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Pedilanthus tithymaloides (L.) Poyt: phytochemical prospection and antimicrobial activity

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Abstract

Most popularly used as an ornamental plant in Brazil, *Pedilanthus tithymaloides* (L.) Poyt (Euphorbiaceae) is also known for its medicinal uses in Asia and Central America. Antihypertensive, antibacterial and antifungal effects were described earlier, but no information is available about the properties of this plant growing in the Amazon region. This study describes the phytochemical prospection and a screening of the antimicrobial effects of *P. tithymaloides* collected in Manaus, Amazonas state, Brazil. Leaves samples were processed to obtain extracts using hexane (Hex), ethyl acetate (EA) and ethanol (EtOH) by maceration. Phytochemical prospection of relevant secondary metabolites was performed as well as antimicrobial evaluation by microdilution method against six pathogenic bacteria and one yeast. Triterpenes, steroids, saponins, tannin and coumarins were detected in the extracts. Hex and EtOH were both effective against *Streptococcus sanguinis* and Hex inhibited *Enterococcus faecalis* with a minimum inhibitory concentration (MIC) of 3 µg mL^{-1}; the extracts also inhibited also *Candida albicans* (6 µg mL^{-1} MIC). No inhibition was detected against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* or *Salmonella enterica*. These findings are in accordance of previous studies and support the potential use of *P. tithymaloides* for future development of antimicrobials against relevant pathogens and that this species maintains its pharmacognostic properties growing in the Amazon region.

Keywords: Euphorbiaceae, phytochemical prospection, antimicrobials, antifungals

Pedilanthus tithymaloides (L.) Poyt: prospecção fitoquímica e atividade antimicrobiana. *Pedilanthus tithymaloides* (L.) Poyt (Euphorbiaceae) é mais conhecida no Brasil como planta ornamental, mas também é utilizada como medicinal na Ásia e na América Central. Seus efeitos anti-hipertensivo, antibacteriano e antifúngico foram descritos anteriormente, mas nenhuma informação sobre as propriedades dessa espécie na região Amazônica foi levantada até momento. O presente estudo descreve a prospecção fitoquímica e uma avaliação da atividade antimicrobiana de extratos de *P. tithymaloides* coletada em Manaus, Amazonas, Brasil. Amostras de folhas foram processadas para obtenção de extratos utilizando hexano (Hex), acetato de etila (EA) e etanol (EtOH) por maceração. Foi realizada a prospecção fitoquímica dos grupos químicos mais relevantes assim como a avaliação de seu efeito sobre 6 bactérias patogênicas e 1 levedura, por método de microdiluição. Triterpenos, esteroides, saponinas, taninos e cumarinas foram detectados nos extratos. Hex e EtOH inibiram *Streptococcus sanguinis* e Hex inibiu *Enterococcus faecalis* com uma concentração inibitória mínima (CIM) de 3 µg mL^{-1}; os extratos também inibiram *Candida albicans* com um CIM de 6 µg mL^{-1}. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* e *Salmonella enterica* não foram suscetíveis. Esses resultados corroboram estudos anteriores quanto ao potencial de *P. tithymaloides* para o desenvolvimento de antimicrobianos contra importantes patógenos, indicando que essa espécie mantém suas propriedades farmacognósticas crescendo na Amazônia.

Key-words: Euphorbiaceae, prospecção fitoquímica, antimicrobianos, antifúngicos

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1. Introduction

Pedilanthus tithymaloides (L.) Poit (Euphorbiaceae) is a succulent shrub well distributed in tropical America and Asia (CORREA, 1984). It has been studied for its chemical composition and potential use for pharmaceutical purposes. Tinctures obtained in ethanol (ABREU et al. 2006) as well as chloroform and methanol extracts (GOSH et al. 2013) were effective as anti-inflammatory on in vivo essays. Later on, antioxidant compounds were identified as kaempferol, quercitrin, isoquercitrin and scopoletin (ABREU et al. 2008). Ethanol extracts were effective against the filariasis vector, *Culex quinquefasciatus*, at all developmental stages (KAMALAKANNAN et al. 2010); as antimicrobial, methanol (VIDOTTI et al. 2006) as well as ethanol extracts (MOHD et al. 2012) were also tested against relevant pathogens. However, no study was dedicated to this plant in Brazil, as it is mostly used in gardening to build live fences. In a survey about medicinal plants on the community of Marudá (Pará state, Brazil), *P. tithymaloides*, called 'coramina', was cited to be used as antihypertensive and heart sedative prepared as tea and maceration of leaves, respectively (COELHO-FERREIRA, 2009). The purpose of the present work was to contribute to the knowledge about *P. tithymaloides* concerning the chemical profile and antimicrobial effects.

