation of its constituents, as the synonym *Psychotria poeppigiana*, which describes the presence

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of scopoletin, esculin, stigmast-4-en-3-one, ergost-5-en-3β-ol and syringaldehyde from the methanolic extract.

Due to the the limited phytochemical studies on *Palicourea tomentosa*, in this work we focused in the isolation and structure elucidation of their specialized metabolites. From this, a carotenoid, three megastigmanes, a terpene lactone, and a mixture of pyrrolizidinoindoline alkaloids were isolated. In addition, other two megastigmanes, one terpene lactone and three carotenoids, besides seven triterpenes and four indole alkaloids were putatively identified through UHPLC-HRMS/MS-based molecular networking dereplication of dichloromethane fraction. The methanol crude extract of *P. tomentosa* and the fractions from its fractionation were evaluated for their antimicrobial and anticholisterase activities.

EXPERIMENTAL

General experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance Neo 300 NanoBay spectrometer (Germany) operating at 300 MHz for ^{1}H NMR and at 75.5 MHz for ^{13}C NMR, using deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d_6) as solvents. The ultra-high performance liquid chromatography (UHPLC) analyses were performed in a Shimadzu Nexera X2 instrument (Japan), equipped with a CBM-20A a system controller, two LC-30AD pumps, a CTO-30A column oven and SIL-30AC autosampler. The mass spectra were recorded on Bruker IMPACT II mass spectrometer (USA), with electrospray ionization source (ESI) in the positive ion mode, quadrupole-time of flight (Q-TOF) analyzer and multichannel plate (MCP) detector. Chromatography separations were performed on silica gel flash (230-400 mesh) and Sephadex LH-20 (Sigma, USA). Thin layer chromatography (TLC) was performed on normal phase pre-coated silica gel 60G or 60GF_{254} (Merck, Germany) plates. Visualization of the compounds on TLC was accomplished by UV irradiation at 254 and 366 nm, and/or by spraying with H_2SO_4:anisaldehyde:acetic acid acid (1:0.5:50 mL) solution followed by heating at 100 °C.

Plant material

Leaves of *Psychotria tomentosa* were collected in Dourados, MS, Brazil (22° 13' 15" S 54° 48' 21" W) in April 2021 and identified by Professor Dr. Zefa Valdevina Pereira. An exsiccate of *P. tomentosa* was deposited in the herbarium of Universidade Federal da Grande Dourados (UFGD) (0006) and the research was registered in SisGen A51F665.

Extraction and isolation of chemical constituents

Air-dried leaves of *P. tomentosa* (315 g) were extracted with methanol by maceration at room temperature. Evaporation of the solvent under reduced pressure provided the methanol crude extract (ME; 23 g). The ME (21 g) was suspended in MeOH:H_2O (1:1, v/v, 400 mL), and partitioned into *n*-hexane, dichloromethane and ethyl acetate. The solvents were removed under reduced pressure to give *n*-hexane (HE; 8.5 g), dichloromethane (DC; 1.2 g), ethyl acetate (EA; 1.3 g) and hydromethanolic (HM; 9.0 g) fractions. The DC fraction was submitted to column chromatography (CC) in silica gel, using *n*-hexane-EtOAc (50 and 75%), EtOAc, and EtOAc-MeOH (2, 5, 10, 30 and 50%) as eluents, affording the sub-fractions DC-1 to DC-28. The sub-fractions DC-7 and DC-11 provided the compound **1** (3 mg), and a mixture of **2** and **3** (9.4 mg), respectively. The sub-fractions DC-15, DC-16 and DC-17 were submitted to column chromatography (CC) in flash silica, eluted with hexane-EtOAc 40, 60 and 80% and EtOAc. The sub-fraction DC-15.5, eluted with *n*-hexane:EtOAc 40%, provided the compound **4** (2.0 mg). The sub-fraction DC-16.5 eluted with *n*-hexane:EtOAc 70% provided the compound **5** (3.2 mg). The DC-17.6 sub-fraction eluted with *n*-hexane:EtOAc 75% afforded the compound **6** (3.0 mg). The EA fraction (1 g) was subjected to purification on Sephadex LH-20 (20.0 g; Ø 2.5 cm) eluted with H_2O, H_2O:MeOH and MeOH, affording the EA-1 to EA-10 sub-fractions. The EA-5 sub-fraction afforded the compound **7** (200 mg). Purification of EA-8 (50.7 mg) on Sephadex LH-20 by using MeOH as eluent afforded the compound **8** (10 mg). The sub-fraction HM (3 g) was subjected to purification on Sephadex LH-20 with H_2O:MeOH and MeOH as eluent, which afforded the compounds **7** (109 mg) and **8** (32.9 mg) previously isolated from the EA fraction.

