

## Antibacterial activity of flavonoid 5.7.4'-trimethoxyflavone isolated from *Praxelis clematides* R.M. King & Robinson

[Actividad antibacteriana del flavonoide 5.7.4'-trimetoxiflavona aislada de *Praxelis clematides* R.M. King & Robinson]

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### Abstract

The flavonoids are a large class of polyphenolic compounds found in plants that are known to exhibit biological effects. In the study, the flavonoid 5.7.4'-trimethoxyflavone (TMF) extracted from *Praxelis clematidea* was evaluated for its antibacterial activity. Microdilution method was used for antibacterial assay of the flavonoid and eleven bacteria strains were used in the study for activities. The results were also compared with the standard drug, Chloramphenicol (100 µg/mL). The results obtained showed activity of the flavonoid against Gram positive and Gram negative bacteria.

**Keywords:** *Praxelis clematidea*, flavonoid, antibacterial activity

### Resumen

Los flavonoides son una clase importante de compuestos polifenólicos encontrados en las plantas que se sabe que presentan efectos biológicos. En el estudio, el flavonoide 5.7.4'-trimetoxiflavone (TMF) extraído de *Praxelis clematidea* fue evaluado por su actividad antibacteriana. Se utilizó el método de microdilución para el ensayo antibacteriano del flavonoide y once cepas de bacterias se usaron en el estudio de las actividades. Los resultados se compararon también con el fármaco estándar, Cloranfenicol (100 µg/mL). Los resultados obtenidos mostraron actividad del flavonoide contra bacterias Gram positivas y Gram negativas.

**Palabras Clave:** *Praxelis clematidea*, la actividad flavonoide, antibacteriano

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## INTRODUCTION

The flavonoids are ubiquitous in photosynthesising cells and therefore occur widely in the plant kingdom (Havsteen, 1983). They are found in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine, propolis and honey, and represent a common constituent of the human diet (Middleton and Chithan, 1993; Grange and Davey, 1990; Harborne and Baxter, 1999). The basic structural feature of flavonoid compounds is the 2-phenyl-benzo[ $\alpha$ ]pyrane or flavane nucleus, which consists of two benzene rings (A and B) linked through a heterocyclic pyrane ring (C) (Brown, 1980).

For centuries, preparations that contain flavonoids as the principal physiologically active constituents have been used by physicians and lay healers in attempts to treat human diseases. Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity (Havsteen, 1983; Harborne and Baxter, 1999; Kaur and Bains, 2012).

The antibacterial activity of flavonoids is being increasingly documented. Crude extracts from plants with a history of use in folk medicine have been screened in vitro for antibacterial activity by many research groups. Flavonoid rich plant extracts from species of *Capsella*, *Chromolaena*, *Piper* and *Gymnema* have been reported to possess antibacterial activity (Aladesanmi et al., 1986; El-Abyad et al., 1990; Fernandez et al., 2012; Wani et al., 2012).

Based on the antimicrobial potential of flavonoids, the present study aimed to investigate the antibacterial activity of the 5,7,4'-trimethoxyflavone isolated from *Praxelis clematidea* R.M. King & Robinson belongs to the *Eupatorieae* tribe of the family *Asteraceae*.

## MATERIALS AND METHODS

### Isolation of 5,7,4'-trimethoxyflavone

The aerial parts of *Praxelis clematidea* R.M. King & Robinson were collected in Lagoa do Paturi, a municipality of Santa Rita, in the state of Paraiba (Brazil), in May 2008. The identification of the botanical material was performed by Prof. Dr. Maria de Fatima Agra (Botany Sector, Laboratory of Pharmaceutical Technology/UFPB "Professor Delby Fernandes de Medeiros"). Exsiccates of the plant were deposited in the Prof. Lauro Pires Xavier (JPB)

Herbarium, Paraiba Federal University (MF Agra et al., 6894 (JPB)).

The process of isolation and identification of the flavonoid 5,7,4'-trimethoxyflavone (TMF) was performed according to Maia et al. (2011).

### Bacterial strains

For antibacterial activity assays, 11 strains of bacteria (*Staphylococcus aureus* - ATCC 13150, *Staphylococcus aureus* - ATCC 25923, *Staphylococcus epidermidis* 12228, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* - P03, *Pseudomonas aeruginosa* - ATCC 25853, *Escherichia coli* - ATCC 25922, *Escherichia coli* - 5, *Salmonella enterica* ATCC 6017, *Salmonella enterica* LM08 and *Shigella sonnei*), were selected. All the microorganism strains were obtained from the Laboratory of Mycology collection of the Federal University of Paraiba. Bacteria 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 dilution of approximately  $10^6$  colony-forming unit per mL (cfu.mL<sup>-1</sup>) adjusted according to the turbidity of 0.5 McFarland scale tube.

