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ORIGINAL ARTICLE

Effect of *Tamarindus indica L.* and *Manihot esculenta* Extracts on Antibiotic-resistant Bacteria

Zenon Machado Lima[1], Lenilson Santos da Trindade[1], Genelane Cruz Santana[2], Francine Ferreira Padilha[1], Marcelo da Costa Mendonça[1], Luiz Pereira da Costa[1,3], Jorge A. López[1], Maria Lucila Hernández Macedo[1]

[1]Program in Industrial Biotechnology- Tiradentes University/ Institute of Technology and Research, [2]Material Science and Engineering- Federal University of Sergipe, [3]Biomaterials and Nanotechnology Laboratory- Technological Institute and Research of the Sergipe State, Aracaju-SE, Brazil

ABSTRACT

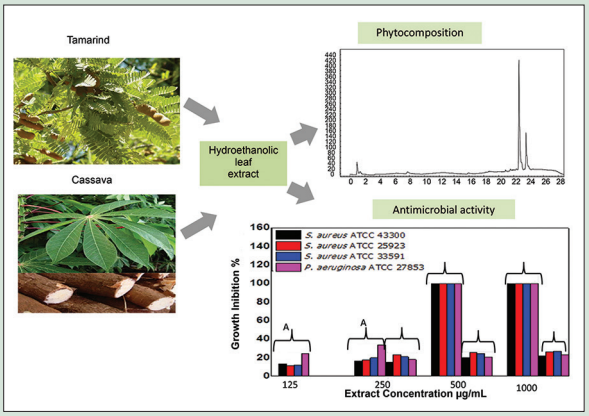
Background: The chemical composition of plants used in traditional medicine exhibits biologically active compounds, such as tannins, flavonoids, and alkaloids and becomes a promising approach to treat microbial infections, mainly with drug-resistant bacteria. **Objective:** The aim of the present study was to evaluate the hydroethanolic leaf extracts of *Tamarindus indica* (*tamarind*) and *Manihot esculenta* (*cassava*) as antimicrobial potential against *Pseudomonas aeruginosa* clinical isolated and Methicillin-resistant *Staphylococcus aureus*. **Materials and Methods:** Hydroethanolic leaf extracts were prepared and characterized by high-performance liquid chromatography/diode array detection, Fourier transform infrared, 1,1-diphenyl-2-picrylhydrazyl, and ultraviolet-visible methods. The antimicrobial activity against four strains of clinical relevance was evaluated by the microdilution method at minimum inhibitory concentrations. **Results:** Phenolic compounds such as flavonoids were detected in the plant extracts. *T. indica* extract at 500 µg/mL showed antimicrobial activity against *S. aureus* and *P. aeruginosa*; however, *M. esculenta* showed only activity against *P. aeruginosa* in this concentration. **Conclusions:** Our results suggested that polyphenols and flavonoids present in *T. indica* leaf extracts are a potential source of antimicrobial compound. The *T. indica* extract showed antibacterial activity against *S. aureus* and *P. aeruginosa* while *M. esculenta* had effect only on *P. aeruginosa* meropenem resistant.

Key words: Antibiotic-resistant bacteria, antimicrobial, plant extract

SUMMARY

- Antibacterial effect of *T. indica* and *M. esculenta* leaf extract was evaluated.
- *T. indica* extract displayed activity against *S. aureus* and *P. aeruginosa* strains.
- *M. esculenta* showed effect on *P. aeruginosa* meropenem resistant.

Abbreviations Used: BHI: Agar brain heart infusion, CAPES: Coordination for the improvement of higher education personnel, DPPH: 1,1-diphenyl-2-picrylhydrazyl, FAPITEC/SE: Foundation for support to research and technological innovation of the state of sergipe, FTIR: Fourier transform infrared spectroscopy, HPLC: High-performance liquid chromatography, KBr: Potassium bromide, MIC: Minimum inhibitory concentration, MRSA: Methicillin-resistant *Staphylococcus aureus*, RSC: Radical scavenging capacity, UV-vis: Ultraviolet-visible.