Acid-base extraction

The crude extract (1.9 g) of *P. tomentosa* was solubilized in a solution of 1 M HCl (100 mL) and extracted with dichloromethane (3 × 20 mL). The acid aqueous layer was alkalinized with sodium carbonate until pH 9.0 and extracted with dichloromethane (3 × 20 mL). The organic fraction was separated, washed with water, dried with calcium chloride and filtered. Evaporation of the solvent under vacuum furnished the alkaloid fraction AF-PT (10 mg).

Molecular networking

The data obtained from the UHPLC-MS/MS analyses were converted to the mzXML format by using the Bruker's data analysis software and introduced and processed on the Global Natural Products Social Networking (GNPS) platform,[19] in order to create a molecular network using the online workflow. The data were filtered by removing all MS/MS peaks within ± 17 Da of the precursor *m/z*, and grouped with MS-cluster with a parent mass and a MS/MS fragment ion tolerance of 2.0 and 0.5 Da, respectively. A network was then created with the edges having cosine score above 0.7 and at least 2 corresponding peaks. The network was searched against the GNPS the spectral libraries of and all the matches were required to have the cosine score above 0.7 and at least 5 matched peaks. The network analysis was exported from GNPS and analyzed in Cytoscape.[19,20]

Microorganisms

The reference strains used in this study were obtained from the Culture Collection of the University of Georgia (Atlanta, GA, USA). Clinical and environmental isolates from the Culture Collection of the Mycology Laboratory (ICB-UFMG) were also used in this study. The yeast and the filamentous fungi isolates were maintained on Sabouraud dextrose agar (SDA, Difco Laboratories, Detroit) and potato dextrose agar (PDA, Difco), respectively, at 4 °C.

Antimicrobial assay

The antimicrobial assays were performed with the methanol extract of *P. tomentosa* (ME) and its hexane (HF), dichloromethane (DF), ethyl acetate (EAF) and hydromethanolic (HMF) against the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and the fungi *Candida albicans*. The minimum inhibitory concentration (MIC) were determined by microdilution method in broth according to standards proposed by document M27-A3 and M7-A6.[21,22]

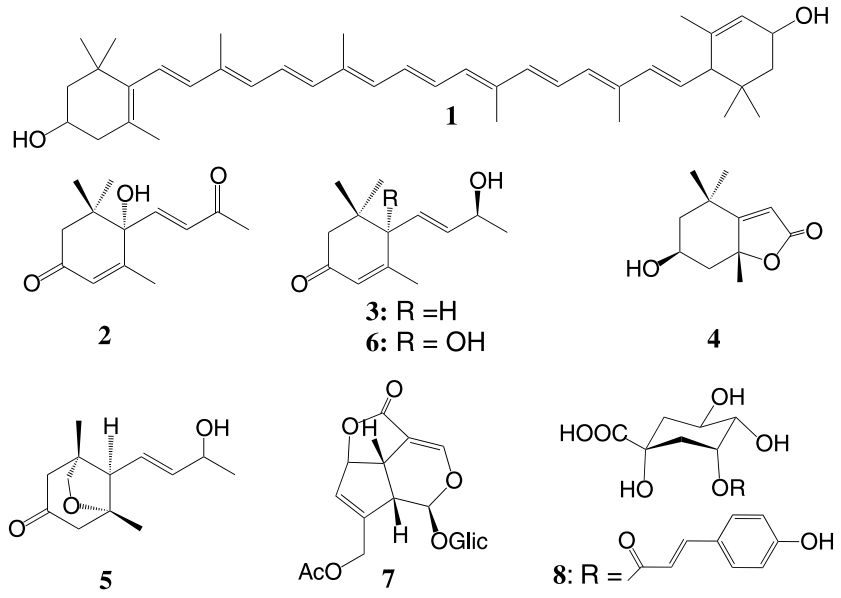

Figure 1. *Structures of compounds* ***1-8*** *isolated from Palicourea tomentosa*