2. Materials and Methods

Specimens of *Pedilanthus tithymaloides* were collected at the Instituto Federal do Amazonas – Campus Zona Leste (3°04'48.3"S 59°56'01.8"W - Manaus, Amazonas, Brazil) and the exsiccate was deposited at the HUAM – Herbarium of the Universidade Federal do Amazonas (voucher HUAM N. 9982). For the purpose of this work, leaf samples were collected. After removing dirt, the selected samples were dried for 48 hours in a circulating air oven at 60 ºC and pulverized to enhance the contact with the solvents. The latter were hexane (Hex), ethyl acetate (EA) and ethanol (EtOH) used for extraction by maceration for a period of nine days with sequential changes of the solvent every three days. The so obtained extracts were filtered and concentrated in a rotary evaporator (model 801, FISATOM, Perdizes – São Paulo, Brazil) and stored at -24 ºC in a freezer.

A rapid and useful colorimetric methodology detailed by Matos (1997), was applied to determine the most relevant groups of secondary metabolites obtained within the extracts. Briefly, the methods should indicate the presence of 1. Steroids and triterpenes, 2. Phenols and tannins, 3. Alkaloids, 4. Coumarins, 5. Saponins, 6. Anthocyanins, Anthocyanidins, Chalcones, Aurones and Flavonoids, and 7. Flavonols, Flavanons, Flavanonols and Xhantones.

Antimicrobial assay

An overnight culture of each microbial strain in brain-heart-infusion broth (BHI) was carried out for the following target organisms: *Enterococcus faecalis* (ATCC29212), *Streptococcus sanguinis* (ATCC15300), *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC8739), *Pseudomonas aeruginosa* (ATCC9027), *Salmonella enterica* (ATCC13076) and *Candida albicans* (ATCC10231).

Each microdilution plate (96 wells) was prepared with 100 µL Mueller-Hinton broth (MH) in each well. Standard solutions of each plant extract – He and EtOH – of 10 mg mL^{-1} in Dimetilsulfoxide 5% (DMSO) were prepared. From each test extract an aliquot of 100 µL was added, so that the final concentration at the first well in a row was 3.33 mg mL^{-1} and a two-fold serial dilution followed until the 12^{th} column. At last, an aliquot of 100 µL of a microbial suspension was inoculated into each well: the inocula concentrations of 10^8 cells mL^{-1} and 10^5 cells mL^{-1} were determined with a Neubauer chamber, respectively, for the bacterial strains (CLSI, 2012) and *C. albicans* (CLSI, 2003). The test was performed in triplicate for each target strain. Following the methodology of Sarker et al. (2007), the bacterial strains were incubated at 35± 2 ºC and *C. albicans* was incubated at 25± 2 ºC for 21h. At this point, 10 µL of a resazurin solution (1.0%) was added to each well and incubated for 3 more hours. A change in color from purple to pink should indicate microbial growth due to the reduction of resazurin. As experimental controls the following solutions were used: DMSO - negative control; chlorhexidine digluconate (CHX 2.0%) - positive control for inhibition of the microbial growth; and sterile MH broth as sterility control. The final results (MIC values) were transformed to µg mL^{-1}.

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3. Results and Discussion

In the present work, leaves of *Pedilanthus tithymaloides* Poit (L.). (Euphorbiaceae) collected at the urban area of Manaus (Brazil) were processed to obtain a profile of the phytochemical composition and the antimicrobial effects of their crude extracts. Leaves were the main plant part used in the cited studies as well as registered by the folk medicine (COELHO-FERREIRA, 2009).

Relevant chemical groups were detected as outlined on Table 1, including steroids, triterpenes, tannins, coumarins and saponins. Although flavonoids and phenolic compounds have been described for this species, under the conditions of this study, they were not detected. The environmental conditions of the different regions were the studies about this plant were conducted before may have influenced the results or another techniques should be applied to enhance the accuracy of the results.

Steroids and triterpenes have been discussed elsewhere as relevant constituents of *P. tithymaloides* leaf extracts, although they have been related to other biological properties (KAMALAKANNAN et al. 2010; PRAKASH et al. 2014).

Coumarins were present in these samples and have been previously described for *P. tithymaloides*, suggested to be used as a specific marker. Sandjo et al. (2012) reported that 9 coumarin derivatives were able to stop the germination of *Magnaporthe orizae*, a phytopathogenic sporulating fungus. Previous studies with isolated coumarins from other species have described their action over a variety of fungi, including *C. albicans* (AYINE-TORA et al. 2016, SONG et al. 2017). In this study, *C. albicans* was susceptible to both extracts with a MIC of 6 µg mL^{-1} (Table 2). These results suggest that future studies should broaden the number of targeted fungi to test the influence of *P. tithymaloides* extracts and/or isolated compounds.

