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**Research Article**

# COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITIES EXTRACTS OF FRUITS, ROOTS AND BARK OF *VITEX DONIANA* (LAMIACEAE) AND *TERMINALIA MANTALY* H. PERRIER (COMBRETACEAE)

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**ABSTRACT**

The objective of this study is to identify the different parts of *Vitex doniana* (Lamiaceae) and *Terminalia mantaly* H. Perrier (Combretaceae) exhibited superior activity against three bacterial strains and one yeast. To do this*, seven aqueous and seven* hydroethanolic extracts (30/70) were extracted, followed by the determination of specific activity in solid media and antibacterial parameters using the liquid dilution method. The results showed that extraction yields were higher with the fruit than with the roots and bark. The determined specific activity (3,337 and 6,596 AU/mg) identified the root of *T. mantaly,* the pulp and seed, and the fruit pulp of *V. doniana* as the parts concentrating the active principles exhibiting inhibitory activity against the studied strains. The lowest MIC values were obtained with the ethanolic extracts of *T. mantaly* and were 0.15 mg/ mL on *E. coli* ATCC 25922, 0.52 mg/ mL on *S. aureus* ATCC 25923 and 0.2 mg/ mL on *S. typhi IPP* 5534 and Vitex seed with MICs of 0.62 mg/ mL, 0.67 ± 0.54 mg/ mL, and 0.52 ± 0.18 mg/ mL respectively on the same strains. The lowest MIC values for *C. albicans* strain ATCC 2091 were obtained with hydroalcoholic extracts of the bark (0.72 ± 0.47 mg/ mL) and pulp + seed, and of *V. doniana.* (0.2±0.09 mg/ mL). The activity of these extracts is either bactericidal or bacteriostatic on the strains studied.

**Keywords:** Pulp, Seed, Fruit, Specific activity, *Vitex doniana*, *Terminalia mantaly*.

## INTRODUCTION

*Vitex doniana* is a plant in the Verbenaceae family, a family very closely related to the Lamiaceae. *V. doniana* is present in a variety of habitats, from forest to savannah, often in humid areas and along waterways This plant is widely used in traditional medicine. The leaves, fruits, roots, bark, and seeds of the plant are used medicinally to treat illnesses (Dadjo, 2014). The roots, trunk bark, and leaves are used in decoctions to treat ulcers, amoebiasis, colic, abdominal pain, stomach aches, leprosy, childhood diarrhea, dysmenorrhea, weakness, body aches, respiratory ailments, and toothaches and headaches (Muanda, 2010). The dried and fresh fruits are consumed as a sedative, digestive regulator, and treatment for eye disorders, kidney problems, diarrhea, dysentery, amoebiasis, jaundice, anemia, and leprosy (Arbonier, 2004; Orwa *et al.,* 2009; Muanda, 2010). Various extracts of leaves, stem bark, and roots have been used to demonstrate the pharmacological activities of *V. doniana.* In general, the species exhibits good antioxidant, antimicrobial, antidiarrheal, anesthetic, antidiabetic, hepatoprotective, anticonvulsant, and antipyretic properties (Dadjo, 2014).

*Terminalia mantaly* H. Perrier, belonging to the Combretaceae family, is a plant species widely distributed in Madagascar and cultivated in several African countries for its ornamental and medicinal qualities (Riviere *et al.,* 2005; Orwa *et al.,* 2009). *T. mantaly* thus contributes to urban biodiversity by providing shelter for numerous pollinating insects and a favorable microclimate. From a medicinal perspective, *T. mantaly* is traditionally used in herbal medicine to treat various conditions such as bacterial infections, malaria, gastrointestinal disorders, and inflammatory diseases (Seguena *et al*., 2013; Majoumouo *et al*., 2019). This plant is among the species explored for

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their richness in secondary metabolites, including tannins, flavonoids, triterpenes, and saponins, known for their bioactive properties (Yunusa *et al.*, 2024). Several studies have highlighted the pharmacological potential of this species, including significant antimicrobial, antioxidant, and antimalarial activities (Tchuenmogne *et al*., 2017; Mariscal *et al*., 2020; Dieng *et al*., 2025).

This study is part of our research on bioactive secondary metabolites from medicinal plants, including *V. doniana* and *T. mantaly*. Previous studies investigating antimicrobial activity have generally focused on the leaves and bark of *V. doniana* and *T. mantaly*. This study, in addition to the bark, also examined the roots and various parts of the fruit of these two plants, focusing on the *in vitro* growth of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella typhi* IPP 5534 and *Candida albicans* ATCC 2091.