Anticholinesterase assay

The inhibitory activity of methanol extract (ME), hexane (HF), dichloromethane (DF), ethyl acetate (EAF), hydromethanolic (HMF), and alkaloid (AF-PT) fractions were determined against acetylcholinesterase (AChE, from *Electrophorus electricus*, type VI-S, lyophilized powder, lot 041M7009V) and butyrylcholinesterase (BuChE, from equine serum, lyophilized powder, lot SLBB2114V), according to the protocol described by Ellman *et al*.[23] The assays were performed in triplicate, at concentrations of 100 μg mL^{-1}. The inhibition percentages were calculated by comparing the control reaction rate with the sample reaction rate using Equation 1:

% Inhibition = (control reaction rate – sample reaction rate)/ (control reaction rate) × 100 (1)

RESULTS AND DISCUSSION

Chemical study

A crude methanol extract of the *P. tomentosa* aerial parts was subjected to partition into *n*-hexane, dichloromethane and ethyl acetate, as well as to acid-base extraction. Purification of dichloromethane and ethyl acetate fractions from solvents partition by using chromatographic techniques afforded compounds **1-8** (Figure 1). From the analyses of ^{1}H NMR, ^{13}C NMR and HRESI-MS/MS data and comparison with those reported, the structures of the isolated compounds were elucidated as lutein (**1**),[24] a mixture of dehydrovomifoliol (**2**) and megastigma-4,7-dien-3-one (**3**),[25,26] loliolide (**4**),[27] 5,13-epoxy-9-hydroxy-megastigma-7-en,3-one (**5**),[28] vomifoliol (**6**),[29] asperuloside (**7**)[30] and 3-*O*-*p*-coumaroylquinic acid (**8**).[31]

The megastigmane **5** was previously isolated from *Centaura salmantica*; however, only its ^{1}H NMR spectra was reported.[28] In this work we report the complete structural NMR assignment of compound **5** (Table 1), through an analysis of their 1D and 2D NMR spectra (^{1}H, ^{13}C, COSY, HSQC, and HMBC). The ^{13}C NMR was consistent whit a megastigmane skeleton due the signals for carbons of ketone group at δ_C 209.4 (C-3), methylene at δ_C 50.1 (C-2) and 49.3 (C-4), methine at δ_C 58.4 (C-6), oxymethine at δ_C 68.6 (C-9), and for carbons of double bond at δ_C 142.0 (C-7) and 122.1 (C-8). The methyl carbons were observed at δ_C 24.3 (C-10), 24.1 (C-11) and 20.6 (C-12). The tetrahydrofuran ring was confirmed by the signals of the methylene, methine and quaternary carbons at δ_C 79.1 (C-13), 58.4 (C-6), 44.2 (C-1) and 83.3 (C-5), respectively. The correlation of the oxymethylene hydrogens H-13a/b with C-1 (δ_C 44.2) and C-5 (δ_C 83.3) confirm that the C-5 and C-13 carbons are connected by a bridge with oxygen, forming a second five-membered cycle. The main correlations observed in HMBC spectra are shown in Figure 2. The relative stereochemistry at C-1, C-5 and C-6 of compound **5** was established by NOE experiment, in which was observed an enhancement of H-6 signal at δ_H 2.34 with the irradiation of H-13a (δ_H 3.63), indicating that these hydrogens are on the same side.

