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Chemical profile and biological activities of *Deguelia duckeana* A.M.G. Azevedo (Fabaceae)

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Deguelia duckeana is popularly known as timbó and used by indigenous people as ictiotoxic. On account of there being no literature pertaining to the chemical profile or biological activity of this plant, the hexane, methanol and aqueous crude extracts from leaves, stems and roots were assayed that presented very high cytotoxic potential against *Artemia salina*, achieving 100% mortality in up to 5.0 μg mL^{-1} concentration, but lower antioxidant potential on 2,2-diphenyl-1-picryl-hydrazyl and Fe^{3+}/Phenanthroline assays. The phytochemical analysis of crude extracts showed the presence of flavonoids and related compounds as major constituents as well as steroids in all of them, and tannins in polar extracts. All the extracts were assayed for antibacterial activity but only the hexane extract of stems showed moderate activity on *Staphylococcus aureus*, which was fractionated and yielded a mixture of 3,5,4′-trimethoxy-4-prenylstilbene, lonchocarpine, 4-hydroxylonchocarpine and derricidine, reported for the first time in *D. duckeana* and other fraction with β-sitosterol and stigmasterol mixture.

Keywords: *Deguelia duckeana*; chalcones; stilbene; flavonoids; antibacterial; *Artemia salina* toxicity; Fabaceae

1. Introduction

Deguelia duckeana is one of the several plants popularly named as timbó and used by indigenous people as ictiotoxic. On account of the earlier reported relationship between cytotoxicity and antitumoral activities (Galotta & Boaventura, 2005), the present work addresses its toxicity on *Artemia salina*, as well as antioxidant and antibacterial potential of its plant extracts.

In developing countries, plants have shown to be major therapeutic resources for the treatment of several illnesses. Bacteria-caused diseases are among the ones that can be treated by these plant extracts. Since antibacterial compounds are becoming less efficient due to the appearance of resistant strains, thorough chemical and biological studies on plant extracts must be performed so as to find new active substances.

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Currently another important biological study is the search for the antioxidant compounds, to be use as food additives, cosmetics, beverages and also on the treatment of degenerative diseases.

Therefore, natural products chemistry plays a major role in plant extract's scientific and therapeutic validation as well as bioprospection of new pharmaceutical compounds (Viegas et al., 2009).

Fabaceae presents a cosmopolitan distribution, including around 650 genera and 18,000 species, representing one of the largest and most economically important Angiosperm families (Souza & Lorenzi, 2005). Several Fabaceae species are reported to have some biological effects, such as *Bauhinia outimouta* for anticonvulsant (Quintans-Júnior et al., 2002); *Senna occidentalis* for antimicrobial, antiparasitic, insecticidal, antitumour, hepatoprotective and laxative (Lombardo, Kiyota, & Kaneko, 2009); *Tetrapleura tetraptera* for moluscicidal; *Erythrina velutina* (Virtuoso et al., 2005), *Derris malaccensis* (Takashima, Chiba, Yoneda, & Ohsaki, 2002), for antibacterial; *Derris indica* for antimicobacterial (Koysomboon, Van Altena, Kato, & Chantrapromma, 2006) and *Derris amazonica* (Alecio et al., 2010) for insecticidal activity.

Deguelia genus belongs to Milletieae tribe (Fabaceae Papilionoideae) and it is a flavonoid and related substances source. This tribe presents taxonomical divergences brought about by different botanist classification, specially between *Lonchocarpus* Kunth, *Derris* Lour. and *Milletieae* genera. Under this context, *Lonchocarpus* subg. *Phacelanthus* Pittier, *Milletieae* and american *Derris* came to be known as *Deguelia*, according to Magalhães, Tozzi, Magalhães, and Moraes (2001) and Tozzi (1989). Due to the similarity in the vegetative genera of this tribe, their flavonoids have been considered to be an important tool for their taxonomical identification (Gomes, Gottlieb, Marini Bettolo, Delle Monache, & Polhill, 1981).

Genus *Deguelia* comprises 20 species, divided in two sections: *Deguelia* sect. *Multiovulis* and *Deguelia* sect. *Deguelia* according to its morphology. This genus is characterised by the presence of isoflavonoids, rotenoids being predominant in the *Deguelia* section, while 4-hydroxy-3-phenylcoumarins are predominant in the *Multiovulis* section (Magalhães et al., 2001).