### Antibacterial assay

The microplate bioassay was used to determine the flavonoid minimum inhibitory concentration (MIC). For this purpose, 96-well plates were prepared by dispensing 100  $\mu$ L of double strength Nutrient Broth (NB) inoculated with the bacteria into each well prior to the assay. An aliquot (100  $\mu$ L) of the flavonoid solutions, at their respective concentrations, was transferred into six consecutive wells. The final volume in each well was 200  $\mu$ L. The highest substance concentration solution was added into the first well and the one having the smallest concentration into the antepenultimate well. The penultimate and the last well, containing 200  $\mu$ L of the NB inoculated with the microorganism suspension and Chloramphenicol (100  $\mu$ g/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 (Viljoen et al., 2003; Sahin et al., 2004).

The antibacterial activity was detected using the colorimetric method by adding 200  $\mu$ L of resazurin staining (0.1 g.100 mL<sup>-1</sup>) aqueous solution

in each well at the end of the incubation period. MIC was defined as the lowest flavonoid 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) (Burt and Reinders, 2003). All experiments were carried out at least twice with consistent results.

## RESULTS

The results for antibacterial activity of the TMF with MIC value are show in Table 1 and the activity was

measured in terms of presence of microorganism growth.

Analyzing this result can be observed that the TMF showed a significant inhibitory effect against species growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, with MIC value equal to 128 µg/mL for both bacterial species (Gram + and Gram -). However, the flavonoid has no effect on the species *Staphylococcus epidermidis*, *Bacillus subtilis*, *Salmonella enteric* and *Shigella sonnei*.

**Table 1**  
Antibacterial activity of the flavonoid TMF

Bacterial strain	TMF (MIC)	Negative control	Positive control (Chloramphenicol)
<i>S.aureus</i> ATCC 13150	128 µg/mL	-	+
<i>S. aureus</i> ATCC 25923	128 µg/mL	-	+
<i>S. epidermidis</i> 12228	-	-	+
<i>B. subtilis</i> ATCC 6633	-	-	+
<i>P. aeruginosa</i> P 03	128 µg/mL	-	+
<i>P. aeruginosa</i> ATCC 25853	128 µg/mL	-	+
<i>E.coli</i> ATCC 25922	128 µg/mL	-	+
<i>E.coli</i> 5	128 µg/mL	-	+
<i>S. enterica</i> ATCC 6017	-	-	+
<i>S. enterica</i> LM08	-	-	+
<i>Shigella sonnei</i>	-	-	+

## DISCUSSION

Flavonoids are phenolic substances widely distributed in all vascular plants. They are a group of about 4000 naturally compounds known, and have been shown to have contribute to human health through our daily diet. They are ubiquitous in plant foods and drinks such as fruits, vegetables, tea, wine, coffee and beer (Giula et al., 1999).

By the observing the results with TMF, it can see that this flavonoid showed a considerable antibacterial effect against the Gram positive and

Gram negative bacteria species studied. Its activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described for quinones. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996).

The antibacterial activity of flavonoids is being increasingly documented. Crude extracts from plants with a history of use in folk medicine have been screened in vitro for antibacterial activity by many research groups. Flavonoid rich plant extracts from

species of *Hypericum*, *Capsella* and *Chromolaena* have been reported to possess antibacterial activity (El-Abyad *et al.*, 1990; Dall'Agnol *et al.*, 2003). Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity (Quarenghi *et al.*, 2000; Süzgeç *et al.*, 2005; Pavithra *et al.*, 2009; Zhao *et al.*, 2011).

The findings of this research are consistent with other studies reported in the literature, which show the antibacterial effect of flavonoid against bacterial species (Gram + and Gram -) (Encarnacion *et al.*, 1994; Sato *et al.*, 1996; Fernandez *et al.*, 2012; Wani *et al.*, 2012) and reaffirms the antimicrobial potency of the TMF, since this flavonoid also showed an antifungal effect against *Candida* species (Filho *et al.*, 2012). This reinforces the importance of continuing to study antibacterial TMF compared to other species of bacteria pathogenic for humans.

## CONCLUSION

The results obtained in this study suggest that the flavonoid presents a considerable antibacterial effect against species of gram positive and gram negative bacteria. Thus, further studies are necessary to explore this effect and discover the mechanism of action of the TMF.

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