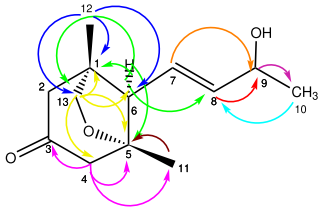

Figure 2. *Main HMBC ($^{1}H \rightarrow {}^{13}C$) correlations of compound* ***5***

The alkaloid fraction from acid-base extraction consists of a mixture, from which was possible to identify the pyrrolidinoindoline-type alkaloids hodgkinsine (**9**), chimonanthine (**10**), and psychotriasine (**11**) together with the isoquinoline alkaloid calycanthine (**12**).[32-39] The identification of pyrrolizidinoindoline alkaloids (Figure 3) was based on their ^{13}C NMR data and in the fragmentation pattern observed in the HRESI-MS/MS spectra (Table 2S, presented in Supplementary Material).

The ^{13}C NMR spectra was characteristic of pyrrolizidinoindoline-type alkaloids[32] due to the signals in the regions of δ_C 51.4-53.8 and δ_C 84.3-86.6, typical of C-2 and C-8a carbons linked to one and two nitrogen atoms, respectively. The signals at δ_C 35.4-39.9, and at δ_C 61.6-62.2 corresponds to the carbons C-3 and C-3a, respectively. The carbons of *ortho*-substituted aromatic rings were observed in the region of δ_C 109.0 to 129.5. The signal at δ_C 150.4 corresponds to the carbons C-7a/C-7a' directly attached to a nitrogen atom. The N-CH_3 groups present in the structures of **9**, **10** and **11** appears at 34.0 to 36.0 ppm. The signals at δ_C 78.0-79.0 are typical of alkaloids

Table 1. ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.5 MHz) of **5** and ^{1}H NMR (CD_3OD, 50 MHz) from the literature for 5,13-epoxy-9-hydroxy-megastigma-7-en,3-one[28]

	Compound **5**			Literature[28]
No.	δ_C	δ_H (mult., *J* in Hz)	HMBC ($^{1}H \rightarrow {}^{13}C$)	δ_H (mult., *J* in Hz)
1	44.2	-	-	-
2α	50.1	2.42 (dd, 17.4 and 2.6)	-	2.2-2.4 (m)
2β		2.29 (d, 17.4)		
3	209.4	-	-	-
4	49.3	2.30-2.46 (m)	3, 5, 6, 11	2.2-2.4 (m)
5	83.3	-	-	-
6	58.4	2.34 (d, 9.6)	1, 5, 7, 8, 11, 13	2.2-2.4 (d)
7	122.4	5.60 (ddd, 15.2, 9.6 and 1.1)	6, 9	5.57 (dd, 15.3 and 9.6)
8	142.0	5.89 (dd, 15.2 and 5.6)	6, 9	5.87 (dd, 15.3 and 5.6)
9	68.6	4.39 (m)	7, 10	4.36 (m)
10	24.1	1.32 (d, 6.4)	8, 9	1.30 (d, 6.4)
11	24.3	1.25 (s)	5, 6	1.23 (s)
12	20.6	1.03 (s)	1, 2, 6, 13	1.00 (s)
13α	79.1	3.74 (d, 8.1)	1, 4, 5, 6	3.72 (d, 8.1)
13β		3.63 (dd, 8.1 and 2.6)	4, 12	3.60 (dd, 8.1 and 2.1)

containing a tryptamine unit linked to the pyrrolidinoindoline by an N-C3a' bond, which supports the presence of the psychotriasine (**11**).[33]

The analysis of HRESI-MS permitted to confirm the presence of compounds **9**, **10**, **11** and **12** in the alkaloid fraction. Hodgkinsine (**9**) was evidenced by the protonated molecular ion (*m/z* 519.3231 $[M + H]^+$), and the ions fragments *m/z* 347.2212 and 173.1065 corresponding to the loss of one and two pyrrolidinoindoline units, respectively, due the cleavage between the carbons C3a' and C3a.[34-36]