## MATERIALS AND METHODS

### Plant material

The plant material composed of the root, bark, and fruit of *V. doniana,* as well as the root and seed of *T. mantaly* wa collected in the Korhogo area. The specimens were then submitted for certification to the National Floristic Centre of the Félix-Houphouët-Boigny University, Abidjan, Côte d'Ivoire. The identification numbers are: UCJ003173 for *T. mantaly* and UCJ017501 for *V. doniana.* The different parts were dried separately in an oven at 50°C (WTB Binder) for two weeks to prevent mold contamination, then ground into powder using an IKAMAG mechanical mill. The resulting powders were used to prepare the various extracts tested.

### Microorganisms used

The target microorganisms are microorganisms frequently encountered in human diseases and are used to detect the antimicrobial activity of extracts. Four microorganisms were used in this study: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella typhi* IPP 5534, and *Candida albicans.* ATCC 2091. All these strains were provided by the National Public Health Laboratory (LNSP). These bacteria are preserved and kept alive by continuous subculturing on various solid and liquid culture media, depending on the species.

### Preparation of extracts from *V. doniana* and *T. mantaly* H. Perrier

The extraction method used was described by Zirihi *et al.* (2003**)**. For the preparation of the different extracts, 50 g of powder from the different parts of the plant are homogenized separately in 500 mL of distilled water or 70% ethanol. After maceration using a magnetic stirrer for 24 hours, the resulting macerate was filtered successively with absorbent cotton and Wattman paper. The aqueous and hydroalcoholic filtrates were evaporated at 50°C for 3 to 5 days and stored at 5°C protected from light before antimicrobial testing.

The yields of the different extracts obtained were determined from the equation below.

$$R = (mf / mi) \times 100$$

R: yield (%); mi: initial mass of powder (mg); mf: mass of the extract after evaporation of the solvent (mg).

### Determination of the specific activity of extracts by the method of diffusion in agar medium

For antimicrobial testing, the DO value at 600 nm obtained with the spectrometer from a preculture allowed for the collection of an amount corresponding to approximately 2 x $10^7$ CFU, depending on the DO/CFU.$mL^{-1}$ ratio each target strain. This inoculum was deposited and homogenized in a Petri dish with 20 mL of supercooled MH or Sabouraud agar at 50°C. Once the agar had solidified, nine 6 mm diameter wells were made using a sterile punch. Subsequently, 80 µL of a pre-prepared extract concentration range from 10 mg/ mL to 0.078 mg/ mL was introduced into each well. The Petri dishes were then incubated for 24 or 48 hours at 37°C or 30°C. After 24 or 48 hours of incubation, the radius of inhibition from the edge of the wells is measured. The values for the different concentrations of each extract allow us to plot the regression line between the dilution of the initial concentration and the radius of inhibition (Figure 1). From this line, we determine the dilution of the initial concentration that results in a 1 mm radius of inhibition, which is an arbitrary unit (AU). This value allows us to calculate the specific activity of the extract, which is expressed in AU/mg. It represents the inverse of the amount of extract (in mg) in a well that induces a 1 mm radius of inhibition from the edge of the well (Zouhir *et al.,* 2011; Moroh, 2013). Thus, comparing the different specific activity values of each extract makes it possible to identify the organ with the highest concentration of the active ingredient and to determine the solvent that best selects this active ingredient.

### Determination of the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC)

The double dilution method in liquid medium was used to determine the values of the antibacterial parameters. Several sets of tubes were prepared with 2 mL of extract at each concentration (20 mg/ mL to 0.156 mg/ mL) and 2 mL of twice-inoculated concentrated broth (2 $10^6$ CFU/ mL) in hemolysis tubes. Two tubes from each set served as a sterility control (4 mL of MH or Sabouraud broth) and a growth control (4 mL of inoculated broth). After incubation at 30°C or 37°C for 18 to 24 hours, the MIC of each extract was determined. After reading the MICs, the MBC and MFC were determined by inoculation from the tube corresponding to the MIC. After 24 hours and 48 hours of incubation at 37°C and 30°C, the MBC or MFC is the concentration at which no visible growth or fewer than three colonies were observed (Doughari, 2006).

