



## FLORAL EXTRACT OF *Tibouchina granulosa* (DESR.) COGN. PHYTOCHEMISTRY PROSPECTION, AND ANTIBACTERIAL ACTIVITY

EXTRATO FLORAL DE *Tibouchina granulosa* (DESR.) COGN. PROSPECÇÃO FITOQUÍMICA, E ATIVIDADE ANTIBACTERIANA

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*Escherichia coli*, *Salmonella spp.*, Gênero *Tibouchina*, *Flavonoides*, *Fenólicos*.

### Keywords:

*Escherichia coli*, *Salmonella spp.*, *Tibouchina Genus*, *Flavonoids*, *Phenolics*.

### Resumo

*Tibouchina granulosa* é uma espécie que apresenta belas floradas anualmente em várias regiões do Brasil. Esta espécie ainda é pouco explorada quanto aos fitocompostos em todos os órgãos desse vegetal, em especial o órgão floral. Flores de *T. granulosa* foram coletadas no município de Rio Verde, Goiás, Brasil, em 2021. O extrato floral hidroetanólico foi preparado por maceração e as análises fitoquímicas qualitativas (reações colorimétricas e formação de sais) e antibacterianas realizadas e os resultados expressos em milímetros de inibição em diferentes concentrações em mg mL<sup>-1</sup>. Foram observadas diversas classes fitoquímicas com resultados positivos, em especial para

alcaloides, fenólicos, oxalatos, saponinas, carboidratos e taninos. Quanto ao ensaio bacteriano, foi observada potencial atividade antibacteriana para todas as cepas bacterianas testadas, exceto para *Salmonella* serovar *Thyphymurium* e serovar *Enteritidis*. Expressivas inibições foram observadas para *Enterococcus faecalis* > *Pseudomonas aeruginosa* > *Staphylococcus aureus* and *Escherichia coli* nas maiores concentrações entre 100-50 mg mL<sup>-1</sup>. O extrato floral de *Tibouchina granulosa* apresentou potencial fitoterápico com a presença de vários grupos fitoquímicos e expressiva atividade antibacteriana.

### Abstract

*Tibouchina granulosa* is a species that blooms annually in several regions of Brazil. This species is still little explored in terms of phytochemicals in all organs of this plant, especially the floral organ. Flowers of *T. granulosa* were collected in the municipality of Rio Verde, Goiás, Brazil, in 2021. The hydroethanolic floral extract was prepared by maceration and qualitative phytochemical (colorimetric reactions and salt formation) and antibacterial analyzes performed and the results expressed in millimeters of inhibition at different concentrations in mg mL<sup>-1</sup>. Several phytochemical classes were observed with positive results, especially for alkaloids, phenolics, oxylates, saponins, carbohydrates and tannins. As for the bacterial assay, potential antibacterial activity was observed for all bacterial strains tested, except for *Salmonella* serovar *Thyphymurium* and serovar *Enteritidis*. Expressive inhibitions were observed for *Enterococcus faecalis* > *Pseudomonas aeruginosa* > *Staphylococcus aureus* and *Escherichia coli* at the highest concentrations between 100-50 mg mL<sup>-1</sup>. The floral extract of *Tibouchina granulosa* showed phytotherapeutic potential with the presence of several phytochemical groups and expressive antibacterial activity.

## INTRODUCTION

The Melastomataceae family is the considered fifth largest group among Angiosperms in Brazil, comprising 5.000 species included in 170 genera, representing one of the most diverse plant families on Earth. Members of this family are common elements of Tropical Savannas, Cloud Forests, and Rainforests, can be where recognised easily by the characteristic leaf acrodromous venation pattern in a numerous anatomical and histological studies (BFG, 2015; REZENDE et al., 2019; REGINATO et al., 2020).

According to Clausing and Renner (2001), Guimarães (2014), and Rezende et al. (2019) *Tibouchina* Aubl., the most representative genus within Melastomataceae family, has approximately 460 species. The greatest diversity of species of *Tibouchina* was described in the Neotropics (ca. 3.000 species), with 929 species native only in Brazil, where 105 are endemic, occurring in the Atlantic Rainforest biome and the Cerrado domain (FLORA DO BRASIL, 2018; REZENDE et al., 2019).

*Tibouchina granulosa* is a semideciduous perennial tree widely used in urban landscapes in Brazilian cities (parks, gardens, streets and avenues). The especie is popularly known as “quaresmeira” that has tolerance to direct light and hardiness, in addition, the species has large, exuberant blooms and purple-hued flowers (CÉZAR et al., 2009; ZAMPIERI et al., 2013; FERREIRA et al., 2014; FREITAS et al., 2017).