Chimonanthine (**10**) and psychotriasine (**11**) presented the same protonated molecular ion (*m/z* 347 $[M + H]^+$) and main fragment ion *m/z* 173, which is originated from the cleavage of C3a-C3a' bond for **10**,[37] and of the N-C3a' bond for compound **11**.[33] The HRESI-MS/MS fragmentation spectrum of compound **10** (Figure 8S, Supplementary Material) showed fragments at *m/z* 173, 144 and 130, consistent with data reported for chimonanthine.[37,38] Besides these fragments, the spectra for compounds **10** and **11** (Figure 9S, Supplementary Material) exhibited an additional fragment at *m/z* 316, which can be associated to psychotriasine (**11**), being originated by the loss of CH_3NH_2 (31 Da) from the protonated molecular ion (*m/z* 347 $[M + H]^+$).[33]

Calycanthine (**12**) was identified in the alkaloid acid-base fraction from the protonated molecular ion *m/z* 347.2230 $[M + H]^+$ and the fragments *m/z* 316.1806, 290.1646 and 285.1385 (Figure 10S, Supplementary Material), corresponding to loss of CH_3NH_2, C_3H_7N and of two units of CH_3NH_2, respectively, from the protonated precursor ion.[39,40]

As previously mentioned, the pyrrolizidinoindoline alkaloids calycanthine, hodgkinsine and psychotriasine have already been putatively identified in *Palicourea tomentosa*. The calycanthine, chimonanthine and hodgkinsine were also isolated from other *Palicourea* species.[41-44] The isolated iridoide asperuloside (**7**) from *P. tomentosa* was reported to occurs in *P. minutiflora* and *P. luxurians*.[7,8] The lutein (**1**) and dehydrovomifoliol (**2**) were isolated also from *Palicourea correae* (as *Psychotria correae*). To our knowledge, this is the first report on the isolation of megastigma-4,7-dien-3-one (**3**), 5,13-epoxy-9-hydroxy-megastigma-7-en,3-one (**5**) and 3-*O*-*p*-coumaroylquinic acid (**8**) from the *Palicourea* genus.

Dereplication of dichloromethane fraction

The dichloromethane fraction (DF) of *P. tomentosa* was analyzed by using UHPLC-HRMS/MS in the positive ionization mode (Figure 11S, Supplementary Material), and the fragmentation data were organized through molecular networking, using the Global Natural Products Social Networking (GNPS) platform. The molecular network was generated from 250 precursor ions, visualized as nodes. Based on the cosine values, a parameter used to verify the similarity profile between the compounds, 19 clusters were formed (Figure 4). Among the clusters formed, we can highlight those of megastigmanes (green nodes), lactones (orange nodes), triterpenes (pink nodes), carotenoids (yellow nodes), pyrrolizidinoindoline alkaloids (purple nodes) and indole alkaloids (red nodes). The putative identification of compounds was based on their fragmentation pattern similarity with the isolated in this work, and by comparison with those of the described in the literature.

For the dereplication of megastigmanes and terpene lactones, the compounds dehydromomyfoliol (**2**), megastigma-4,7-dien-3-one (**3**), loliolide (**4**), 5,13-epoxy-9-hydroxy-megastigma-7-en,3-one (**5**) and vomifoliol (**6**), isolated and characterized in the present study, were used as standard. This strategy allowed the putative annotation of two other megastigmanes, 4-hydroxy-β-ionone (**13**) and corchoionol A (**14**), and the lactone dihydroactinidiolide (**15**). The fragmentation data obtained and those of literature for **3**-**6** and **13**-**15** are presented in Table 3S (Supplementary Material).

The dereplication of triterpenes was performed by comparing the fragmentation data of triterpenes isolated from *Palicourea*, which allowed the putative annotation of the compounds 5-olean-12-en-28-oic acid (**16**), ursolic acid (**17**), sumarecinolic acid (**18**), masticadienonic acid (**19**), pomholic acid (**20**), rotungenic acid (**21**)

