Antimicrobial activity of active principles from *Distephanus garnierianus*

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ABSTRACT

Distephanus garnierianus is an endemic species of Madagascar belonging to the Asteraceae family. It is traditionally used in ethnopharmacology for its antidiarrheal properties. Microbiological tests were conducted on crude extracts, liquid-liquid partition fractions (hexane, ethyl acetate, dichloromethane DCM, intermediate, and aqueous), and four pure molecules: two flavone-type compounds named HAI13-23 and HAI13-25, and two terpenoids named HAI32 (oleanolic acid) and HAI4399 (alpha-amyrin). Antimicrobial activity against eight bacterial strains (*Escherichia coli* ATCC 25922, *Klebsiella oxytoca* ATCC 8724, *Salmonella enteritidis*, *Enterobacter cloacae* ATCC 70323, *Staphylococcus aureus* ATCC 11632, *Bacillus cereus* ATCC 13061, *Streptococcus pneumoniae* ATCC 6301, *Listeria monocytogenes* and *Candida albicans,* yeast strain, was evaluated using solid medium culture (MUELLER-HINTON) incubated at 37 °C for 24 h with a disc load of 2000 µg of extract. Among the tested extracts, the DCM fraction yielded the most notable results, demonstrating strong activity against Gram-positive bacteria, including *Staphylococcus aureus* ATCC 11632, *Streptococcus pneumoniae* ATCC 6301, *Bacillus cereus* ATCC 13061, and *Listeria monocytogenes*, with inhibition zone diameters of 20, 14, 16, and 13 mm, respectively. Gram negative bacteria showed lower sensitivity, while the yeast strain *Candida albicans* exhibited resistance to all plant-derived products. Regarding the pure molecules (HAI13-23, HAI13-25, HAI32, and HAI4399), high sensitivity of *Staphylococcus aureus* ATCC 11632 was observed, with inhibition zones of 22, 24, 20, and 18 mm, respectively. These results support the ethnobotanical use of *Distephanus garnierianus* in the traditional treatment of diarrhea.

Keywords: *Distephanus garnierianus*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, antibacterial assay

I. INTRODUCTION

In Madagascar, medicinal plants play an essential role in daily life. The vast majority of the population, in both urban and rural areas, relies on traditional medicine to treat common ailments (Ralainirina, 2019).

According to ethnobotanical surveys conducted among traditional healers, *Distephanus garnierianus* is frequently used to treat diarrhea. Plant preparations are empirical, mainly consisting of decoctions administered alone or in combination with other plants, although specific proportions are not strictly defined.

However, in Madagascar, diarrhea accounts for 22% of deaths in children under five years of age annually, according to findings from the 2012–2013 National Millennium Development Goals (MDG) Monitoring Survey.

Seven pathogenic diarrheic bacterial strains were investigated to determine the antimicrobial activity of *Distephanus garnierianus*, which could contribute to reducing infant mortality associated with bacterial diarrhea.

II. MATERIALS AND METHODS

II.1. Plant Material

The plant material studied is *Distephanus garnierianus* (formerly *Vernonia garnieriana*), locally known by the vernacular names "Kijejalahy", "Ninginingana", and "Tsiparapandy". It is an endemic shrub of Madagascar belonging to the Asteraceae family and the genus *Distephanus*, which comprises 35 species : 2 present in Southeastern Africa, 1 in Mauritius, and 32 endemic to Madagascar (SCHATZ, 2001).

Raw material collection was performed during the vegetative period on the Central Highlands of Madagascar, in the Alaotra-Mangoro region, Moramanga district, Ambohibary commune, within the Ambatomainty forest, at an altitude of 999 m (latitude: 18°52’29.8’’ S, longitude: 048°17’37.7’’ E).

In accordance with recommendations provided by traditional healers during the ethnobotanical survey, leaves were used for the traditional treatment of diarrhea and selected for these analytical studies.