Some species of the *Tibouchina* genus have phytotherapeutic use by the local population, being a genus rich in phytochemicals of great pharmaceutical, agronomic, biological and biotechnology interest. Although little is known about this remarkable genus, Rezende et al. (2019) performs a compilation with in studies report several biological activities such as anti-inflammatory, and antioxidant (JIMÉNEZ et al., 2015), antinociceptive (DIAS et al., 2016), antibacterial (DOS SANTOS et al., 2012; TRACANNA et al., 2015),

antifungal (NIÑO et al., 2003; KUSTER et al., 2009), antiparasitic (SINGHA et al., 1992; CUNHA et al., 2009; TRACANNA et al., 2015), anticancer and antitumoral (JONES et al., 1981) principal activities observed in leaf extracts in several species.

The lack of studies evaluating the various organs of a plant has a negative impact on prior knowledge of the possible biological potential for pharmaceutical use in the development and production of new phytopharmaceuticals such as antibiotics. The indiscriminate use of these mostly synthetic antibacterial agents is currently presenting a serious problem regarding the low sensitivity to numerous bacterial strains and isolates with high pathological potential, making these microorganisms resistant to the usual recommended doses, requiring higher doses to combat inflammatory and systemic processes that these microorganisms produce in an infection, especially in patients in the intensive care unit (ICU) bed (SILVAL et al., 2018; RODRIGUES et al., 2018; GÓMEZ-RÍOS; RAMÍREZ-MALULE, 2019).

Thus, it is necessary to constantly research for new natural sources that produce biomolecules capable of presenting certain growth inhibition activity for the groups of Gram-positive and Gram-negative bacteria, based on classical assays for the floral organ of *T. granulosa*, the beginning of this process of obtaining scientific information capable of providing the advent and even the synthesis of new molecules for therapeutic use in human and animal medicine.

The study aimed to evaluate the qualitative phytochemical composition and the antibacterial activity of the floral hydroethanol extract of *Tibouchina granulosa*.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals and instruments

All P.A-ACS grade reagents and equipment described in the subtopics.

## 2.2. Sample collection

*T. granulosa* flowers, were collected in May, 2021, in Rio Verde city, GO, Brazil, (17°46'59.0"S and 50°58'05.8"W). The plant material was identified by Ms<sup>o</sup>. Antonio Carlos Pereira de Menezes Filho, and deposited in the Herbarium that belongs to the Goiano Federal Institute, Campus Rio Verde, GO, Brazil. (exsiccate n° 12037).

## 2.3. Extract production

About 150 g of the floral sample was meticulously weighed and kept in three 500 ml beakers each containing 200 mL of 70% hydroethanolic solution for 48 h. The samples were filtered in quantitative filter paper and the filtrate were concentrated with a rotary evaporator at 68 °C. The obtained floral extract were stored in a refrigerator at -12°C until the performance of analysis described by Oladeji et al. (2019) modified.

## 2.4. Preliminary phytochemical screening

The concentrated floral extract were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures described by Prakash; Vedanayaki (2019). Alkaloids, flavonoids, phenolics, oxylates, terpenoids, saponins, tannins, carbohydrates, steroids, oil and resins, anthraquinone, coumarins, glycoside, proteins and amino acids, phlobatannins, cardiac-glycosides, emodine and fatty acids. Cross test: (-) negative, (+) weak positive, (++) moderate positive, and (+++) strong positive by according Marinho et al. (2021). The phytochemical test was performed in triplicate.

## 2.5. Antibacterial assay

The bacterial strains were obtained from the Technological Chemistry Laboratory of the Goiano Federal Institute, Campus Rio verde, GO, Brazil. The microbiological test followed as described by Vieira et

al. (2021) adapted, using the paper disc diffusion technique, and the results expressed in (mm). Were used of strains from *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (LB29212), *Salmonella serovar* Enteritidis (ATCC 13076) and *Salmonella serovar* Typhimurium (ATCC 14028).

The activation of microorganisms was carried out in a sterile solution of NaCl conc. 0.85% until reaching the degree of 0.5 on the scale MacFarland conc. ( $1 \times 10^6$  CFU mL<sup>-1</sup>). Petri dishes (10 cm<sup>2</sup>) were prepared with Mueller Hinton Agar (MHA) after sterilization. The Petri plates containing specific medium were inoculated using a sterile swab soaked with a microbial suspension, and spread across the plate. Filter paper discs with a diameter of 7 mm were impregnated with 100 µL of the extract in different concentrations (100, 50, 25, 5 and 2,5 mg mL<sup>-1</sup>), as a negative control, the saline solution used with 10% DMSO was used (*v/v*), and as positive control discs with antimicrobial agents, for bacteria Azithromycin (15 µg), Cephalexin (30 µg), Tigecycline (15 µg) and Amikacin (30 µg). The plates were incubated at 36 °C with an interval between 24-36 h, after that period, the halo of antibiosis when present was measured with a digital caliper. The test were carried out in triplicate.