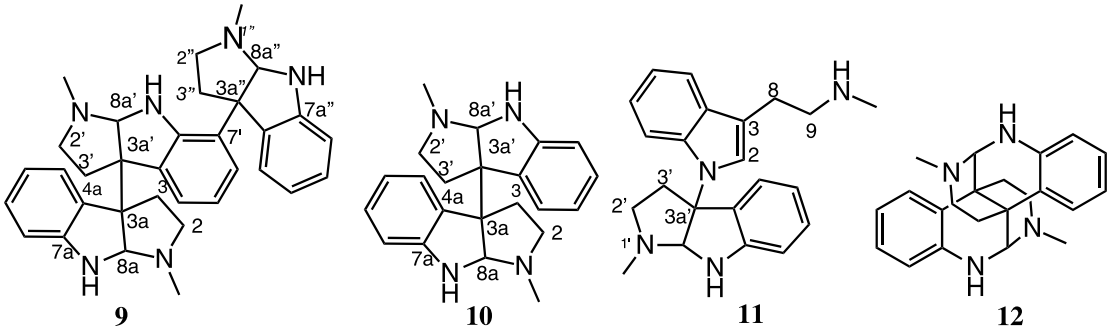

Figure 3. *Structures of alkaloids hodgkinsine (**9**), chimonanthine (**10**), psychotriasine (**11**) and calycanthine (**12**) identified in the acid-base extract of Palicourea tomentosa*