Deguelia duckeana (*Deguelia* section) is an endemic Brazilian plant known as cipó-cururu or timbó (Tozzi, 1989). It is one of the several plants that is used by indigenous people as ictiotoxic. Since there is no literature, as far as we know, pertaining to the chemical profile or biological activity of this species, this work was carried out by preparing organic and aqueous extracts of *Deguelia duckeana* and assaying them on 2,2-diphenyl-1-picryl-hydrazyl (DPPH), *Artemia salina* and the following bacteria of clinical interest: *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Aeromonas hydrophila*.

2. Results and discussion

The phytochemical screening revealed flavonoids to be rich in all the extracts, while tannins were observed only in methanol and aqueous extracts of stems and the aqueous extract of roots. None of the extracts showed the presence of alkaloid, but steroids were found in all of them. The TLC analysis confirmed some of the metabolite classes and also the presence of several UV-fluorescents substances, emphasising the possibility of aromatic compounds presence, such as flavonoids. The ^{1}H-NMR spectra analysis confirmed the presence of flavonoidic substances and related compounds, such as: flavones, flavanones, flavonols and chalcones, as well as the singlet between 12 and 14 ppm, indicated as the main characteristic in the flavonoidic skeleton, the presence of a chelated hydroxyl by

intramolecular hydrogen binding, which happens to be very frequent in the flavonoids isolated from the *Deguelia* species.

All hexane, methanol and aqueous extracts from *D. duckeana* (stems, leaves and roots) were assayed on *P. aeruginosa*, *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *A. hydrophila* in order to screen their antibacterial profile. Only the hexane extract from stems showed a medium activity against *S. aureus*, presenting an inhibition halo of 7 mm.

This extract was submitted to successive chromatographic fractionations so as to isolate its active compounds. The ^{1}H-NMR mixture spectrum of the less polar fraction showed some steroidal skeleta characteristic signals: a methyl group at δ_H 0.66, a multiplet at δ_H 3.50 (2H) characteristic of H-3, a doublet at δ_H 5.35 (2H, $J = 4.0$ Hz), 5.16 (dd, $J = 15.5$; 9.0 Hz) and 5.03 (dd, $J = 15.5$; 9.0 Hz,) which are characteristics of the H-22 and H-23 of stigmasterol. All these data corroborate the β-sitosterol and stigmasterol mixture of 1 : 1 ratio.

Another apolar fraction was submitted to open chromatographic column using silica gel 60 as a stationary phase and eluted with hexane/ethyl acetate and ethyl acetate/methanol gradients, resulting in one rich flavonoid mixture. The phytochemical and spectroscopic analyses and standard comparison allow us to characterise the mixture of compounds: 3,5,4′- trimethoxy-4-prenylstilbene (**1**), lonchocarpine (**2**), 4-hydroxylonchocarpine (**3**) and derricidine (**4**) (Figure 1), already reported in the literature (Braz-Filho, Gottlieb, Mourão, Rocha, & Oliveira, 1975; Mourão, 1975). The presence of these classes of compounds is in accordance to the chemistry of *Deguelia* genus (Fabaceae).

The chalcone mixture was determined by ^{1}H-NMR data spectra analysis. The first sign noted were the singlets above δ_H 13.0 (1H, s, 2′-OH), characteristic of the chelated hydroxyl group and of the pairs of doublets with $J = 15.5$ Hz [δ_H 7.43 (1H, d, $J = 15.5$ Hz, H-β) and δ_H 7.83 (1H, d, $J = 15.5$ Hz, H-α)], typical of the *trans*-cinnamic system of chalcones. The presence of the 2,2-dimethylchromene ring was characterised by signals at 1.46 (6H, s, H-7″, 8″), 5.59 (1H, d, $J = 10.0$ Hz, H-5″) and 6.75 (1H, d, $J = 10{,}0$ Hz, H-4″)], while the 3,3-dimethylalyl group was identify by the multiplet at δ_H 5.48 (1H, m, H-2″), the doublet at δ_H 4.57 (2H, d, $J = 6.8$ Hz, H-1″) and by the singlets at δ_H 1.76 (3H, s) and δ_H 1.81 (3H, s), characteristics of the methyl hydrogens. The stilbene was identified by the two doublets pairs with a large coupling constant (16.0 Hz) near δ_H 7.0 ppm (1H), due to the presence of signals referents to a 3,3-dimethylalyl group [δ_H 3.37 (2H, d, $J = 7.5$ Hz, H-7′); 5.25 (1H, m, H-8′); 1.80 (3H, s, CH_3-10′) and 1.65 (3H, s, CH_3-11′)] and by the singlets at δ_H 3.88 (6H) and δ_H 3.74 (3H), characteristics of aromatic methoxyls.