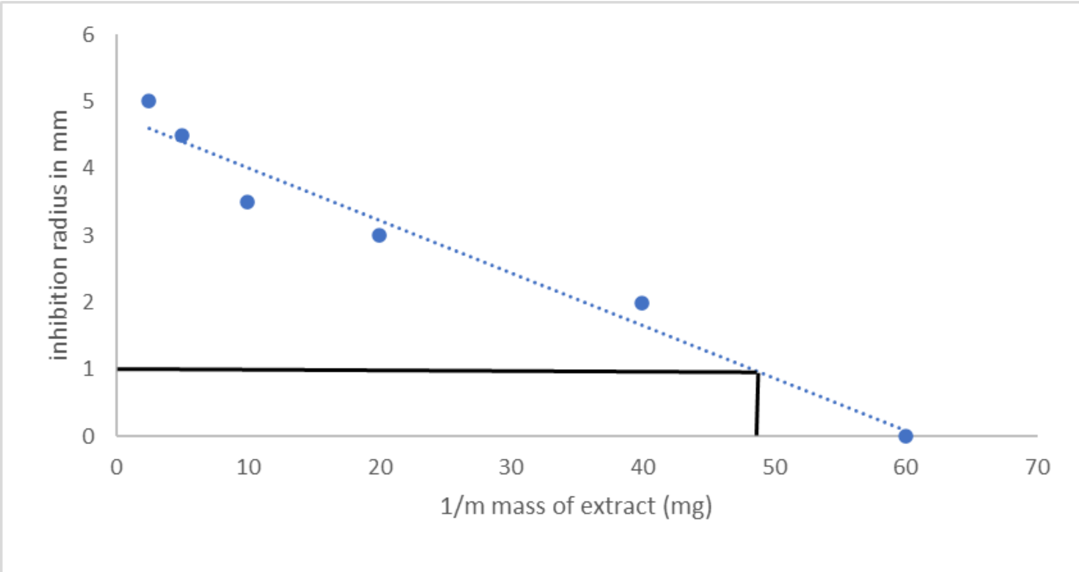



**Figure 1**. Détermination de l'activité spécifique en (UA/mg).

**Statistical analysis**

One-way analysis of variables (ANOVA) and the Newmann- Keuls test were performed using IBM Statistics software to understand the variables measured across the different parameters studied. Differences were considered significant for p-values < 0.05 ($p < 5\%$). This software also allowed for the calculation of means and standard deviations of the analyzed parameters. The figures were created using Excel 2016.

**RESULTS AND DISCUSSION**

To evaluate the antibacterial activity of *V. doniana* and *T. mantaly,* several extracts were prepared from the bark, root, and fruit. Extracts from the fruit of *V. doniana* and *T. mantaly* yielded the best results compared to those from the root and bark, with the highest yields (26.4% and 36.76%, respectively) from the pulp of *V. doniana.* Aqueous maceration provided the best yields (12.82% to 36.76%) with the fruit of *T. mantaly* and the various parts of the fruit of *V. doniana,* except for the root of both plants, where hydroalcoholic maceration yielded the best results. According to our results, the yields of the extractions obtained depend primarily on the part of the plant used (root, bark, pulp, and seed) and the extraction power of the solvents (Figures 2 and 3). This contradicts the results of other studies which have shown that hydroalcoholic maceration yields better results than aqueous maceration (Dieye and *al*., 2021). However, the extraction methods do not allow for the extraction of specific chemical compounds. Therefore, the yields cannot be correlated with the measured biological activity values (Diatta *et al.*, 2019). The antibacterial activity test in agar medium allowed for activity screening. This method made it possible to determine the specific activity of each extract. The specific activity values of the aqueous extracts ranged from 0 to 5.3 AU/mg. The extract from which activity was observed was the aqueous extract of the root of *T. mantaly* against all strains. Thus, the best activities were obtained against the *S. typhi strains.* IPP 5534 (5.3 AU/mg) and *C. albicans* ATCC 2091 (5.22 AU/mg). Regarding hydroalcoholic extracts, the values of specific activities vary between 0 and 6.596 AU/mg. The best specific activities were observed with the hydroalcoholic extract of the root of *T. mantaly* against all strains. The root of *T. mantaly* is therefore the organ with the highest concentration of antimicrobial active compounds. Extracts of the seed of *T. mantaly* showed no activity against the strains studied, while extracts of the fruit pulp of *V. doniana* showed activity against these same strains. Thus, the best specific activity was obtained with the hydroalcoholic extracts of the pulp and the pulp and seed. The specific activity values ranged from 4.637 AU/mg to 5.297 AU/mg (Figure 4). The inhibitory activity of the fruit of *V. doniana* has already been demonstrated by the work of Dah- Nouvlessounon *et al.* (2023) on bacterial and fungal strains. According to these authors, the ethanolic extract of *V. doniana* fruit inhibits 80% of the strains studied, while the ethanolic extract of the leaves inhibits 60%. Overall, hydroalcoholic extracts exhibited antibacterial and antifungal activity, unlike aqueous extracts (except for the aqueous extract of *T. mantaly)*. The hydroalcoholic mixture (30/70) provided the best extraction of inhibitory compounds from *V. doniana,* whereas with *T. mantaly,* both solvents allowed for the extraction of active compounds from the root.