II.2. Microbiological studies

II.2.1. Tested extracts and compound

The antibacterial activity of the plant was evaluated by determining the susceptibility of microorganisms to:

- The crude extract and five fractions obtained by liquid-liquid partition of the crude extract: hexane, dichloromethane (DCM), ethyl acetate, intermediate, and aqueous fractions.
- Four pure compounds isolated and purified from the DCM extract: two flavones belonging to the flavonoid family (HAI13-23 and HAI13-25), and two triterpenoids (HAI32 / HAI E33, oleanolic acid, and HAI4399, alpha-amyrin).

II.2.2. Bacterial strains

The investigated microorganisms included ATCC reference strains as well as local clinical isolates, which were purified, identified, and preserved at the laboratory of the National Center for Environmental Research (*Centre National de Recherche sur l'Environnement*, CNRE).

Among these, four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella oxytoca* ATCC 8724, *Salmonella enteritidis*, *Enterobacter cloacae* ATCC 70323), four Gram-positive bacteria (*Staphylococcus aureus* ATCC 11632, *Bacillus cereus* ATCC 13061, *Streptococcus pneumoniae* ATCC 6301, *Listeria monocytogenes*), and *Candida albican* yeast strain were tested. Authentication of local non-ATCC strains was performed using a MALDI-TOF mass spectrometer (Matrix-Assisted Laser Desorption/Ionization - Time of Flight), a method based on the identification of specific ribosomal protein profiles of microorganisms.

II.2.3. Disk diffusion antibacterial assay

The assay was conducted over three consecutive days as follows:

- **Day 1:** Each pure strain was inoculated into nutrient broth and incubated at 37 °C for 24 h to reactivate bacterial growth.
- **Day 2:** The reactivated culture of each strain was diluted to obtain a bacterial concentration of 10^3 cells/ml (inoculum). One milliliter of this inoculum was uniformly spread using the flooding technique onto Petri dishes containing Mueller-Hinton agar (4 mm thickness). The plates were kept at room temperature for 1 min to allow bacterial adhesion to the agar surface, then dried in an incubator at 37 °C for 15 min. Sterile filter paper disks (previously autoclaved) were impregnated with 20 µL of extract (corresponding to a load of 2000 µg of extract per disk) and placed onto the agar surface using sterile fine forceps. The plates were incubated at 37 °C for 24 h. Each assay was performed in duplicate.
- **Day 3:** Following incubation, the diameters of the inhibition zones formed around each disk were measured, and mean values were calculated.

The criteria used for interpreting agar diffusion test results are summarized in Table 1. These criteria were established by the Antibiogram Committee of the French Society for Microbiology (CA-SFM, 1998), based on the recommendations of the WHO Expert Committee on Biological Standardization (1977).

Table 1: Standard scale used for interpreting disk diffusion assay results.

Inhibition Zone Diameter x	Susceptibility level	Symbol
x < 7 mm	Resistant / Insensitive	-
7 mm ≤ **x** < 8 mm	Moderately sensitive	+
8 mm ≤ **x** < 9 mm	Sensitive	+ +
x ≥ 9 mm	Highly sensitive	+ + +

III. RESULTS

III.1. *Microbiological analyses*

As a reminder, the antimicrobial activities of the plant were evaluated by measuring the degree of susceptibility of eight bacterial and yeast strains (*E. coli* ATCC 25922, *S. aureus* ATCC 11632, *C. albicans*, *S. enteritidis*, *K. oxytoca* ATTC 8724, *B. cereus* ATCC 13061, *S. pneumoniae* ATTC 6301, and *L. monocytogenes*) toward six extracts (crude, hexane, DCM, ethyl acetate, aqueous, intermediate) and four pure compounds (HAI13-23, HAI13-25, HAI E33, and HAI4399). The obtained results are summarized in Table 2.

Table 2: Antimicrobial activity (inhibition zone diameters in mm and susceptibility degree) of extracts and isolated compounds from *Distephanus garnierianus*.