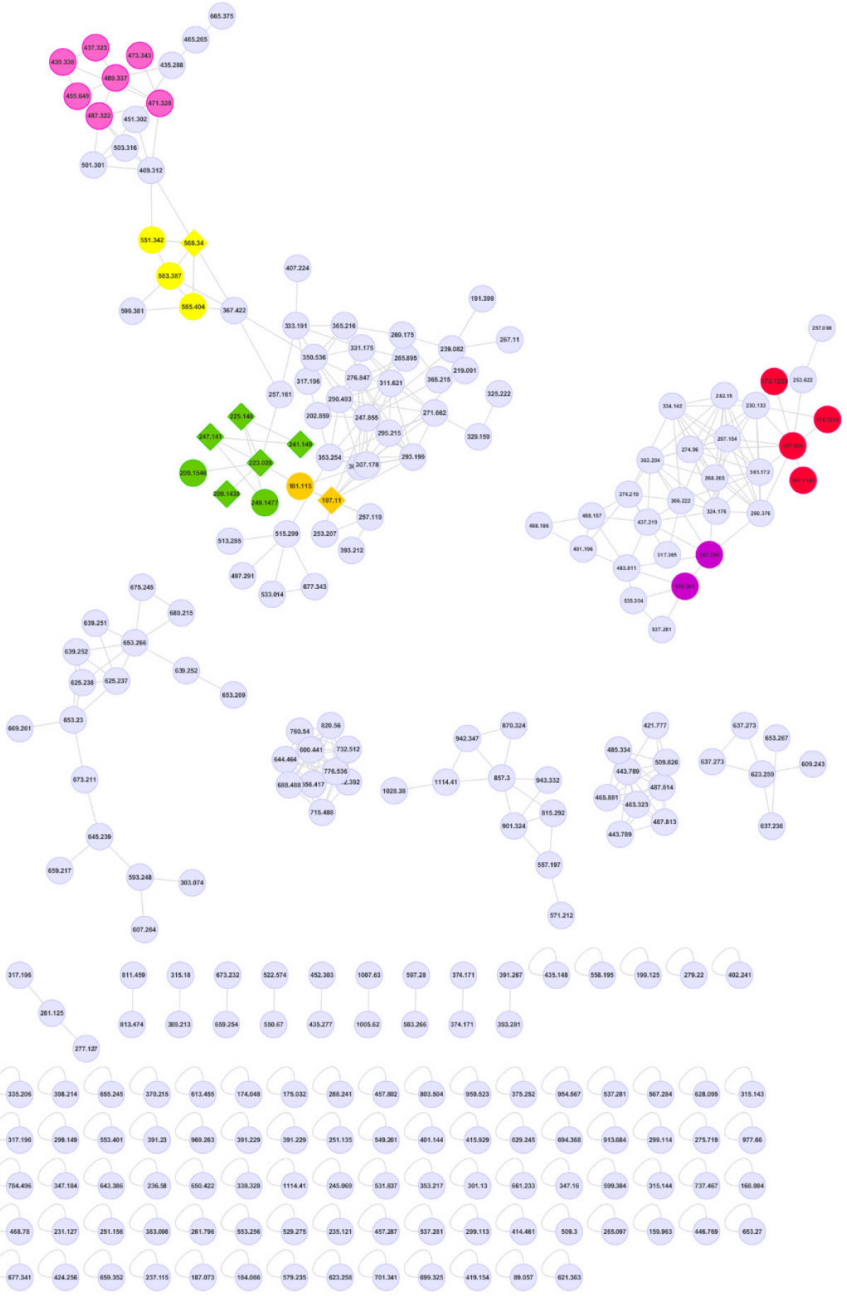

Figure 4. *Molecular networking for the dichloromethane fraction of Palicourea tomentosa, using UHPLC-HRMS/MS in the positive ionization mode. The nodes represent identified compounds and are colored according to the class of specialized metabolites: megastigmanes (green nodes); lactones (orange nodes); triterpenes (pink nodes); carotenoids (yellow nodes); pyrrolizidinoindoline alkaloids (purple nodes) and indole alkaloids (red nodes). Diamond nodes represent the isolated compounds in the present work, and circular nodes represent putatively identified compounds*

and alisol C (**22**) (Table 4S, Supplementary Material).

The lutein (**1**), isolated and characterized by NMR and UHPLC-HRMS/MS, was used as standard for the putative annotation of three other carotenoids: equinenona (**23**), adoxanthin (**24**) and anteraxanthin (**25**). The fragmentation data obtained and those of literature are presented in Table 5S (Supplementary Material).

The dereplication studies led also to the putative annotation of the indole alkaloids tetrahydro-β-carboline (**26**), *N*-methyl-tetrahydro-β-carboline (**27**), *N*-methyltryptamine (**28**) and alline (**29**), and of pyrrolizidinoindoline alkaloids chimonanthine (**10**) and hodgkinsine (**9**) (Figures 34S to 39S, Supplementary Material). The fragments *m/z* 157, 144, 130, 119 and *m/z* 91 were observed for compounds **26**-**29**. Compounds **9** and **10** presented the fragments ions *m/z* 347, 173, 172, 143 and 130, consistent with those described for pyrrolidinoindoline alkaloids. The fragmentation data obtained for the putatively identified compounds and data from the literature are presented in Table 6S (Supplementary Material).

The putatively annotated indole alkaloids were isolated from other *Palicourea* species. *N*-Methyl tetrahydro-β-carboline (**27**) was found in *P. marcgravii*,[45] *N*-methyltryptamine (**28**) and alline (**29**) were reported to occurs in *P. sessilis*.[46]

Biological assays

Antimicrobial activity

From the antimicrobial assays results it was observed that the

crude extract (ME) and HF fraction did not inhibit the growth of the microorganisms tested. On the other hand, the fractions DF, EAF and HMF were able to inhibit *Candida albicans* with minimum inhibitory concentrations (MICs) of 125, 250 and 125 μg mL^{-1}, respectively. These results show that the species *P. tomentosa* has promising potential as a source of metabolites with antifungal activity. This is the first report of antifungal activity of this species.

The antifungal activity presented by the fractions of *P. tomentosa* can be related to the presence of megatigmanes and alkaloids in the dichloromethane (DF), and of the iridoid (**7**) and chorogenic acid (**8**) in the ethyl acetate (EAF) and hydromethanolic (HMF) fractions. The megatigmane vomifoliol, isolated from *Treculia acuminata* (Moraceae), showed antifungal activity (MIC of 78 μg mL^{-1}) against *Candida albicans*.[47] The alkaloid calycanthine, isolated from the seeds of *Chimonanthus praecox*, showed inhibitory activity against plant pathogenic fungi, with greater susceptibility to *Bipolaris maydis* with EC_{50} 29.3 μg mL^{-1}.[39] Vomifoliol (**5**) and calycanthine (**12**) were identified in the present study from the dichloromethane fraction.

Anticholinesterase activity

The anticholinesterase assay results are summarized in Table 2.

