

Antimicrobial Activity and Phytochemical Screening of Extracts from Leaves of *Croton polyandrus* Spreng

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ABSTRACT

Aromatic plants have been used in folk medicine as antimicrobial agents since ancient times. The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and flavonoids. The antimicrobial activity of extracts was studied using the microdilution method and determination of minimal inhibitory concentration (MIC) value. Investigations on the phytochemical screening of *Croton polyandrus* leaves extracts revealed the presence of saponins, steroids and tannins. The results obtained with *Croton polyandrus* ethyl acetate and dichloromethane extracts showed promising antifungal activity against *Candida albicans* (ATCC 90028) with MIC of 64 µg/mL and 128 µg/mL, respectively.

Key words: *Croton polyandrus*, extracts, antimicrobial activity.

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being¹.

Aromatic plants have been used in folk medicine as antimicrobial agents since ancient times^{2,3}. Although approximately 20% of the world plants have been submitted to pharmacological or biological test, it could be concluded that natural products from plant origin are an important source to discover new leads with economical and pharmaceutical importance and great possibilities to be developed as drugs, dyes, fragrances and pesticides, among others⁴. To obtain novel and promissory substances many plant extracts have to be assayed. For example, Mendes et al. (2011)⁵ assayed 2 plant extracts and found extracts with strong antimicrobial activity.

Furthermore, the screening of plant extracts as antimicrobial agents is necessary to go insight into medicinal flora and get the molecules responsible for this activity and add value to natural resources from tropical areas⁶.

Many Euphorbiaceae are well known in different parts of the world as toxic and/or medicinal plants. The high diversity of the described effects is a reflex of the high chemical diversity of this plant group. *Croton* is a large genus of Euphorbiaceae, comprising around 1,300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of both hemispheres⁷.

Based on the medicinal properties of plants of *Croton* genus, the present study aimed to investigate the antimicrobial activity of the extracts isolated from *Croton polyandrus* belongs to the family Euphorbiaceae.

MATERIALS AND METHODS

Preparation of plant extract: The leaves of *Croton polyandrus* were collected in the town of Santa Rita, Paraiba, Brazil. The botanical material was identified by Dr^a. Maria de Fatima Agra of the Botany Section of the Laboratory of Pharmaceutical Technology "Prof. Delby Fernandes de Medeiros". Voucher specimens of these plants are deposited in the Herbarium Prof. Lauro Pires Xavier (JPB), exsicata Agra & Gois 1446 (JPB), Federal University of Paraiba.

The powder aerial parts - leaves (3000 g) was sprayed dried and subjected to maceration with hexane, dichloromethane, ethyl acetate and ethanol in a stainless steel container for 72 hours each extraction. After extraction, the extraction solutions were concentrated in a rotary evaporator under reduced pressure at a temperature of 45 ° C to yield extracts: hexane (40.2 g), dichloromethane (46.5 g), ethyl acetate (59.8 g) and ethanol (194.4 g).

Phytochemical analysis of the plant extracts: The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and flavonoids in accordance with Trease et al. (1989)⁸ and Harborne (1998)⁹ with little modification.

Table 1 - Phytochemical analysis of the extracts from *C. polyandrus*.

Extracts/Secondary metabolites	Saponins	Steroids	Tannins	Alkaloids	Flavonoids
EtOH extract	+	+	+	-	-
Acoet extract	-	+	-	-	-
Hexane extract	-	+	-	-	-
Dichloro extract	-	+	-	-	-

(+) presence and (-) absence

Table 2 – Antibacterial activity of the extracts from *C. polyandrus*.

Substance/Bacteria strain	EtOH extract (MIC)	Acoet extract (MIC)	Hexane extract (MIC)	Dichloro extract (MIC)	Negative control	Positive control (Chloramphenicol)
<i>S.aureus</i> ATCC 13150	1024 µg/mL	1024 µg/mL	-	-	-	+
<i>S. aureus</i> ATCC 25923	-	-	-	-	-	+
<i>P. aeruginosa</i> P 03	-	-	-	1024 µg/mL	-	+
<i>P. aeruginosa</i> ATCC 25853	1024 µg/mL	1024 µg/mL	-	1024 µg/mL	-	+
<i>E.coli</i> ATCC 25922	-	-	-	-	-	+
<i>E.coli</i> 5	-	-	-	-	-	+

(-) no growth inhibition (+) growth inhibition

Table 3: Antifungal activity of the extracts from *C. polyandrus*.

Substance/Bacteria strain	EtOH extract (MIC)	Acoet extract (MIC)	Hexane extract (MIC)	Dichloro extract (MIC)	Negative control	Positive control (Nistatin)
<i>C. albicans</i> ATCC 90028	1024 µg/mL	64 µg/mL	-	128 µg/mL	-	+
<i>C. albicans</i> LM-109	-	-	-	-	-	+
<i>C. tropicalis</i> ATCC 13803	-	-	-	-	-	+
<i>C. tropicalis</i> LM-P20	-	-	-	-	-	+
<i>C. krusei</i> LM-13	-	-	-	-	-	+
<i>C. krusei</i> LM-08	1024 µg/mL	-	-	1024 µg/mL	-	+