The antibacterial activity found in the extract could be related to the presence of these chalcones, which were significant in this extract and very common in the *Deguelia* genus, and have shown antibacterial activity against *S. aureus* (Mbaveng et al., 2008;

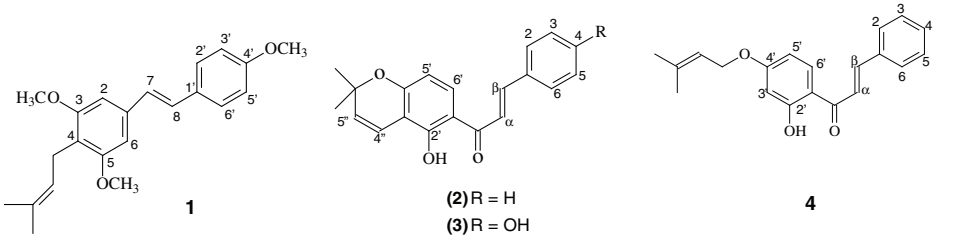

Figure 1. Substances identified from the hexane extract of *D. duckeana* stems.

Ngameni et al., 2006). The antibacterial activity of flavonoids and related compounds might be due to their property for binding with the bacterial cell wall (Cowan, 1999) and so inhibit the bacterial growth.

All plant part extracts were assayed against *A. salina* and showed to be 100% lethal at $1000\,\mu g\,mL^{-1}$ concentration. Several dilutions were assayed and even in $5.0\,\mu g\,mL^{-1}$ concentration they still remained 100% active. These findings indicate their high toxicity, which can be attributed to the presence of flavonoids that happen to be the major constituents.

The antioxidant potential of hexane, methanol and aqueous extracts from the stems, leaves and roots of *D. duckeana* was assessed. The findings of the assays are presented as ascorbic acid equivalents, so values near 1 are the most active, because it can be inferred that 1 g of extract could have the same activity of 1 g of ascorbic acid (Table 1, Figures S1 and S2). Among them, only the methanolic extract of leaves and the aqueous extract of stems and roots showed a low antioxidant activity when assayed for Fe^{3+}/phenanthroline, while the rest were found inactive. These findings corroborate the TLC qualitative analyses with DPPH. The yellow-white spots indicating the antioxidant substances were observed in the more polar extracts (MeOH and H_2O) through TLC analyses. Normally this activity is correlated to the presence of phenolic substances. In our case, even though several flavonoid compounds, which commonly possess antioxidant activity, were identified in the extracts, they did not show antioxidant potential as high as expected. Yet, in regards to the three flavonoid compounds identified in the *D. duckeana* stem's hexane extract, two of them (**2** and **4**) possess only one hydroxyl group which is chelated, and only one of them (**3**) has one hydroxyl group able to act as antioxidant. Hence, species might produce almost exclusive flavonoid compounds with chelated hydroxyl groups or even other compounds that are not phenolic.

3. Experimental

3.1. *Plant material*

Roots, leaves and stems of *D. duckeana* were collected in Praia Dourada, Manaus (AM). A voucher specimen (50410) was deposited at the Herbarium of the Botanical Research Coordination of the National Research Institute of Amazonia (INPA).

Table 1. DPPH and Fe^{3+}/PHenanthroline results, as ascorbic acid equivalent, of *D. duckeana* extracts.