## 2.6. Statistical analysis

All the chemical analyses were done in triplicate. The treatment was carried out in quadruplicate and the experimental design was thoroughly randomized. Data were submitted to the analysis of variance (ANAVA) and the means of the treatments were evaluated by the Scott-Knott test at 5% significance level by the ASSISTAT software (Free Edition, Brazil).

## 3. RESULTS AND DISCUSSION

The metabolites analyzed include alkaloids, flavonoids, phenolics, oxylates, saponins, tannins

(hydrolyzable or gallic), carbohydrates, anthraquinone, cardiac-glycosidic and emodine. Several studies have been carried out on the phytochemical compounds of vegetables to investigate their medicinal therapeutics. Alkaloids in medicinal plants are reported as an important antimicrobial, antiparasitic and analgesic drug (SILVA et al., 2019; BATIHA et al., 2020). Phenolic compounds constitute a major bioactive compound in plants, and they are known to have antimicrobial, antiparasitic,  $\alpha$ -glucosidase inhibition, anti-allergic, antifungal, anti-inflammatory, antioxidants, anticarcinogenic and antitumoral properties (GOMES et al., 2017; NGUYEN et al., 2020; BATIHA et al., 2020).

Flavonoids were reported to contain antimicrobial compounds, and allelopathic and

antiparasitic effects (BARCELOS et al., 2017; SAI et al., 2019; COSTA et al., 2019; BATIHA et al., 2020; SIQUEIRA et al., 2020). Tannins were studied and reported to draw tissues together, thus restricting the flow of blood, which helps to heal wounds, and anti-inflammatory activity (KUMARI et al., 2017; ZEVALLOS, 2018). The saponins foam and hemolytic is found in abundance in this could be responsible for its application in detergent industries as foaming agents (KUMARI et al., 2017; OLADEJI et al., 2019; SIQUEIRA et al., 2020), and regulate blood cholesterol and treatment of cardiovascular diseases, and antimicrobial activity (NGUYEN et al., 2020; BATALINI et al., 2020). Results of preliminary phytochemical screening are summarized in (Table 1).

**Table 1.** Qualitative phytochemical screening of floral extract of *Tibouchina granulosa*.

Phytochemicals	Hydroethanolic floral extract
Alkaloids	+++
Flavonoids	++
Phenolics	+++
Oxylates	+++
Terpenoids	-
Saponins	+++
Tannins	Blue
Carbohydrates	+++
Steroids	-
Oil and Resins	-
Anthraquinone	++
Coumarins	-
Glycoside	-
Proteins and Amino Acids	-
Phlobatannins	-
Cardiac-Glycosides	++
Emodine	++
Fatty Acids	-

Cross test: (-) negative, (+) weak positive, (++) moderate positive, and (+++) strong positive. Source: Authors, 2021.

Phytochemical screening showed in of floral extract of *T. granulosa*, the absent phytochemical groups for terpenoids, oils, resins, coumarins, glycoside, proteins and amino acids phlobatannins and fatty acids.

Several phytochemical groups are shared between species of the same genus and family, this can be seen in several studies that assess not only one, but

several organs of the plant. In the literature, it is possible to verify some works on phytochemical analysis in *Tibouchina* genus, although scarce, the plant organ needs attention, as, as observed in this study, it presents an exuberant amount of phytochemical groups.

For *T. granulosa*, phytochemicals are described for the main groups, such as anthocyanins, flavonols and phenolic acids (3-*O*-glucuronide, isorhamnetin 3-*O*-diglucoside, isorhamnetin 3-*O*-rutinoside, quercetin 3-(*O*-galloyl)-hexoside/ $\beta$ -type procyanidin monomer, dimer, trimer and pentamer) for leaf and flower extracts (REZENDE et al., 2019). Tracanna et al. (2019) isolated a phenolic derivative 2,8-dihydroxy-7H-furo[2,3-*f*]chromen-7-one in the aerial parts extract of *T. paratropica*. A study by Thilakarathna and Hettihewa (2021) also observed the presence of flavonoids, isoflavonoids and anthocyanins predominant floral pigments in other species of *Tibouchina* (*T. urvilleana*).

Sobrinho et al. (2017) verified that the leaf extract of *T. granulosa* has an anti-inflammatory action due to the presence of proanthocyanidins and flavonoids. Similar results were described by Guilhon et al. (2017) in experimental model of endometriosis for the ethanol leaves extract and ethyl acetate fraction of *T. granulosa*.