(-) no growth inhibition (+) growth inhibition

Bacterial and fungal strains: For antibacterial activity assays, were selected six strains of bacteria (*Staphylococcus aureus* - ATCC 13150, *Staphylococcus aureus* - ATCC 25923, *Pseudomonas aeruginosa* - P03, *Pseudomonas aeruginosa* - ATCC 25853, *Escherichia coli* - ATCC 25922 and *Escherichia coli* - 5) and for antifungal activity assays were selected 6 strains of fungi (*Candida*

albicans – ATCC 90028, *Candida albicans* – LM 109, *Candida tropicalis* - ATCC 13803, *Candida tropicalis* – LMP 20, *Candida krusei* – LM 13 and *Candida krusei* – LM 08). All the microorganism strains tested belong to the collection of the Mycology Laboratory, Federal University of Paraíba. Bacteria and fungi were kept on Nutrient Agar

(NA) slants at 4 °C. Inocula were obtained from overnight cultures grown on NA slants at 37 °C and diluted in sterile saline solution (NaCl 0.85% w/v) to provide a final concentration of approximately 10⁶ Count Forming Unit per mL (CFU.mL⁻¹) adjusted according to the turbidity of 0.5 McFarland scale tube.

Antimicrobial assay: The microplate bioassay was used to determine the minimum inhibitory concentration (MIC)

of *Croton polyandrus* extracts. The 96-well plates were prepared by dispensing 100 µL of double strength Nutrient Broth (NB) inoculated with the bacterium inoculum into each well prior to the assay. An aliquot (100 µL) of the extracts solutions at their respective concentrations was transferred into seven consecutive wells. The final volume in each well was 200 µL. The solution having the highest extracts concentration was added into the first well and the one having the smallest concentration was added into the antepenultimate well. The penultimate and the last well, containing 200 µL of the NB inoculated with the microorganism suspension and Chloramphenicol (100 µg/mL) or Nistatin (100 UI/mL), were used as the negative control and positive control, respectively. The microplate was aseptically sealed, followed by mixing on a plate shaker (300 rpm) for 30 seconds and incubated at 37 °C for 24 hours^{10,11}.

The antibacterial and antifungal activities were detected using the colorimetric method by adding 200 µL of resazurin staining (0.1 g.100 mL⁻¹) aqueous solution in each well at the end of the incubation period. MIC was defined as the lowest essential oil concentration able to inhibit the bacterial growth as indicated by resazurin staining (dead cells were not able to change the staining

color by visual observation – blue to red)¹². All experiments were carried out at least twice with consistent results (Table 2 and Table 3).

RESULTS

Phytochemical screening of *Croton polyandrous* leaves ethanolic extracts revealed the presence of saponins, steroids and tannins. Yet the phytochemical screening of *Croton polyandrous* leaves hexane, dichloromethane and ethyl acetate extracts revealed the presence of steroids (Table 1).

The results for antimicrobial activity of the extracts with MIC value are show in Table 2 and Table 3. The activity was measured in terms of presence of microorganism growth. the extracts from *Croton polyandrus* show no antibacterial activity against either gram (+) or gram (-) bacteria. However, results obtained from the *in vitro* antifungal assay showed that the ethyl acetate (Acoet) and dichloromethane (Dichloro) extracts show antifungal activity against *Candida albicans* (ATCC 90028) with MIC of 64 µg/mL and 128 µg/mL, respectively. Furthermore, these extracts showed low antifungal activity against *Candida krusei* (LM 08) with MIC of 1024 µg/mL.

DISCUSSION

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency^{13,14}. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils¹⁵, as well as in flavonoid¹⁶.

Investigations on the phytochemical screening of *Croton polyandrous* leaves ethanolic extracts revealed the presence of saponins, steroids and tannins. Yet the phytochemical screening of *Croton polyandrous* leaves hexane, dichloromethane and ethyl acetate extracts revealed the presence of steroids. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *Croton polyandrous*. These secondary metabolites exert antimicrobial activity through different mechanisms¹.

Steroidal compounds present in *Croton polyandrous* leaves ethyl acetate extract are of importance and interest due to their relationship with various anabolic hormones including sex hormones¹⁷. Quinlan *et al.* (2000)¹⁸ worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann *et al.* (2004)¹⁹ also confirmed the antiviral property of steroids.

Analyzing this result obtained from the *in vitro* antibacterial assay showed that the extracts from *Croton polyandrus* show no antibacterial activity against either gram (+) or gram (-) bacteria. However, results obtained from the *in vitro* antifungal assay showed that the ethyl

acetate (Acoet) and dichloromethane (Dichloro) extracts show promising antifungal activity against *Candida albicans* (ATCC 90028) with MIC of 64 µg/mL and 128 µg/mL, respectively. Furthermore, these extracts showed low antifungal activity against *Candida krusei* (LM 08) with MIC of 1024 µg/mL. The different behavior observed between strains of the same species could be justified by the existence of genetic variability among different strains²⁰.

This antifungal activity against *Candida albicans* of extracts from *Croton polyandrus* has been observed in other studies with extracts of plant species of the family Euphorbiaceae²¹.

CONCLUSION

Based on these results it can be stated that the *Croton polyandrous* extracts have an important antifungal activity against *Candida* species, which highlights the need for further studies with other fungal species to investigate the immense therapeutic potential of this plant species and with his isolated secondary metabolites.

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