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# Antibacterial activity of *Minquartia guianensis* extracts and phytochemical evaluation

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**Abstract:** The species *Minquartia guianensis* (Olacaceae) is found in the Amazon region and also in Nicarágua, Panamá and Costa Rica. Indigenous people from Ecuador use the bark infusion for intestinal infections treatment caused by parasites, against muscular pain and cutaneous irritations. For this reason, the aim of this work was to evaluate the antibacterial activity of *M. guianensis* extracts over Gram-negative (*Shigella flexneri* M90T*, Salmonella choleraesuis* 6958*, Escherichia coli* E2348/69) and Gram-positive bacteria (Methicillin-Resistant *Staphylococcus aureus* 33591*,* Methicillin-Sensible *Staphylococcus aureus* 25923, *Bacillus cereus* 9634*, Bacillus liquefaciens* clinical isolated). These bacteria are diarrhea related, which causes several child death in tropical regions. The active extract is under fractionation (leaf DCM) and until now, four triterpene were isolated lupeol, taraxerol, lupenona and squalene, but it was not possible yet to assay the substances, because of their small amount.

**Key words:** Antibacterial Activity, *Minquartia guianensis*, Olacaceae, Gram-positive bacteria, Gram-negative bacteria

# نشاط المضادات البكتيرية لمستخلص نبات Minquartia guianensis وتقيم محتويات النبات الكيميائية

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**الملخص**: تنتشر بعض انواع نبات Minquartia guianensis التابع للفصيلة Olacaceae في مناطق الامازون كما تتواجد أيضا في نيكارغوا وبنما وكوستاريكا. يستخدم سكان الإكوادور الاصليين منقوع لحاء هذا النبات لعلاج الالتهابات المعوية الناجمة عن الطفيليات ، كما يستخدم ايضا لعلاج آلام العضلات والتحسسات الجلدية. ولهذا السبب، فان الهدف من هذه الدراسة هو تقييم نشاط المضادات البكتيرية لمستخلص نبات Minquartia guianensis على البكتيريا السالبة لصبغة جرام (Shigella flexneri M90T, Salmonella choleraesuis 6958, Escherichia coli E2348/69)والبكتيريا الموجبة لصبغة جرام (Methicillin-Resistant Staphylococcus aureus 33591, Methicillin-Sensible Staphylococcus aureus 25923, Bacillus cereus 9634, Bacillus liquefaciens).ولهذه البكتيريا علاقة مباشرة بالإسهال ، والذي يتسبب في وفاة العديد من الاطفال في المناطق المدارية. يجري العمل في الوقت الحاضر لفصل المواد الفعالة من مستخلص هذا النبات ، وحتى الان تم عزل اربعة من مركبات التريتربين (triterpene) هما lupeol ، taraxerol، lupenonaو squalene.ولم يكن بالمقدور حتى الآن تحليل هذه المواد نظرا لصغر حجم العينات المعزولة.

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## Introduction

Several diseases are affecting people around the world, among them intestinal infections are common. Studies with children in the hospital in João Pessoa, PB, Brazil, showed a significant number of children are under problems with intestinal infection (Tôrres et al., 2005). This problem is widespread all over tropical regions and some of them are so acute that causes children death. The main causes of diarrhea are related to bacterial infections (Viswanathan et al., 2008).

Plant species are producers of several chemical metabolites and in the available literature, there are several examples of plants and their metabolites that showed antibacterial activity. For example, ethanolic extracts *Ocotea duckei* showed activity against *Escherichia coli* (Antunes et al., 2006), methanolic extract from *Caesalpinia bonducella* and triterpenes α-amiryn, β-amyrin, lupeol, and lupeol acetate showed activity against *Staphylococcus aureus, Escherichia coli, Shigella flexneri* (Saeed et al., 2001). The acetyl aleuritolic acid isolated from *Spirostachys africana* was active against *Escherichia coli, Staphylococcus aureus*, *Vibrio cholera, Salmonella typhi*, *Shigella dysentery, S. flexnerii* and *S. boydii* (Mathabe et al., 2008).