Correspondence:
Dr. Maria Lucila Hernández Macedo,
Tiradentes University, Institute of Technology and Research, Av. Murilo Dantas, 300, Bairro Farolândia, CEP: 49032-490, Aracaju/SE, Brazil.
E-mail: lucyherma@gmail.com
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INTRODUCTION

Ethnobotanical and experimental evidence supports the use of plants due to their wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial activity,[1-4] displaying even synergistic effects with existing antimicrobial drugs.[5]

Thus, due to the growing problem of antibiotic resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, there is a great interest in plants considering their phytochemicals as potential therapeutic agents.[6,7] In this context, *Tamarindus indica* L. and *Manihot esculenta* have significant importance in traditional medicine for different medicinal purposes.[8-12]

The *T. indica* L. and *M. esculenta* phytochemical studies have correlated their antimicrobial activities to several metabolites. *T. indica* leaf extract revealed the presence of many compounds such as ascorbic acid, β-carotene, polyphenols, and flavonoids (e.g., apigenin, catechin, epicatechin, and naringenin).[13]

Regarding *M. esculenta* leaf phytocomposition, it indicated the flavonoids, tannins, ascorbic acid, alkaloids, and saponins presence,[14] which display antimicrobial, antioxidant, and anti-inflammatory effects.[15]

Overall, phenolic compounds display a structural diversity which could be used for therapeutic intervention due to their biological broad spectrum.[16,17] These bioactivities are attributed to polyphenol

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interactions with proteins, lipids, and carbohydrates. These interaction induced cell permeability, causing membrane disruption.[18]

Therefore, the chemical composition of *T. indica* L. and *M. esculenta* leaf extracts was evaluated, as well as their antimicrobial activity against *P. aeruginosa* clinical isolated and Methicillin-resistant *S. aureus* (MRSA).

MATERIALS AND METHODS

Plant material and leaf extract preparation

T. indica L. and *M. esculenta* were collected in Porto da Folha, Sergipe, Brazil. The plants species were identified by Prof. Marla Ibrahim Uehbe de Oliveira and deposited in the Tiradentes University herbarium as AJU154 and AJU155, respectively. The leaves were previously oven-dried at 65°C for 2 days and powdered before extract preparation according to Sultana *et al.*[19] In brief, 10 g of each dried leaves was mixed with 100 mL of hydro-alcohol (20:80) and ultra-sounded for 1 h. Then, the obtained aqueous phases were concentrated and lyophilized. The dried crude concentrated extracts were stored at −4°C, until used for analyses.

Determination of total phenolic compounds

Total phenolic extract content was determined by Folin-Ciocalteu method.[20] The absorbance was measured in triplicate at 745 nm (DR 5000™ ultraviolet-visible [UV-vis] Spectrophotometer) and results expressed as mg of gallic acid equivalents per 100 mg of extract.

Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl radicals

The extract for antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Experiments were carried out according to Shaker *et al.*[21] with some modification. Briefly, 3 mL of each hydroethanolic extract (50–250 μg/mL) was mixed with 750 μL of ethanolic solution of DPPH 400 mM. The reaction mixture was shaken thoroughly and incubated in the dark at room temperature for 15 min. The absorbance was measured in triplicate at 517 nm (DR 5000™ UV-vis Spectrophotometer). The free radicals percentage inhibition was calculated using the following formula: % RSC = $(A_0 - A)/A \times 100$, where RSC = radical scavenging capacity (%), A_0 = absorbance of control sample (t = 0 h), and A = absorbance of a tested sample at the end of the reaction (t = 1 h).

