SHORT REPORT

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Chemical Constituents of *Jacaranda oxyphylla* and their Acetylcholinesterase Inhibitory and Antimicrobial Activities

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Abstract: This study evaluated chemical composition of *Jacaranda oxyphylla*, acetylcholinesterase inhibitory and antimicrobial activities of the isolated compounds. Phytochemical investigation of leaves extract yielded three classes of substances: fatty compounds, sterols and triterpenes. Butyl hexadecanoate (**1**), fatty alcohol (**2**), 2-(4-hydroxyphenyl)ethyl triacontanoate (**3**), *β*-sitosterol (**4**), sitosterol-3-O-*β*-D-glucoside (**5**), 6'-palmitoyl-sitosterol-3-O-*β*-D-glucoside (**6**), oleanolic acid (**7**), ursolic acid (**8**) and corosolic acid (**9**) were obtained from *n*-hexane, $CHCl_3$ and EtOH extracts of *J. oxyphylla*. It was found a pronounced acetylcholinesterase inhibitory activity for the fatty compounds **1-3** and sterols **5** and **6**, with values between 60 to 77%. Substances **7-9** presented a high antibacterial action against *Bacillus cereus* and *Salmonella typhimurium*, with values of growth inhibition in the range of 84 to 90%.

Keywords: Bignoniaceae; *Jacaranda oxyphylla*; acetylcholinesterase inhibition; antibacterial activity.

1. Plant Source

Jacaranda oxyphylla Cham. is found in the Brazilian Cerrado region, popularly known as "caroba-de-São-Paulo" and it is used in folk medicine to treat microbial infections[1,2]. Due to some similarities, *J. oxyphylla* has been previously identified as a variety of the medicinal plant *J. caroba*. However, these species can be differentiated by analysis of their respective leaflets, which are elliptic-lanceolate with 7-9 secondary veins in *J. oxyphylla*[3].

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The aerial parts of *J. oxyphylla* were collected in São João da Chapada, near Diamantina city in July 2012. Plant material was identified by Dr. L. H. Y. Kamino (Institute of Biological Science, Universidade Federal de Minas Gerais, Brazil) and a voucher specimen (No. 170.970) was deposited in BHCB *Herbarium* of the same university.

2. Previous Studies

Fatty materials have been found in the extracts of *Jacaranda* species[4]. For example, 2-(4-hydroxyphenyl)ethyl triacontanoate (**3**) was isolated from the stem of *J. filicifolia* and showed inhibitory activity against the 5-lipoxygenase enzyme[5].

Phytosterols are metabolites widespread in plant species and can be found as free alcohols, esterified to fatty acids or as glycosides[6]. *β*-sitosterol (**4**) was previously isolated from *J. filicifolia*[5], *J. mimosifolia*[7] and *J. caroba*; sitosterol-3-O-*β*-D-glucoside (**5**) was identified from the stem bark of *J. mimosifolia*[8]. Plant sterols have drawn attention due to biological activities featured by them. There is evidence that some phytosterols are effective in preventing cardiovascular diseases[9].

Acid triterpenes of different skeleton have been isolated from *Jacaranda* species[4]. Oleanolic acid (**7**) was previously isolated from *J. mimosifolia*[7] and *J. caroba*; ursolic acid (**8**) was identified in *J. filicifolia*[5], *J. caroba*, *J. copaia*[10] and *J. decurrens*[11]; corosolic acid (**9**) was previously isolated from *J. caucana*[12]. Several biological effects are associated with triterpenes, such as antitumor, anti-inflammatory, antimicrobial and anti-HIV activities[13,14].

3. Present Study

After drying at room temperature, leaves and twigs of *J. oxyphylla* were separated and powdered. Dried leaves of *J. oxyphylla* (1.2 kg) were extracted successively with *n*-hexane, $CHCl_3$ and EtOH by maceration. Extracts were prepared at room temperature, followed by filtration. *n*-Hexane, $CHCl_3$ and EtOH extracts were concentrated under vacuum using a rotary evaporator to afford crude extracts as follows: *n*-hexane extract (13 g), $CHCl_3$ extract (56 g) and EtOH extract (215 g).