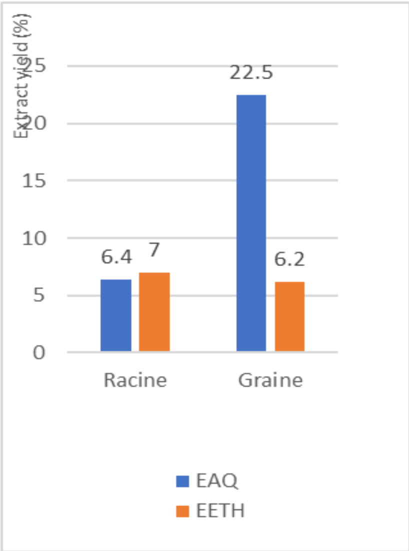



**Figure 2.** Rendement des extraits de *T. mantaly.*

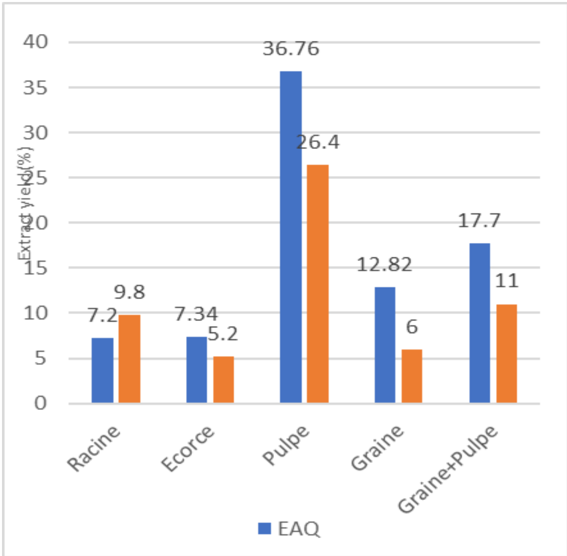
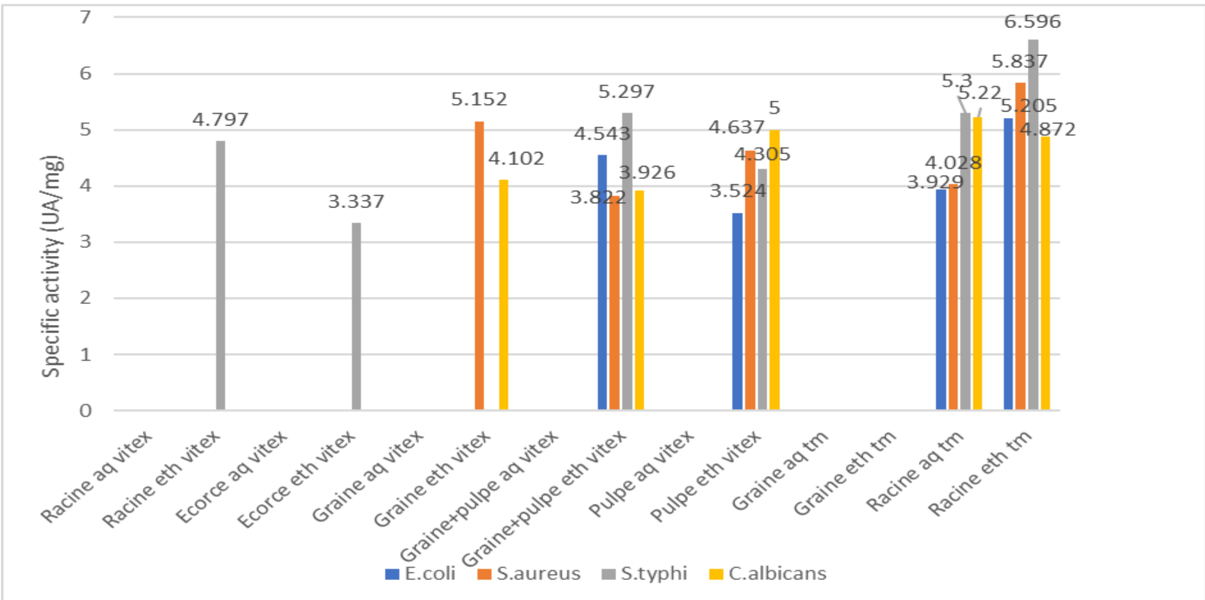