Part plant	Extract	DPPH (mg extract/mg ascorbic acid)	Standard deviation	Fe^{3+}/phenanthroline (mg extract/mg ascorbic acid)	Standard deviation
Leaves	Hexane	68.8	5.1	15.4	2.3
	Methanol	27.2	1.1	9.3	0.4
	H_2O	42.7	9.8	6.0	1.3
Stems	Hexane	65.8	12.6	14.0	2.7
	Methanol	27.0	1.1	11.9	1.8
	H_2O	29.0	4.9	8.4	0.0
Roots	Hexane	74.8	14.1	12.3	0.6
	Methanol	37.1	7.8	12.0	0.6
	H_2O	60.7	0.0	6.9	4.0

3.2. *Extract preparation and fractionation*

Plant material was dried, powdered and then extracted with hexane using ultrasound bath for 20 min and then filtered. The extraction procedure was repeated thrice. The plant material was then dried and extracted with methanol and finally with water, by using the same ultrasound procedure. Organic extracts were concentrated on rotaevaporator and aqueous extracts by lyophilisation.

The hexane extract (2.0 g) from stems was submitted to a chromatographic column fractionation on silica gel, eluted with gradients of hexane/ethyl acetate and ethyl acetate/methanol, yielding 112 fractions with 50 mL each. Fraction 25 (180 mg) obtained in hexane : ethyl acetate 9 : 1 was submitted to a column chromatographic fractionation on silica gel and eluted with gradients of hexane, dichloromethane and methanol, obtaining 44 fractions of 50 mL. Fraction 10 (5.0 mg) showed the presence of a yellow solid that after NMR analyses showed to be constituted by the mixture of the compounds **1–4**. Fractions 34–36 (87 mg) were eluted with the hexane/ethyl acetate 8 : 2 system mixed and fractionated in chromatographic column using neutral alumin (Al_2O_3) as a stationary phase and eluted with the gradients of hexane/ethyl acetate and ethyl acetate/methanol. From this column, fractions 1–9 yielded a 20 mg mixture of the steroids: β-sitosterol and stigmasterol. Structures of all substances obtained from the phytochemical fractionation were determined by their ^{1}H-NMR data analyses, as well as literature data comparison.

Stilbene: 3,5,4′-trimethoxy-4-prenylstilbene (1): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.66 (3H, s, Me), 1.77 (3H, s, Me), 3.37 (2H, d, $J = 7.2$ Hz, H-1′), 3.83 (9H, s, OMe), 5.19 (1H, m, H-2″), 6.65 (2H, d, $J = 2.0$ Hz, H-2 and 6), 6.90 (1H, d, $J = 16$ Hz, H-7), 6.91 (2H, d, $J = 8.8$ Hz, H-3′ and 5′), 7.04 (1H, d, $J = 16$ Hz, H-8), 7.41 (2H, d, $J = 8.8$ Hz, H-2′, 6′).

Chalcone: lonchocarpine (2): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.47 (6H, s, H-7″, 8″), 5.60 (1H, d, $J = 10.0$ Hz, H-5″), 6.39 (1H, d, $J = 9.0$ Hz, H-5′), 6.75 (1H, d, $J = 10.0$ Hz, H-4″), 7.40 (3H, m), 7.57 (1H, d, $J = 15.6$ Hz, H-β), 7.60 (2H, m), 7.73 (1H, d, $J = 9.0$ Hz, H-6′), 7.88 (1H, d, $J = 15.6$ Hz, H-α), 13.80 (1H, s, 2′-OH).

Chalcone: 4-hydroxy-lonchocarpine (3): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.46 (6H, s, H-7″, 8″), 5.59 (1H, d, $J = 10.0$ Hz, H-5″), 6.38 (1H, d, $J = 9.0$ Hz, H-5′), 6.75 (1H, d, $J = 10.0$ Hz, H-4″), 6.88 (2H, d, $J = 8.5$ Hz, H-3, 5), 7.43 (1H, d, $J = 15.5$ Hz, H-β), 7.56 (2H, d, $J = 8.5$ Hz, H-2, 6), 7.71 (1H, d, $J = 9.0$ Hz, H-6′), 7.83 (1H, d, $J = 15.5$ Hz, H-α), 13.68 (1H, s, 2′-OH).

Chalcone: derricidine (4): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.76 (3H, s, Me), 1.81 (3H, s, Me), 4.57 (2H, d, $J = 6.8$ Hz, H-1″), 5.48 (1H, m, H-2″), 6.48 (1H, d, $J = 2.8$ Hz, H-3′), 6.50 (1H, dd, $J = 8.8$, 2.8 Hz, H-5′), 7.40 (3H, m), 7.59 (1H, d, $J = 15.6$ Hz, H-β), 7.60 (2H, m), 7.83 (1H, d, $J = 8.8$ Hz, H-6′), 7.89 (1H, d, $J = 15.6$ Hz, H-α), 13.44 (1H, s, 2′-OH).