Part of the crude *n*-hexane, $CHCl_3$, and EtOH (10, 20, and 20 g, respectively) extracts were submitted to silica gel 60 column chromatography (*n*-hexane, $CHCl_3$, EtOAc and MeOH as eluents, in order of increasing polarity). Fractions of 150 mL were collected and concentrated under vacuum in a rotary evaporator. After thin layer chromatography analysis, similar fractions were pooled in groups. Successive column chromatography purifications and recrystallizations were used for isolation and final purification of compounds **1**-**9**, that belong to different classes of phytochemicals (Figure 1). The purified compounds were characterized using 1D and 2D NMR techniques, UV spectroscopy, mass spectrometry analysis and comparison with previously reported spectral data[15-20].

From the *n*-hexane extract, it was identified butyl hexadecanoate (**1**) (eluting with *n*-hexane-$CHCl_3$ 7:3; 87 mg), fatty alcohol (**2**) (eluted with *n*-hexane-$CHCl_3$ 1:1 and recrystallized with *n*-hexane; 68 mg), *β*-sitosterol (**4**) (eluting with $CHCl_3$-EtOAc from 9:1 to 7:3; 94 mg) and oleanolic acid (**7**) (eluted with $CHCl_3$-EtOAc from 9:1 to 0:1 and recrystallized with EtOH; 11 mg). From the $CHCl_3$ extract, there were isolated the following compounds: 2-(4-hydroxyphenyl)ethyl triacontanoate (**3**) (eluted with $CHCl_3$-EtOAc 9:1 and recrystallized with *n*-hexane; 14 mg), *β*-sitosterol (**4**) (eluting with $CHCl_3$-EtOAc 9:1; 9 mg), sitosterol-3-O-*β*-D-glucoside (**5**) (eluted with EtOAc-MeOH from 9:1 to 1:1 and recrystallized with $(CH_3)_2CO$; 7 mg), 6'-palmitoyl-sitosterol-3-O-*β*-D-glucoside (**6**) (eluting with $CHCl_3$-EtOAc 1:9; 50 mg), oleanolic acid (**7**) and ursolic acid (**8**). Oleanolic and ursolic acids have very similar structures and the separation of these compounds is not easy. The $CHCl_3$ extract was subjected to column chromatography over silica gel and eluted gradient with $CHCl_3$-EtOAc from 1:0 to 3:7. It was obtained a fraction with oleanolic acid (156 mg), an intermediate fraction with oleanolic and ursolic acids mixed (570 mg) and another fraction with ursolic acid (601 mg). These substances were purified by recrystallization with EtOH. From the EtOH extract, ursolic acid (**8**) (740 mg) and corosolic acid (**9**) (624 mg) were isolated over silica gel eluting with $CHCl_3$-EtOAc 3:2 to EtOAc-MeOH 1:1. These acid triterpenes were purified by recrystallization with EtOH.

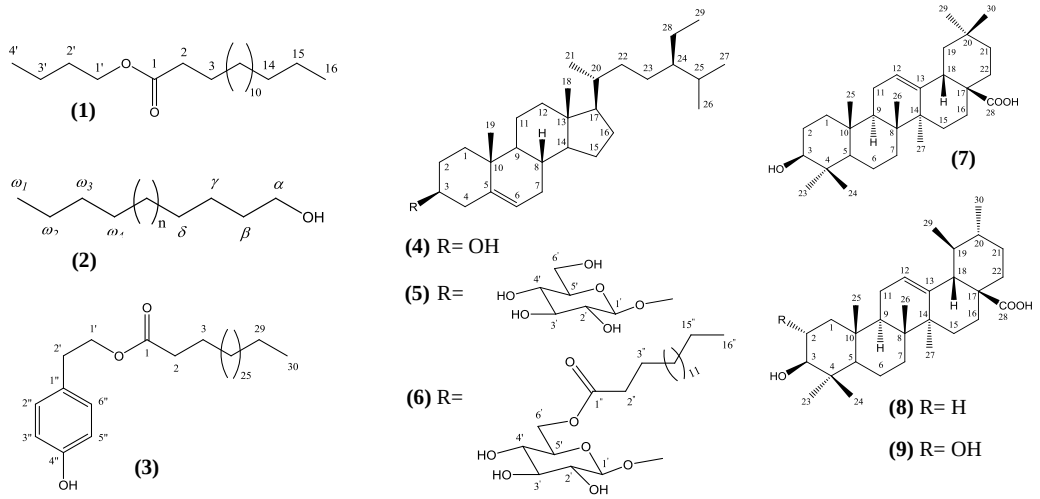

Figure 1. Structures of compounds **1-9** isolated from leaves of *J. oxyphylla*.