**Figure 3.** Rendement des extraits de *V. doniana.*




aq: aqueous; eth: ethanolic; tm: *T. mantaly*

**Figure 4.** Specific activity (UA/mg) of the different extracts of *Vitex doniana* and *Terminalia mantaly* on different microorganisms.

The antibacterial parameters, namely the MIC, MBC, and MFC, were determined by the liquid-based method using extracts that exhibited inhibitory activity against the *in vitro growth* of the studied strains. All extracts showed inhibitory activity against all strains at varying concentrations. The lowest MIC values for the bacterial strains were obtained with the ethanolic extract of *T. mantaly*. Thus, we obtained an MIC of 0.15 mg/ mL on *E. coli* ATCC 25922, and an MIC of 0.52 mg/ mL on *S. aureus*. ATCC 25923 and 0.2 mg/ mL on *S. typhi* IPP 5534. The results obtained in this work with the ethanolic extract of the root of *T. mantaly* are better than those obtained with the ethanolic extract of the bark on the *in vitro growth* of *E. coli* ATCC 25922 (0.3125 mg/ mL). However, on the *S. aureus strain* ATCC 25923, the ethanolic extract of the bark showed the best activity (Kokora *et al*., 2013). Regarding the extracts of *V. doniana*, the extract that showed a low MIC value against the bacterial strains was the ethanolic extract from the seed, with MIC values of 0.62. mg/ mL, 0.67 ± 0.54 mg/ mL, and 0.52 ± 0.18 mg/ mL, respectively, were observed on the same strains. However, the lowest activity (MIC = 5 mg/ mL) was obtained with the pulp extract, while in solid medium, this extract exhibited better activity against the strains than the root, bark, and seed extracts. Thus, the inhibitory activity of the extracts varies depending on the medium (solid or liquid) and the microorganisms. This could be explained by the availability of the active substances in the medium, the migration rate of these compounds, and their sites of action on the microorganisms studied. On the *E. coli strain* ATCC 25922, the inhibitory activity (0.21±0.09 mg/ mL) of the ethanolic extract of the fruit (seed + pulp) of *V. doniana* was higher than those of the other extracts. However, our results on the bacterial strains are better than those obtained by Dah- Nouvlessounon *et al.* (2023) on the strains of *S. aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Micrococcus luteus* ATCC 10240, *Streptococcus oralis*, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *Proteus mirabilis* A24974, *Proteus vulgaris* A25015 and *Pseudomonas aeruginosa* ATCC 27853.

**Table 1.** Minimum inhibitory concentration (MIC) of extracts of *V. doniana* and *T. mantaly* H. Perrier.

| Extracts | CMI | | | |
|---|---|---|---|---|
| | ***E. coli*** **ATCC 25922** | ***S. aureus*** **ATCC 25923** | ***S. typhi*** **IPP 5534** | ***C. albicans*** **ATCC 2091** |
| Root eth vitex | 0.88±0.63 [a] | 1.77±1.26 [a] | 2.08 ± 0.72 [bc] | 1.66 ± 0.72 [bcd] |
| Vitex bark | 2.5 [b] | 2.08±0.72 [ab] | 1.87±1.08 [bc] | 0.72 ± 0.47 [ab] |
| Eth vitex seed | 0.62 [a] | 0.67±0.54 [a] | 0.52 ± 0.18 [ab] | 1.04 ± 0.36 [abc] |
| Vitex pulp | 5 [c] | 5 [c] | 5 [c] | 2.5 [d] |
| Seed + pulp eth vitex | 0.21±0.09 [a] | 1.041 ± 0.36 [a] | 1.45 ± 0.95 [abc] | 0.2 ± 0.09 [a] |
| Racine aq tm | 5 [c] | 4.16 ± 1.44 [bc] | 0.72 ± 0.47 [ab] | 2.5 [d] |
| Racine eth tm | 0.15 [a] | 0.52 ± 0.18 [a] | 0.2 ± 0.09 [a] | 2.5 [d] |

aq: aqueous; eth: ethanolic; tm: *T. mantaly*

Means assigned the same letter in the same column are not statistically different at the 5% level.