Table 2. Inhibition against AChE and BuChE for crude extract and fractions of *Palicourea tomentosa*

Sample	Inhibition / %	
	AChE	BuChE
Crude extract (ME)	37.80 ± 1.47	63.09 ± 0.94
Hexane fraction (FH)	31.55 ± 4.08	25.18 ± 0.19
Dichloromethane fracion (DF)	21.75 ± 1.05	60.39 ± 1.36
Ethyl acetate fraction (EAF)	19.19 ± 3.70	38.36 ± 2.31
Hydromethanol fraction (HMF)	44.54 ± 1.46	36.42 ± 1.07
Alkaloid fraction (AF-PT)	32.09 ± 2.45	56.22 ± 3.06

AChE: acetylcholinesterase; BuChE: butyrilcholinesterase.

Analysis of Table 2 data showed that the crude extract and all fractions from its fractionation exhibited a weak AChE inhibition (less than 50%), with inhibition percentage in the range of 19.19 to 44.54, at 100 μg mL^{-1}. A weak BuChE inhibitory activity was also observed for hexane, ethyl acetate and hydromethanol fractions. On the other hand, the crude extract (ME), dichloromethane (DF) and alkaloid (AF-PT) fractions showed moderate inhibitions towards BChE with percentages of 63.09, 60.39 and 56.22%, respectively. From all fractions tested, these three were the most active and selective to BChE. The activity presented by AF-PT fraction can be related to the presence of isolated and/or annotated alkaloids. Besides alkaloids, the megastigmanes, lactones and triterpenes isolated and/or putatively identified may contribute for anticholinesterase activity of dichloromethane fraction (DF). Studies demonstrated[45] that the compounds alline and *N*-methyltryptamine, which were annotated in this work from dereplication, exhibited a moderate inhibitory effect of acetylcholinesterase (AChE) at percentages of 53.7 ± 2.8 and 49.8 ± 2.4%, respectively, at 50 μM.

The inhibition of the AChE in the hippocampus by *Palicourea tomentosa* extracts (syn. *Psychotria poeppigiana*) has already been described.[17] As showed in our previous work,[11] the oral administration of 30 and 100 mg kg^{-1} of methanolic extract of *P. tomentosa* significantly inhibited AChE in the frontal cortex, and reduced inflammation and hyperalgesia in mice.

CONCLUSIONS

Phytochemical investigation of *Palicourea tomentosa* led to isolation of lutein (**1**), a mixture of dehydrovomifoliol (**2**) and megastigma-4,7-dien-3-one (**3**), loliolide (**4**), 5,13-epoxy-9-hydroxy-megastigma-7-en,3-one (**5**), vomifoliol (**6**), asperuloside (**7**), 3-*O*-*p*-coumaroylquinic acid (**8**), and a mixture of the pyrrolizidinoindoline alkaloids hodgkinsine (**9**), chimonanthine (**10**) and psychotriasine (**11**). From the dereplication studies, megastigmanes, terpene lactones, carotenoids, triterpenes, pyrrolizidinoindoline and indole alkaloids were putatively annotated. The presence of pyrrolizidinoindoline alkaloids are in agreement with previous chemical studies and can contribute with the chemotaxonomy of the *Palicourea* genus. This is the first report on the isolation of compounds **3**, **5** and **8** from the *Palicourea* genus. The antifungal activity against *Candida albicans*, and anticholinesterase effect towards butyrilcholinesterase (BuChE) presented by *Palicourea tomentosa* demonstrated its importance as source of biological active compounds.

SUPPLEMENTARY MATERIAL

NMR spectra for mixture of **2** and **3**, compound **5** and alkaloid fraction; HRESI-MS/MS spectra for all isolated and annotated compounds; molecular networking and Tables with fragmentation data of putatively annotated compounds are available free of charge in the supplementary material at http://quimicanova.sbq.org.br/ as PDF file.

ACKNOWLEDGMENTS

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, finance code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT), Financiadora de Estudos e Projetos (FINEP, code 04.18.0011.00) and Universidade Federal da Grande Dourados for financial support, and to Complexo de Centrais de Apoio à Pesquisa da Universidade Estadual de Maringá (COMCAP-UEM) for the facilities. We also thank Dr. Z. V. Pereira for collecting and identifying the plant material, and Dr. L. Pizzuti and Dr. C. S. Schwalm for obtaining the NMR spectra.

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