Among plant kingdom, Olacaceae is a family with 24 genera and 150 species, and in Brazil there are 12 genera and 60 species (APG II, 2008). Several compounds were isolated from this family, such as: flavonoids, alkaloids, terpenes and saponins (Forgacs, 1981; Polonsky, 1984; Haron, 1997; Wiart, 2001).

The plant species chosen for this work is *Minquartia guianensis* Aubl. which is found in the Amazon region and also in Nicaragua, Panama and Costa Rica (MOBOT, 2010). Their popular names are: acariquara, acariquara-roxa, acari, acapu, acaximba and arariuba (Camargo, 2005). There are few previous chemical work realized with this species. Rasmussen et al. (2000) described the minquartinoic acid which showed activity against malaria and leishmaniasis. Other compounds isolated from this species were triterpenes, xantones, acetylenic acid (El-Seedi et al., 1994) and other triterpenes as squalene, lupe-3-one, taraxerone and lupeol (Cursino et al., 2009).

This article describes antibacterial activity and phytochemical evaluation of *M. guianensis* extracts.

## Materials and Methods

### Plant material

*Minquartia guianensis* Aubl. (Olacaceae) was collected in Reserva Florestal Ducke, Manaus, AM, in April/2005. A voucher specimen (179.806) was deposited at INPA's Botanical Research Coordenation Herbarium.

The leaves and branches were dried, grounded and extracted by using dichloromethane (DCM), methanol (MeOH) and water ($H_2O$), each extraction performed 3 times by using ultra-sound for 20 min.

### Assays

### Bacterial strains

The extracts were tested against Gram-negative (*Shigella flexneri* M90T, *Salmonella choleraesuis* 6958, *Escherichia coli* E2348/69) and Gram-positive bacteria (Methicillin-Resistant *Staphylococcus aureus* 33591, Methicillin-Sensible *Staphylococcus aureus* 25923, *Bacillus cereus* 9634, *Bacillus liquefaciens* clinical isolated). The bacteria were kept on the defined bacterium medium which contains proteose peptone, sodium chloride, meat extract, bacteriological grade agar and distilled water, at room temperature.

### Agar-Well diffusion method

The assay was conducted by agar well diffusion method (Perez et al., 1990). The bacterial strains grown on nutrient agar at 37°C for 24 h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards ($10^8$ CFU/mL). The suspension was used to inoculate 90 mm diameter Petri. Wells (6 mm diameter) were punched in the agar and filled with 100 μL of 20 mg/mL extracts. The dissolution of the organic extracts (DCM and MeOH) was aided by 1% (v/v) DMSO and that of the aqueous extracts with water, which did not affect the growth of microorganisms, in accordance with our control experiments. Plates were incubated in air at

37°C for 24 h. The experiments were conducted thrice. DMSO was taken as control for the organic extracts. Sterile distilled water was taken as control for aqueous extracts. Imipenem was used as standard antibiotic (32 µg).

In order to visualize the activity, 8 mL of triphenyltetrazolium chloride 0,1% with bacteriological agar 1% were add after 18 h of incubation and incubated again for 30°C. Antibacterial activities were evaluated by measuring inhibition zone diameters after 4 h.

**Resazurin MIC assay**

The method used is the proposed by Mann and Markham (1998), with some modifications. Serial three-fold dilutions starting at 20 mg/mL of each extract were prepared by vortexing in sloppy agar at room temperature. The resazurin assay medium, LB, was inoculated with the test organism to yield a final cell density ca. 1 log cycle lower than the cell density required to reduce resazurin (usually 2.5 x $10^5$ cell/mL). The inoculum density was confirmed by plate count.