**Table 2.** Minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) of extracts of *V. doniana* and *T. mantaly* H. Perrier

| Extracts | CMB | | | |
|---|---|---|---|---|
| | ***E. coli*** **ATCC 25922** | ***S. aureus*** **ATCC 25923** | ***S. typhi*** **IPP 5534** | ***C. albicans*** **ATCC 2091** |
| Root eth vitex | 5b | 5 [c] | 5 [c] | 2.5 [ab] |
| Vitex bark | 5b | 5 [c] | 5 [c] | 4.17±1.44 [ab] |
| Eth vitex seed | 5b | 2.5 [b] | 5 [c] | 2.5 [a] |
| Vitex pulp | 5b | 5 [c] | 5 [c] | 5b |
| Seed + pulp eth vitex | 5b | 5 [c] | 4.17±1.44 [c] | 2.5 [a] |
| Racine aq tm | 5b | 5 [c] | 1.25 [b] | 2.5 [a] |
| Racine eth tm | 1.67±0.72 [a] | 0.83 ± 0.36 [a] | 0.26±0.09 [a] | 2.5 [a] |

aq: aqueous; eth: ethanolic; tm: *T. mantaly*

Means assigned the same letter in the same column are not statistically different at the 5% level.

On the *C. albicans* ATCC 2091, all extracts showed inhibitory activity. However, this was particularly true with the hydroalcoholic extracts of the bark (0.72 ± 0.47 mg/ mL) and the seed and pulp: fruit (0.2 ± 0.09 mg/ mL) of *V. doniana.* that the best activities were obtained. The highest MIC values were obtained with the aqueous and hydroalcoholic extracts of *T. mantaly and C. albicans* ATCC 2091 is therefore more sensitive to extracts of *V. doniana* than *T. mantaly.* The inhibitory activity of the fruit of *V. doniana* could be explained by its richness in polyphenols, tannins, sterols, triterpenes, steroids, sugars (carbohydrates), saponins of polyphenolic compounds, flavonoids, and anthraquinones (Bouquet., 1972; Suleiman and Yusuf., 2008; Dah- Nouvlessounon *et al.,* 2023). The hydroalcoholic extract of *T. mantaly root and the hydroalcoholic extracts of V. doniana* root and bark are bacteriostatic against *E. coli* and *C. albicans, respectively.* ATCC 2091 and bactericidal against other strains. Hydroalcoholic extracts of the pulp and seed, and of the seed of *V. doniana,* are also bacteriostatic against *E. coli* ATCC 25922 and *S. aureus ATCC* 25923, respectively and *S. typhi* IPP 5534 but bactericidal on other strains. Only aqueous extracts of *T. mantaly* and hydroalcoholic pulp of *V. doniana* demonstrated bacteriostatic activity on strains of *E. coli* ATCC 25922, *S. aureus* ATCC 225923, *S. typhi* IPP 5534 and *C. albicans* ATCC 2091 (Table 1 and 2). The antimicrobial activity of extracts from these two plants has been demonstrated several times by several authors, including Ngouana *et al.,* (2015); Soumahoro *et al.,* (2016); Bunu *et al.,* (2021); Owolabi *et al.,* (2022); Dah - Nouvlessounon *et al.* (2023).

## CONCLUSION

Different extracts of the root, bark, and fruit of *Vitex doniana* and *Vitex mantaly inhibited the in vitro growth* of three bacterial strains and one yeast strain using solid and liquid diffusion methods. The plant parts that best concentrated the active compounds were found to be the root of Vitex mantaly and the pulp and pulp + seed (fruit) of Vitex doniana . While better inhibitory activity was obtained with the root and bark extracts of both plants, low MIC values were obtained with the different parts of the *Vitex doniana* fruit.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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable


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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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