3.3. *Phytochemical screening*

Phytochemical screening was performed following the methodology described by Matos (1997), followed by TLC analyses (using silica with UV_{254} fluorescence detector on aluminum support), eluted with appropriated systems and revealed with UV light ($\lambda = 254$ and 365 nm), DPPH, anisaldehyde, ceric sulphate IV, ferric chlorite and aluminum chlorite. Each extract was also analysed by ^{1}H-NMR (400 MHz).

3.4. *Assays*

3.4.1. *Antibacterial assay*

Each extract was assayed against the following bacteria of clinical interest: *P. aeruginosa* (ATCC 10145), *B. cereus* (ATCC 14579), *B. subtilis* (ATCC 6051), *S. aureus* (ATCC 12600), *S. epidermidis* (ATCC 14990), *K. pneumoniae* (ATCC 13883) and *A. hydrophila* (ATCC 7966). Antibacterial activity was determined by the agar diffusion method proposed by Bauer, Klrby, Sherris, and Turck (1966), in duplicate. Each microorganism was inoculated in Petri dishes with the agar Müeller–Hinton culture medium (Difco), then 6 mm holes were made and 20 μL of the extracts (20 mg mL^{-1} concentration) were dropped in each. Dimethylsulphoxide (DMSO) was used as a negative control and oxytetracycline as a positive control. Then, dishes were incubated for the time and temperature proper for each microorganism. At the end of the incubation period, the halos formation was measured and those presenting diameters of 1–6 mm were classified as low active, those with 7–13 mm as medium active and above 13 mm as high active.

3.4.2. *Antioxidant assay*

Qualitative extracts antioxidant assay was performed on TLC using 0.2% of DPPH in methanol, which is capable of quenching free radicals. Occurrence of yellow-white-coloured spots following 30 min application indicated the presence of antioxidant compounds (Cuendet, Hostettman, Potterat, & Dyatmiko, 1997).

In a quantitative antioxidant assay, all the extracts were assayed to determine their ability to discolour the oxidant DPPH that is purple; and in the presence of a reductor, the colour changes to yellow-pale, and it can be measured on 517 nm (FENTOM, model Cirrus 80ST). The results were compared with ascorbic acid, used as the standard. The reaction started by the addition of 990 μL of DPPH (30 μg mL^{-1} in methanol) and 10 μL of each extract sample (0.5 mg mL^{-1}) in triplicate, readings were taken after 30 min. The negative control used 10 μL of methanol with 990 μL of DPPH solution.

3.4.3. *Brine shrimp assay*

Brine shrimp assay was performed using *A. salina* eggs. At first, artificial sea water was prepared (38 g L^{-1}) and 10 mg of eggs were added; after 48 h, with strong aeration and under a continuous light regime at 25–28°C temperature, hatching occurs. Then 10 larvae were collected and put into a 24-well plate. All extracts were initially tested on 1000 μg mL^{-1} concentration, following the Meyer et al. (1982) methodology, and the solvent used to dissolve the extract was used as a negative control. All experiments were made in triplicate. After 24 h of incubation, the number of survivors in each well were counted and noted. To be sure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation, we compared the dead larvae in each treatment to those in control.

4. Conclusions

All extracts were toxic against *A. salina* even in very low concentration (as 5.0 μg mL^{-1}). Only the methanol extract from leaves showed a low antioxidant activity. In the antibacterial assay, only the hexane extract from stems showed a medium activity against *S. aureus*, presenting an inhibition halo of 7 mm. The chemical fractionation of the hexane extract from the stems of *D. duckeana* allows to identify a mixture of 3,5,4′-trimethoxy-4-prenylstilbene (**1**), lonchocarpine (**2**), 4-hydroxylonchocarpine (**3**) and derricidine (**4**),

reported for the first time in *D. duckeana* and another fraction with the mixture of β-sitosterol and stigmasterol.

Supplementary material

Supplementary figures S1 and S2 relating to this article are available online.

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