A sterile 96-well microtitre tray was set up with each of the tested bacteria as follows: 100 µL inoculum + 100 µL extract dilution; 100 µL inoculum + 100 µL extract diluent (positive control); sterile resazurin assay medium plus 100 µL extract diluent (negative controls). The well contents were thoroughly mixed. Two trays were prepared for each organism and incubated at 37°C for 3 h. After incubation, 30 µL resazurin solution (6 µg/mL) was added to all except negative control, to which 30 µL distilled water was added. After a second incubation for 4 h at 37°C, the wells were assessed visually for colour change, with the highest dilution remaining blue, indicating the MIC.

**Results and Discussion**

**Agar-Well diffusion method**

Some authors define an active extract when it shows an inhibition halo > 13 mm (Vieira, 2010). But it is more interesting to compare the inhibition halo against the antibiotic halo used as standard.

So it is need to define what activity is, because if we consider the first thought (> 13 mm) to define it then we can consider that the extracts: $H_2O$ leaves, MeOH and $H_2O$ branch extracts active against *Shigella flexneri*, DCM and MeOH leaf extracts and MeOH branch extract against *Bacillus cereus* and DCM and MeOH leaf extracts and MeOH branch extract against *Bacillus liquefaciens*, and MeOH leaf extract against Methicillin-Resistant *Staphylococcus aureus.*

By other hand if only extracts that showed at least half of inhibition antibiotic halo are considered so the active one will be: DCM leaf extract against *Salmonella choleraesuis,* $H_2O$ leaves, MeOH and $H_2O$ branch extracts active against *Shigella flexneri*, DCM and MeOH leaf extracts and MeOH branch extract against *Bacillus cereus*, DCM leaf extract against Methicillin-Sensible *Staphylococcus aureus*, which now is the most active extract.

So, in the second analyses, the most active extract is the DCM leaf extract against Methicillin-Sensible *Staphylococcus aureus*, which sowed the same inhibition as that the antibitic used. And the extracts which showed activity against *Bacillus liquefaciens*, in the first analysis, now classified as medium active.

The MeOH and $H_2O$ leaf extract and $H_2O$ branch extracts showed a not defined halo, called intermediary halo, which can be infered as bacteriostatic (Table 1).

The results obtained from *M. guianensis* leaf extracts (Table 1) showed a mild tendency of the active extracts to be more active against Gram-positive bacteria. This can be explained through difference of bacterial wall. As Gram-positive organisms have a thick layer of peptidoglycan in the cell wall (murein), these extracts can have some murein inhibitor synthesis in their composition. As there is a huge mixture of substances in the extract composition, it is not possible yet to explain the action mechanism.

As the leaves and branches extracts of *Minquartia guianensis* were active against *Staphylococcus aureus* (Table 1), which is also related to cutaneous irritations it corroborates the folk uses of their barks in Ecuador (Marles et al., 1989).

**Table 1. Antibacterial inhibition halos found to *M. guianensis* extracts.**

| Bacteria | Inhibiton halo (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Imipenem | Leaves Extracts | | | Branches Extracts | | |
| | | DCM | MeOH | $H_2O$ | DCM | MeOH | $H_2O$ |
| **Gram-negative bacteria** *Salmonella choleraesuis* 6958 | 20 | 10 | 4 | - | 3 | 4 | 3 |
| *Shigella flexneri* M90T | 30 | 9 | - | 20 IH | - | 20 | 20 IH |
| *Escherichia coli* E2348/69 | 10 | 3 | - | - | - | - | - |
| **Gram-positive bacteria** *Bacillus cereus* 9634 | 30 | 23 | 16 | 7 | 8 | 16 | 7 |
| *Bacillus liquefaciens* clinical isolated | 50 | 21 | 20 | 6 | 11 | 21 | - |
| Methicillin-Sensible *Staphylococcus aureus* ATCC: 25923 | 10 | 10 | 5 | 1.5 | 3 | 5 | - |
| Methicillin-Resistant *Staphylococcus aureus* ATCC:33591 | 40 | - | 19 IH | - | - | - | - |

-: No active

IH: Intermediary halo

**Resazurin MIC assay**

The minimum inhibitory concentration (MIC) obtained to the leaves DCM extract was 10 mg/mL against Methicillin-Sensible *Staphylococcus aureus*, 5 mg/mL against *Bacillus liquefaciens*, and 2.5 mg/mL against *B. cereus* but this extract was inactive against Methicillin-Resistant *Staphylococcus aureus* (Table 2).