Qualitative phytochemical characterization

High-performance liquid chromatography/diode array detection

The hydroethanolic leaf extract composition was performed by reversed phase high performance liquid chromatography according to Aznar *et al.*[22] Dry leaves extract samples (5 mg/mL) were dissolved in methanol and 9 μL was injected into the HPLC (Varian ProStar HPLC system, Walnut Creek, Au). The samples were eluted using 0.1% formic acid and acetonitrile as mobile phase for gradient elution (flow rate = 1 mL/min) through the column Phenomenex C18 (4.6 mm × 100 mm, 2.6 μ particle size, Torrance, CA, USA). The peaks were detected at 280 nm. The compounds identification was done by comparison of their retention's time and UV absorption spectrum with those of the standards.

Ultraviolet-visible and fourier transform infrared

To detect *T. indica* and *M. esculenta* phenolic compounds, the plant extracts were scanned by UV-vis in the wavelength ranging from 200 to 800 nm using a LAMBDA™ 45 UV-vis Perkin Elmer spectrophotometer. Fourier transform infrared (FTIR) analysis was performed using 10 mg of dried powder of each plant extracts, encapsulated in 100 mg of KBr, and loaded in FTIR spectroscope (Spectrum BX, Perkin Elmer), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Microorganisms

Microorganism strains (*P. aeruginosa* ATCC 27853, *S. aureus* [MRSA] ATCC 25923, *S. aureus* [MRSA] ATCC 33591, and *S. aureus* [MRSA] ATCC 43300) were supplied by the National Institute for Quality Control in Health (INCQS) from the Oswaldo Cruz Foundation (FIOCRUZ). The bacteria maintenance was carried out on Agar Brain Heart Infusion (BHI) (stationary culture) for 37°C at 24 h.

Minimum inhibitory concentration Determination

The microdilution method was used to estimate the minimum inhibitory concentration (MIC).[23] Briefly, 96-well microplate was coated with 100 μL BHI, 100 μL leaves extract (0, 1 g/mL), and 20 μL of microorganisms suspensions previously diluted at 0.5 McFarland turbidity standard. The microplates were incubated at 37°C for 24 h and microbial growth inhibition was determined at 595 nm (Microplate Reader Model 550, Bio Rad, USA). Vancomycin and meropenem were used as standard positive drug against *S. aureus* and *P. aeruginosa*, respectively. Experiments were done in triplicate.

Statistical analysis

All experimental results were centered using three parallel measurements of the mean ± standard deviation. Analysis of variance was performed followed by Tukey's test as applicable, using the statistical software R (R Development Core Team, 2008). $P < 0.05$ was considered statistically significant.

RESULTS

Phenolic content and antioxidant activity of the extracts

The total phenolic content of *T. indica* and *M. esculenta* extracts showed 132.85 ± 1.43 μg/100 mg and 121.64 ± 1.71 μg/100 mg of phenolic compounds, respectively, expressed as gallic acid equivalents.

The RSC evaluated by DPPH method is presented in Figure 1. Both extracts were able to reduce the DPPH in a concentration-dependent manner; however, *T. indica* showed better antioxidant capacity than *M. esculenta* compared to silymarin control, ranged from 18% to 75% of RSC.

Qualitative phytochemical composition

The chromatographic profiles of *M. esculenta* leaf extract showed two major peaks whose absorption spectrum matching to 353 nm (band I) and 256 nm (band II) and for *T. indica* 212 and 269 nm [Figures 2 and 3].

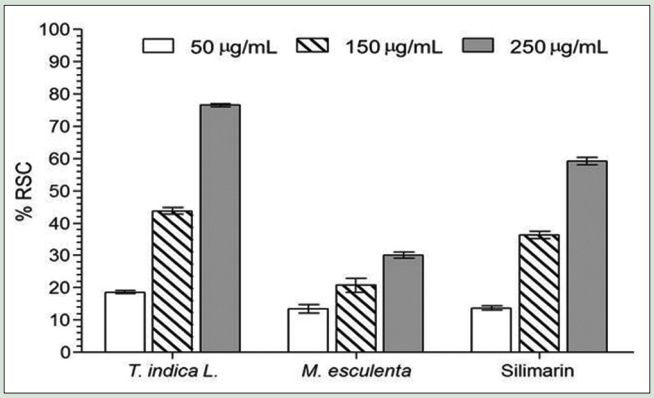

Figure 1: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of the *Tamarindus indica* and *Manihot esculenta* extracts. Values are means ± standard deviation (n = 3). RSC: Radical scavenging capacity