EXTRAIT / SOUCHES	BRUT	HEXANE	DCM	ACEOT	ACQUEUX	INTERMEDIAIRE	HAI 13-23	HAI 13-25	HAI E33	HAI 4399
Echerichia coli ATCC 25922	6 -	6 -	7 +	7 +	6 -	6 -	8 ++	8 ++	6 -	7 +
Staphylococcus aureus ATCC 11632	16 +++	6 -	20 +++	6 -	6 -	6 -	22 +++	24 +++	20 +++	18 +++
Candida albicans	6 -	6 -	6 -	6 -	6 -	6 -	6 -	6	6 -	6 -
Salmonella enteridis	7 +	6 -	7 +	7 +	6 -	6 -	7 +	7 +	8 ++	8 ++
Klebsiella oxytoca ATCC 8724	11 +++	6 -	14 +++	6 -	6 -	6 -	14 +++	15 +++	8 ++	6 -
Enterobacter cloacae ATCC 70323	7 +	6 -	7 +	6 -	6 -	6 -	6 -	6 -	6 -	7 +
Bacillus cereus ATCC 13061	14 +++	6 -	16 +++	6 -	6 -	6 -	16 +++	19 +++	17 +++	15 +++
Streptococcus pneumoniae ATCC 6301	16 +++	6 -	20 +++	6 -	6 -	6 -	20 +++	22 +++	13 +++	10 +++
Listeria monocytogenes	13 +++	6 -	19 +++	6 -	6 -	6 -	16 +++	20 +++	10 +++	8 ++

(Note: Compounds HAI13-23 and HAI13-25 are flavone-type flavonoids, while HAI E33 and HAI4399 are pentacyclic triterpenes identified as oleanolic acid and alpha-amyrin, respectively).

- Gram-negative bacteria *Escherichia coli* ATCC 25922 were sensitive to pure molecules HAI13-23 and HAI13-25 with Inhibition Zone Diameters (IZD) of 8 mm, and moderately sensitive to DCM and ethyl acetate extracts (IZD = 7 mm). They showed no sensitivity to crude, hexane, intermediate, or aqueous extracts.
- *Staphylococcus aureus* ATCC 11632 was highly sensitive to the crude extract, DCM extract, and pure compounds HAI13-23, HAI13-25, HAI E33, and HAI4399, with IZDs of 16, 20, 22, 24, 20, and 18 mm, respectively. Highest sensitivity was recorded for compound HAI13-25, whose chemical structure features hydroxylated groups.
- *Salmonella enteritidis* was sensitive to triterpenoid compounds HAI E33 and HAI4399 (IZD = 8 mm) and moderately sensitive to crude, DCM, and ethyl acetate extracts, as well as HAI13-23 and HAI13-25 (IZD = 7 mm).

- *Klebsiella oxytoca* (8724) showed high sensitivity to crude extract, DCM extract, and pure compounds HAI13-23 and HAI13-25 with IZDs of 11, 14, 14, and 15 mm, respectively, and was sensitive to HAI E33 (IZD = 8 mm).
- *Enterobacter cloacae* ATCC 70323 was moderately sensitive to crude extract, DCM extract, and HAI4399 (IZD = 7 mm), remaining resistant/insensitive to all other tested fractions.
- *Bacillus cereus* ATCC 13061 was highly sensitive to crude extract, DCM extract, and pure compounds HAI13-23, HAI13-25, HAI E33, and HAI4399, with IZDs of 14, 16, 19, 17, and 15 mm, respectively.
- *Streptococcus pneumoniae* (6301) displayed high sensitivity to crude extract, DCM extract, and pure compounds HAI13-23, HAI13-25, HAI E33, and HAI4399, with IZDs of 16, 20, 20, 22, 13, and 10 mm, respectively, while remaining insensitive to other tested products.
- *Listeria monocytogenes* exhibited high sensitivity to crude extract, DCM extract, and pure compounds HAI13-23, HAI13-25, and HAI E33 (IZDs of 13, 19, 16, 20, and 10 mm, respectively), was sensitive to HAI4399 (IZD = 8 mm), and showed no sensitivity to other fractions.
- The yeast *Candida albicans* was completely insensitive (resistant) to all extracts and isolated compounds from *Distephanus garnierianus*.