The MIC leaves MeOH extract was 10 mg/mL against Methicillin-Sensible *Staphylococcus aureus,* 20 mg/mL against Methicillin-Resistant *S. aureus*, and 5 mg/mL for both *Bacillus cereus* and *B. liquefaciens*.

The MIC branches MeOH extract was 2.5 mg/mL against Sensible *Staphylococcus aureus,* 20 mg/mL against Methicillin-Resistant *S. aureus*, and 5 mg/mL for both *Bacillus cereus* and *B. liquefaciens*.

**Table 2. MIC found to *M. guianensis* extracts.**

| Bacteria | MIC (mg/mL)* | | | | | |
|---|---|---|---|---|---|---|
| | Leaves Extracts | | | Branches Extracts | | |
| | DCM | MeOH | $H_2O$ | DCM | MeOH | $H_2O$ |
| **Gram-positive bacteria** *Bacillus cereus* 9634 | 2.5 | 5 | NA | NA | 5 | NA |
| *Bacillus liquefaciens* clinical isolated | 5 | 5 | NA | NA | 5 | NA |
| Methicillin-Sensible *Staphylococcus aureus* ATCC: 25923 | 10 | 10 | NA | NA | 2.5 | NA |
| Methicillin-Resistant *Staphylococcus aureus* ATCC:33591 | - | 20 | NA | NA | 20 | NA |

-: No active

NA: Not assayed

## Phytochemical evaluation

In order to determine which chemical classes are present in the leaves extracts, a standard phytochemical screening was made, following classical assays (Matos, 1997; Bessa et al., 2007).

There were found phenols, tannins, flavonoids, saponins and triterpenes in MeOH leaf extract and MeOH and $H_2O$ branch extract.

Phenols, tannins and saponins were found in the $H_2O$ leaf extract.

Both DCM extracts showed positive reaction to triterpenes and only DCM leaf extract showed coumarins. Alkaloids, anthocyanins, anthocyanidins, chalcones and aurons were not found in any extract analyzed (Table 3).

**Table 3. Phytochemical prospection of *M. guianensis* extracts.**

| | Leaves Extracts | | | Branches | | |
|---|---|---|---|---|---|---|
| **Phytochemical detected** | **DCM** | **MeOH** | **$H_2O$** | **CM** | **MeOH** | **$H_2O$** |
| Phenols and tanins | - | +++ | ++ | - | +++ | +++ |
| Alkaloids | - | - | - | - | | |
| Saponins | - | +++ | +++ | - | + | ++ |
| Coumarins | + | - | - | - | | |
| Anthocyanins and anthocyanidins | - | - | - | - | - | - |
| Chalcones and aurons | - | - | - | - | - | - |
| Flavonoids | + | +++ | - | - | | +++ |
| Triterpenes | +++ | + | - | | | + |

-: Negative; +: Low; ++: Medium; +++: Strong

As the most active extract was the DCM leaf extract against several strains and showed reaction when analyzed to triterpenes, it was chosen to perform a phytochemical fractionation. By now, four triterpenes were isolated: lupeol, taraxerol, lupenone and squalene (Cursino et al., 2009). As there are some reports about antibacterial activity of the triterpenes previously isolated, we believe that part of the DCM leaves extract activity is due to them. Further assays are needed to prove it. As the amount of these triterpenes is not enough to perform the MIC, a new fractionation of the leaves DCM extract is going on to get more mass to perform these assays.

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