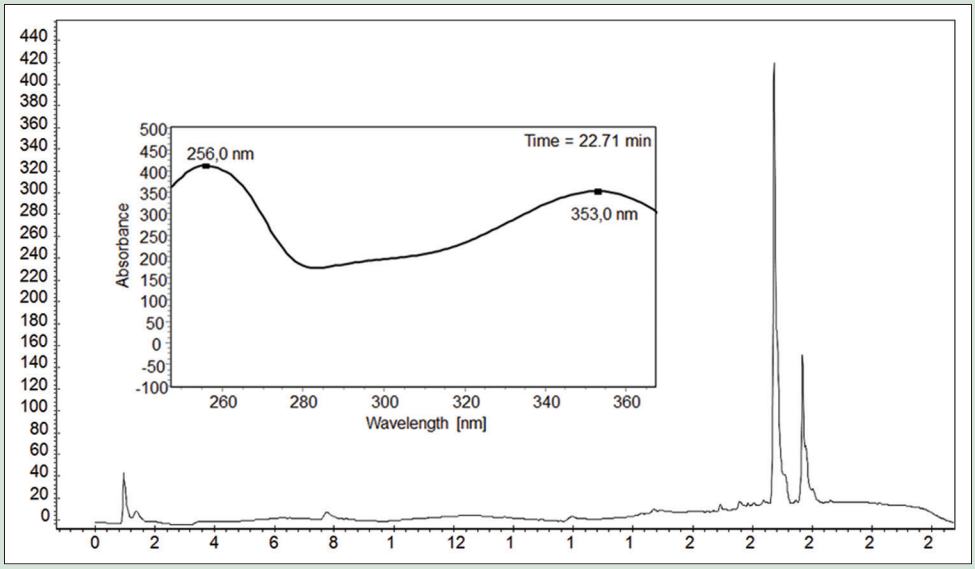

Figure 2: Chromatographic profile of *Manihot esculenta* leaf extract used to investigate the qualitative content of polyphenols

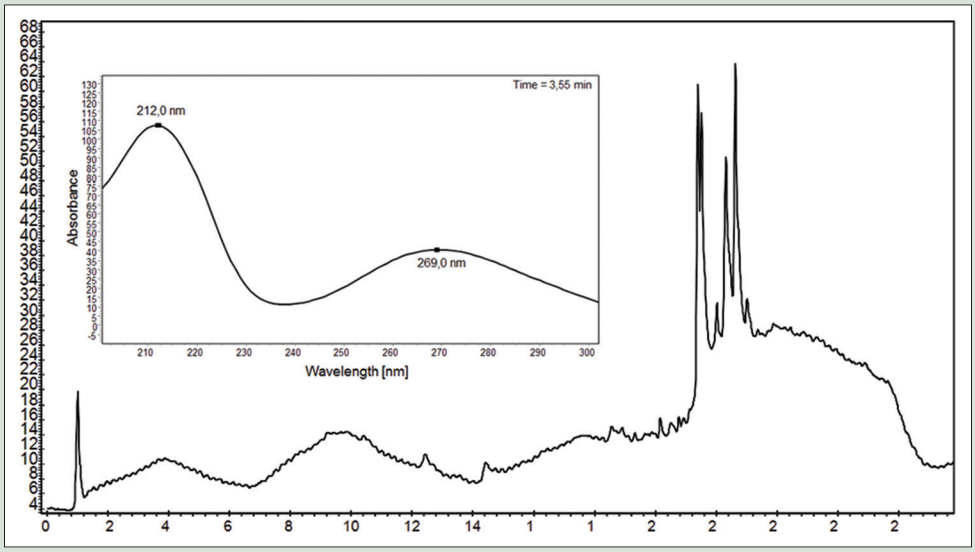

Figure 3: Chromatogram of the extract of leaves of *Tamarindus indica* used to investigate the presence of flavonoids

Ultraviolet-visible and fourier transform infrared analysis

With respect to UV-vis analyses, the extracts showed bands at 283 and 327–328 nm for *T. indica*, whereas bands at 286 and 309–321 nm were observed for *M. esculenta* extract [Figure 4].