Diterpenoids were characterized in the latex of *P. tithymaloides*, which was effective against *Mycobacteryum tuberculosis* (MIC 12.5 µg mL^{-1}), but has not affected *C. albicans* at a concentration of 50 µg mL^{-1} (MOGKOLVISUT and SUTTHIVAIYAKIT 2007). Considering our results, this might indicate a difference on the chemical composition between the latex and the substances reserved in the leaf structures. Also the hypothesis that a synergistic effect of some specific compounds may act together in the crude extracts should not be disregarded.

Table 1. Phytochemical groups detected on extracts* of *Pedilanthus tithymaloides* leaves.

Chemical classes	Hex	EA	EtOH
Steroids	+	-	+
Triterpenes	+	-	-
Phenols	-	-	-
Tannins	-	-	+
Alkaloids	-	-	-
Coumarins	-	+	-
Saponins	+	-	+
Anthocyanins, Anthocyanidins, Chalcones, Aurones and Flavonoids	-	-	-
Flavonols, Flavanons, Flavanonols and Xanthones	-	-	-

*Extracts obtained in hexane (Hex), ethyl acetate (EA) and etanol (EtOH).

Microdilution assays were conducted by Vidotti et al. (2006). The authors found 2 new compounds obtained from the ethanolic extract to be effective against *S. aureus*, *Bacillus subtilis*, *E. coli* and *P. aeruginosa* in very low concentrations. Testing the methanolic extracts of leaf samples, Vu *et al*. (2016) found *P. tithymaloides* to pursue a broad antimicrobial spectrum, since it was effective in avoiding growth of gram negative and gram positive species. By *E. coli* and *P. aeruginosa* the MIC achieved was much higher (2.000 µg mL^{-1}) than by the gram positive strains, which were far more sensitive (MIC 250 µg mL^{-1}), the 2 of them belonging to the genus *Bacillus* and *S. aureus*. The latter was also included in our study, but was not susceptible. The MIC values achieved by our crude extracts other gram positive strains were much lower: *S. sanguinis* 3.0 µg mL^{-1} by both extracts, and *E. faecalis* 3.0 and 6.0 µg mL^{-1} by EtOH and Hex, respectively.

Further studies are needed for a better approach to the chemical composition and the antimicrobial effects of the isolated compounds in a perspective of correlating the results with those obtained from the crude extracts.

4. Conclusion

This work showed that *P. tithymaloides* constituents include steroids, triterpenes, tannins, coumarins and saponins reserved in the leaves and

that *S. sanguinis*, *E. faecalis* and *C. albicans* are susceptible to the crude extracts in low concentrations.

Table 2. Minimum inhibitory concentration (MIC µg mL^{-1}) of *Pedilanthus tithymaloides* extracts against six pathogenic bacterial strains and *Candida albicans*

Extract	SS	EF	AS	EC	PA	SE	CA
Hex	3.0	6.0	-	-	-	-	6.0
EtOH	3.0	3.0	-	-	-	-	6.0
CHX	0.2	0.2	0.2	0.3	0.3	0.3	0.2
DMSO	-	-	-	-	-	-	-

SS: *Streptococcus sanguinis*; EF: *Enterococcus faecalis;* SA*: Staphylococcus aureus;* EC: *Escherichia coli;* SE: *Salmonella enterica;* PA: *Pseudomonas aeruginosa;* CA: *Candida albicans*; Hex: extract obtained in hexane; EtOH: extract obtained in ethanol; CHX: Chlorhexidine digluconate solution; DMSO: dimetilsulphoxide solution.

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Divulgação

Este artigo é inédito e não está sendo considerado para qualquer outra publicação. Os autores e revisores não relataram qualquer conflito de interesse durante a sua avaliação. Logo, a revista *Scientia Amazonia* detém os direitos autorais, tem a aprovação e a permissão dos autores para divulgação, deste artigo, por meio eletrônico.

References

ABREU, P.; MATTHEW, S.; GONZÁLEZ, T.; COSTA, D.; SEGUNDO, M.A.; FERNANDES, E. Anti-inflammatory and antioxidant activity of a medicinal tincture from *Pedilanthus tithymaloides*. **Life Sciences,** n. 78, 2006, 1578-1585.

ABREU, P.M.; MATTHEW, S.; GONZÁLEZ, T.; VANICKOVA, L.; COSTA, D.; GOMES, A.; SEGUNDO, M.A.; FERNANDES, E. Isolation and identification of antioxidants from *Pedilanthus tithymaloides*. **Journal of Natural Medicine,** n. 62, 2008, 67–70.