Acetylcholinesterase inhibition: Chemical constituents (**1-9**) isolated from *J. oxyphylla* leaves were screened on a quantitative assay for measuring acetylcholinesterase inhibition (iAChE), based on Ellman's method[21]. It was found a potential acetylcholinesterase inhibitory activity for the fatty materials **1-3** and sterols **5** and **6** with values between 60.9 to 77.7% of inhibition, as presented in Table 1. It was observed that the presence of glycosides in the structure of sterols **5** and **6** makes these compounds at least eight times more potent if compared to their precursor, compound **4**. The hydroxyl moieties present in **5** and **6** could be involved in hydrogen bonding with the amino acid residues of the active site of the acetylcholinesterase enzyme[22].

Table 1. *In vitro* antiacetylcholinesterase activity (iAChE) and growth inhibition of microorganism induced by compounds **1-9** isolated of *J. oxyphylla.*

Compound	iAChE*	Microorganism growth inhibition*				
		B. cereus ATCC 1778	*S. aureus* ATCC 29212	*E. coli* ATCC 25922	*S. typhimurium* ATCC 14028	*C. albicans* ATCC 18804
1	60.9 ± 1.4	23.6 ± 1.4	17.8 ± 0.7	0	9.1 ± 0.8	5.8 ± 1.1
2	77.7 ± 1.2	23.3 ± 1.6	31.1 ± 0.8	11.8 ± 0.8	11.6 ± 1.2	14.7 ± 1.6
3	75.4 ± 1.3	28.1 ± 1.5	38.2 ± 1.2	16.8 ± 0.7	18.2 ± 1.4	35.7 ± 1.5
4	8.0 ± 1.0	19.0 ± 1.0	26.7 ± 1.3	10.6 ± 0.8	8.3 ± 0.9	37.1 ± 1.4
5	65.0 ± 1.3	20.9 ± 2.0	33.1 ± 0.9	17.7 ± 0.8	18.3 ± 1.3	31.0 ± 1.4
6	72.8 ± 1.5	35.3 ± 1.3	12.9 ± 0.8	0	45.8 ± 1.5	24.0 ± 1.2
7	0	90.3 ± 1.4	30.4 ± 1.0	49.6 ± 0.9	87.1 ± 1.1	36.6 ± 1.6
8	0	88.7 ± 1.5	27.6 ± 0.8	40.1 ± 0.7	85.8 ± 0.9	33.7 ± 1.1
9	0	85.8 ± 1.1	27.7 ± 0.9	41.1 ± 0.8	84.5 ± 0.9	52.1 ± 1.4
Standard**	87.8 ± 0.7	98.5 ± 0.5	99.3 ± 0.4	99.4 ± 0.3	97.8 ± 1.1	97.9 ± 1.1

*Results are mean values of quintuplicate assays ± standard deviation (expressed as % inhibition); **eserine for acetylcholinesterase, ampicillin for bacteria and nystatin for yeast; compounds were assayed in concentration of 100 μg mL^{-1}.

Antimicrobial screening: Compounds **1-9** obtained from leaves of the *J. oxyphylla* were subjected to antimicrobial assay by broth microdilution method[23]. Gram-positive bacteria *Bacillus cereus* and

Staphylococcus aureus, Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* and the yeast *Candida albicans* were tested. Substances **7-9** presented a high antibacterial action against *B. cereus* and *S. typhimurim*, with values of growth inhibition in the range of 84.5 to 90.3%. Moreover, triterpene **9** presented a moderate activity against *C. albicans* (52.1%). The overall results of the antimicrobial assay are shown in Table 1.

This study reported the isolation of nine compounds from the leaves of *J. oxyphylla*, a species without chemical and biological studies in the literature. *β*-sitosterol and its glycosides derivatives (compounds **4-6**) were the phytosterols obtained and the triterpenoid acids isolated were olean-12-ene or urs-12-ene derivatives (compounds **7-9**). It was obtained a high quantity of ursolic acid (3.7% of EtOH extract), corosolic acid (3.1% of EtOH extract) and oleanolic acid (0.8% of $CHCl_3$ extract). Thus, *J. oxyphylla* revealed to be a natural source of these triterpenes, which exhibited a high antibacterial activity. This is the first report on the isolation of compounds **2** and **6** in Bignoniaceae family. These fatty compounds have potential inhibitory activity towards acetylcholinesterase and could be useful as lead for developing alternative drugs to the treatment of Alzheimer's disease.

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