On the other hand, the FTIR spectra obtained from *T. indica* and *M. esculenta* extracts showed bands between 3.500 and 3.000 cm^{-1} assigned to OH group stretching vibration (alcohol and water), and also bands ranged from 2.950 to 2800 cm^{-1} indicating CH groups vibrations (methyl and methylene) were observed. The peak obtained at 1.680–1.630 cm^{-1} showed stretching vibration of the C=O (carbonyl) groups while bands between 1.150–1.050 cm^{-1} and 900–1.300 cm^{-1} corresponding to C-OH and CH groups, respectively [Figure 5].

Antimicrobial activity

The antibacterial activity of the plant extracts is shown in Table 1. The results indicated that extracts showed antibacterial activities at variable degrees against clinical isolates (*P. aeruginosa*) and MRSA bacteria, with MICs values varying from 125 to 1000 μg/mL. *T. indica* extracts displayed the most important spectrum of activity at 500 and 1000 μg/mL, by inhibiting 100% growth of all microorganisms, whereas *M. esculenta* at 500 μg/mL had satisfactory antibacterial activity only against *P. aeruginosa*. Vancomycin at 1.95 μg/mL inhibited the *S. aureus* growth while meropenem had no activity against *P. aeruginosa* at 500 μg/mL (data not shown).

DISCUSSION

Total phenolic content in plant extracts and their antioxidant activity are strongly related. Phenols have ability of eliminating free radicals due to its hydroxyl groups; therefore, the presence of these compounds in plants may directly contribute to their antioxidant and antimicrobial actions.[24-27] In this study, the phenolic content of *T. indica* leaf extract was high than in *M. esculenta*. Although the presence of polyphenols in *M. esculenta* was observed, its antioxidant activity is decreased due to glycosides substitutions in their molecular structure, interfering

Table 1: Minimal inhibitory concentration of *Tamarindus indica* and *Manihot esculenta* extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. (–) No inhibition

Leaves extract Concentration (µg/mL)	MIC mean+-SD (%)							
	***S. aureus* 43300**		***S. aureus* 25923**		***S. aureus* 33591**		***P. aeruginosa* 27853**	
	T. indica	***M. sculenta***	***T. indica***	***M. sculenta***	***T. indica***	***M. sculenta***	***T. indica***	***M. sculenta***
125	17	-	15	-	16	-	24	-
250	18	17	19	22	20	21	37	20
500	100	23	100	26	100	27	100	100
1000	100	30	100	31	100	33	100	100

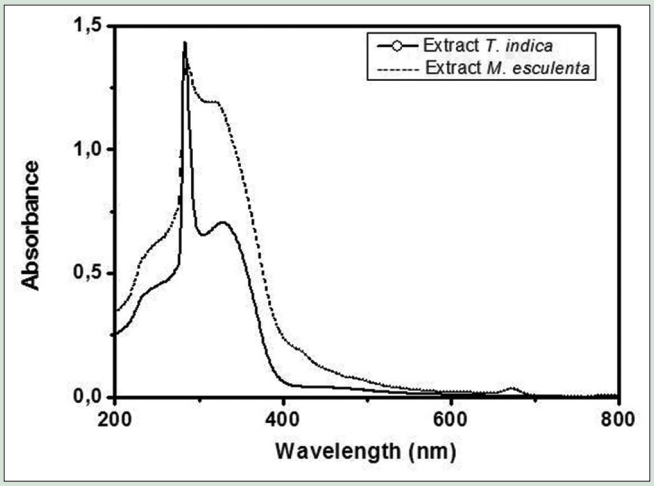

Figure 4: Ultraviolet-visible spectrum of *Tamarindus indica* and *Manihot esculenta* leaf extract