AYINE-TORA, D. M.; KINGSFORD-ADABOH, R.; ASOMANING, W. A.; HARRISON, J. J. E. K.; MILLS-ROBERTSON, F. C.; BUKARI, Y.; SAKYI, P. O.; KAMINTA, S.; REYNISSON, J. Coumarin antifungal lead compounds from *Millettia* thonningii and their predicted mode of action. **Molecules** n. 21, 2016, 1369, doi:10.3390/molecules21101369.

CLSI - Clinical and Laboratory Standards Institute. M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. ninth edition Wayne, PA, USA: CLSI; 2012, 12.

CLSI - Clinical and Laboratory Standards Institute. M27-A2. Method for antifungal susceptibility tests of yeasts; approved standard. second edition Wayne, PA, USA: CLSI; 2003, 19.

COELHO-FERREIRA M. Medicinal knowledge and plant utilization in an Amazonian coastal community of Marudá, Pará State (Brazil). **Journal of Ethnopharmacology**, n. 126, 2009, 159-175.

GOSH, S.; CHATTOPADHYAY, D.; MANDAL, A.; KAITY, S.; SAMANTA, A. Bioactivity guided isolation of anti-inflammatory, analgesic, and antipyretic constituents from leaves of *Pedilanthus tithymaloides* (L.). **Medicinal Chemistry Research**, n. 22, 2013, 4347. doi:10.1007/s00044-012-0449-4

KAMALAKANNAN, S.;MADHIYAZAGAN, P.; DHANDAPANI, A.; MURUGAN, K.; BARNARD, D. *Pedilanthus tithymaloides* (Euphorbiaceae) leaf extract phytochemicals: toxicity to the filariasis vector *Culex quinquesfasciatus* (Diptera: Culicidae). **Vector-Borne and Zoonotic Diseases**. N. 8, 2010, 817-820.

MATOS, F.J.A. Introdução à fitoquímica experimental. Fortaleza: Edições UFC, Brasil. 1997.

MOGKOLVISUT, W.; SUTTHIVAIYAKIT, S. Antimalarial and antituberculosis poly-o-acylated jatrophane diterpenoids from *Pedilanthus tithymaloides.* **Journal of Natural Products.** 70, 2007, 1434-1438.

MOHD AKR, NORHAYATI AH, EMYNUR SS, MOHAMAD FG, AZLINA M, TAJUL ZM, AHMAD ZAL. Screening of seven types Terengganu herbs for their potential antibacterial activity against selected food microorganisms. **Borneo Science.** 31, 2012, 11-27.

PRAKASH, N. K. U.; RANJITHKUMAR, M.; SRIPRIYA, N.; LAKSHMI, R. P.; DEEPA, S.; BHUVANESWARI, S. Antioxidant, free radical scavenging activity and CG-MS studies on *Pedilanthus tithymaloides* (L.) Poit. **International Journal of Pharmacy and Pharmaceutical Sciences**, n. 6, 2014, 284-287.

SANDJO, L.P.; FOSTER, A.J.; RHEINHEIMER, J.; ANKE, H.; OPATZ, T.; THINES, E. Coumarin derivatives from *Pedilanthus tithymaloides* as inhibitors of conidial germination in *Magnaporthe orizae*. **Tetrahedron Letters,** 53, 2012, 2153-2156.

SARKER, S.D.; NAHAR, L.; KUMARASAMY, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. **Methods**, n. 42, 2007, 321-324.

SONG, P-P.; ZHAO, J.; LIU, Z-L.; DUAN, Y-B.; HOU, Y-P.; ZHAO, C-Q.; WU, M.; WEI, M.; WANG, N-H.; LV, Y.; HAN, Z-J. Evaluation of antifungal activities and structure-activity relationships of coumarin derivatives. **Pest Management Science**, n. 73, 2017, 94-101. doi: 10.1002/ps.4422.

VIDOTTI, G.J.; ZIMMERMANN, A.; SARRAGIOTTO, M.H.; NAKAMURA, C.V.; DIAS FILHO, B. P. Antimicrobial and phytochemical studies on *Pedilanthus tithymaloides*. **Fitoterapia** 77, 2006, 43-46.

VU, T. T.; KIM, H.; TRAN, V. K.; DANG, Q. L.; NGUYEN, H. T.; KIM, H.; KIM, I. S.; CHOI, G. J.; KIM, J. C. **BMC Complementary and Alternative Medicine**, 16:32, 2016, doi: 10.1186/s12906-016-1007-2.