III.2. *Mechanism of action*

- Flavonoids : The presence of flavonoids in the plant extract significantly contributes to the observed antibacterial activity (Kumar & *al*., 2013). Flavonoids are known to inhibit nucleic acid synthesis, disrupt cytoplasmic membrane function, impair energy metabolism, prevent adhesion and biofilm formation, target outer membrane porins, induce membrane permeability alterations, and attenuate bacterial pathogenicity (Cushnie & Lamb, 2011). Furthermore, flavones have been reported to inhibit essential enzymes such as DNA gyrase / topoisomerase IV (blocking DNA replication), as well as beta-glycosidases and other hydrolases critical to bacterial energy metabolism (Santos-Buelga & *al*., 2019).
- Pentacyclic triterpenes : Triterpenes disrupt cytoplasmic membranes by integrating into the lipid bilayer, leading to increased membrane permeability and intracellular ion leakage. They also inhibit peptidoglycan synthesis via interaction with cell wall enzymes, inhibit efflux pumps (thereby potentiating antibiotic efficacy), and disrupt intracellular enzymatic activities (Dzubak & *al*., 2006; Fontanay & *al*., 2008; Cushnie & Lamb, 2011; Kurek & *al*., 2012).

IV. DISCUSSION

Regarding antibacterial activity, among the six tested extracts (crude, hexane, ethyl acetate, DCM, intermediate, and aqueous), only the dichloromethane (DCM) extract demonstrated significant inhibitory activity against the tested microbial strains, showing pronounced efficacy against Gram-positive bacteria. This finding aligns with results reported for *Distephanus polygalifolius* by Rafalimanana (2020). Conversely, for *Distephanus glutinosa*, Rajosefa (2014) reported that the leaf ethyl acetate extract displayed notable inhibitory activity against *Escherichia coli* (14 mm), whereas *Staphylococcus aureus* was highly sensitive to the leaf essential oil (17 mm). These comparisons highlight the intraspecific and interspecific variability of antimicrobial profiles within the genus *Distephanus*.

Compared to *Dissotis rotundifolia* Triana (Melastomataceae), another medicinal plant known for its antidiarrheal properties which yielded inhibition zone diameters against *Staphylococcus aureus* of 16 mm for the ethanolic extract and 10 mm for the ethyl acetate extract (Abere & *al*., 2010). *Distephanus garnierianus* demonstrated superior antibacterial potency.

These experimental data support the traditional ethnobotanical use of *Distephanus garnierianus* as an antidiarrheal remedy. Out of seven pathogenic diarrheic bacterial strains tested, five (*E. coli*, *S. aureus*, *K. oxytoca*, *E. cloacae*, and *L. monocytogenes*) exhibited clear susceptibility to its extracts and isolated

compounds. This biological potential could be leveraged to address antimicrobial resistance (AMR), which the World Health Organization has designated as one of the top ten global public health threats (WHO, 2021).

V. CONCLUSION

Distephanus garnierianus is an endemic plant species of Madagascar belonging to the Asteraceae family, traditionally used in ethnopharmacology for treating diarrhea.

Microbiological assays conducted on crude and DCM extracts, as well as on purified compounds, demonstrated potent antibacterial activity against Gram-positive bacteria (*Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 11632, *Streptococcus pneumoniae* ATCC 6301, and *Listeria monocytogenes*). Gram-negative bacteria (*Enterobacter cloacae* ATCC 70323, *Salmonella enteritidis*, and *Escherichia coli* ATCC 25922) showed lower susceptibility, whereas the yeast strain *Candida albicans* was resistant to all plant-derived products.

These findings validate the traditional therapeutic use of *Distephanus garnierianus* in managing infectious diarrhea. It represents a promising candidate for the development of standardized phytomedicines targeting bacterial diarrhea, offering a safe and effective therapeutic alternative to reduce childhood mortality associated with gastrointestinal infections. As a prospective follow-up, further investigations will focus on evaluating the antiviral activity of compounds isolated from *Distephanus garnierianus* to address viral causes of pediatric diarrhea.

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