The hydroethanolic floral extract of *T. granulosa* showed no activity in front of Gram-negative bacteria, *S. serovar* Thyphimurium and *serovar* Enteritidis. As for *S. aureus*, *P. aeruginosa*, *E. faecalis* and *E. coli*, a potential growth inhibition action was observed (Table 2), pointing to the importance of research to provide the search for new antibiotics. Since many microorganisms exhibit resistance to some medications marketed.

The *T. granulosa* floral extract proved to be effective in inhibiting *E. faecalis* with bactericidal activity between 19-6 mm, where at the highest concentrations there was no significant difference by the Scott-Knott test with 5% significance. Important inhibition activities were also observed for *P. aeruginosa* with an inhibition rate ranging from 17-5 mm; *S. aureus* between 15-7 mm and for *E. coli* between 10-6 mm where they presented a significant difference when compared to the reference antibacterials.

**Table 2.** Antimicrobial activity of the hydroethanolic floral extract of *Tibouchina granulosa*.

Microorganisms	Inhibition concentrations in mm zone*							
	100	50	25	2.5	1.25	Disc 15 $\mu$ g <sup>a</sup>	Disc 30 $\mu$ g <sup>b</sup>	Disc 15 $\mu$ g <sup>c</sup>
<i>P. aeruginosa</i>	17b	14b	9c	5d	0e	21a	23**a	nd
<i>E. coli</i>	10b	6b	0c	0c	0c	19a	21*a	nd
<i>S. aureus</i>	15b	14b	7c	0d	0d	23a	26*a	nd
<i>E. faecalis</i>	19a	17a	12b	6c	0d	22a	nd	23a
<i>S. Enteritidis</i>	-	-	-	-	-	27	25*	nd
<i>S. Typhimurium</i>	-	-	-	-	-	26	24*	nd

\*Concentration of floral extract of *T. granulosa* determined in mg mL<sup>-1</sup>.

(-) There was no halo formation. (Nd) Not determined.

Azithromycin disco<sup>a</sup>. Cephalexin disco<sup>b\*</sup>. Amikacin disco<sup>b\*\*</sup>. Tigecycline disco<sup>c</sup>.

Equal letters on the same line do not differ from each other by the Scott-Knott test with 5% significance.

Source: Authors, 2021.

Table 2 shows satisfactory results of antibacterial activity promoted by the crude floral extract of *T. granulosa*, suggesting that this inhibition of bacterial growth is closely linked with the main phytochemical groups present in the floral extract, such as alkaloids, phenolics and saponins. Future work should evaluate concentrations higher than those in this study, in order

to know the maximum antibacterial activity it has compared to the reference antibacterials. Thus, it will be possible to determine maximum doses of extract on each species of bacteria, and, in addition, to determine by high-efficiency liquid chromatography which phytochemicals are present and their quantitative content. Thus, it will be possible to say which or which

molecules have the bactericidal activity for the strains tested.

Comparative studies with species of *Tibouchina* were verified in the literature, where they showed moderate efficiency in inhibiting bacteria and fungi. Santos et al. (2012) observed high efficiency in compounds isolated from aerial organ extracts of *Tibouchina candolleana*, isolated compounds such as ursolic and oleanolic acids showed effective bacteriostatic action against *Bacteroides fragilis*, while the ursolic and oleanolic acid isolates proved to be effective for most of the bacteria tested *Actinomyces naeslundii*, *Porphyromonas gingivalis*, *Prevotella nigrescens*. The flavonoids genistein and luteolin isolated from the extract did not demonstrate efficacy as antibacterial agents. Tracanna et al. (2014) evaluated the extract of aerial parts of *T. paratropica* where they found good inhibition efficiency for *P. aeruginosa* and *E. faecalis* and for *Candida albicans*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans* fungi. Bomfim et al. (2020) evaluated extracts from the aerial part of *Tibouchina lhotzkyana* and *Tibouchina francavillana* where they did not observe inhibition activity for *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Micrococcus luteus* and *Bacillus subtilis*, and for the fungus *Aspergillus niger*, the observed lack of inhibition, can be explained by the usual low concentrations (3.0 to 0.045 mg mL<sup>-1</sup>) lower than in this study.

## CONCLUSIONS

This study observed a series of groups of phytomolecules in the qualitative assay, as well as important bacterial inhibition results for important bacteria of human interest from the hydroethanolic floral extract of *Tibouchina granulosa*.

Future studies should evaluate this partitioned extract as well as an accurate analysis of liquid chromatography to better understand the phytoconstituents, their quantitative contents, possible

approaches to isolation of molecules and higher dosages for the antibacterial assay.

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