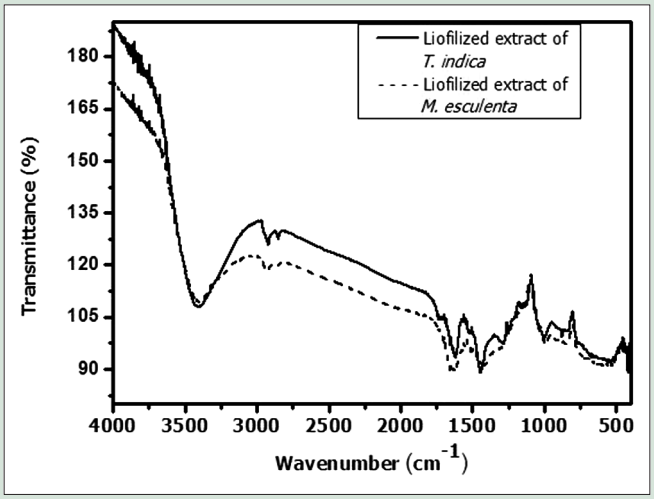

Figure 5: Fourier transform infrared of *Tamarindus indica* and *Manihot esculenta* extract

in this activity.[28] This may explain the lower value of the DPPH with *M. esculenta* extract despite its phenols content. Similar results were obtained by Rahiman *et al.*[29] by analyzing the antioxidant activity and total phenolic content of plants used as home remedies.

Thus, the better antioxidant capacity of *T. indica* extract can be explained by a variety of flavonoids and phenolic compounds presence, which act as reducing agents, stabilizing the free radical DPPH.[21]

The extract UV-vis spectra revealed two bands at 310–350 and 250–290 nm, indicating the presence of flavones and flavonols, respectively.[30,31] This result is relevant considering the antimicrobial activity of these compounds.

UV-vis and FTIR data are supported by chromatographic profiles of *M. esculenta* and *T. indica* leaf extracts that indicated the phenolic compounds and flavonoids presence, suggested by two typical bands of flavonoids structure and functional groups present (e.g., acids, carbonyl, alcohols, aldehydes, alkanes, and ethers) in these compounds; this is consistent with several data described in scientific literature.[15,32]

According to Jakobek,[18] the detected flavonoids and phenolic compounds in plant extracts act as antimicrobial agents against various human pathogens. The polyphenols mechanism of action on microorganisms are still poorly understood, and some authors suggest that polyphenol acts by reducing the iron availability, inhibiting protein expression, changing the cell membrane permeability, and fluidity.[33-35]

Although antimicrobial compounds present in *T. indica* leaves are not well elucidated, our results are in line with Cuban and Puerto Rican plant extract studies from the same species, which showed antimicrobial potential probably due to the presence of phenolic compounds.[36,37]

T. indica extract inhibited the *S. aureus* and *P. aeruginosa* growth at 500 and 1000 µg/mL. MIC values between 50 and 500 µg/mL indicate strong activity while 600–1500 µg/mL values denote a moderate antimicrobial action,[38] showing that *T. indica* extract has activity against the bacteria studied.

Although literature reports that Gram-negative bacteria are more resistant than Gram-positive to polyphenols due to the cell wall chemical composition,[39] this resistance was not observed in our results, since the *T. indica* extract showed an inhibitory capacity against *P. aeruginosa*.

On the other hand, *M. esculenta* extract has inhibition at 500 µg/mL only against *P. aeruginosa* meropenem resistant, evidencing the antimicrobial potential of the extract.. The low inhibitory effect of *M. esculenta* on *S. aureus* is probably due to attachment of carbohydrates in polyphenols and flavonoids compounds that decreased their antioxidant and antimicrobial activities.[18,33,40]

CONCLUSIONS

In the present study, two plant extracts (*T. indica L.* and *M. esculenta)* were studied and their antioxidant and antimicrobial capacities were evaluated. Biomolecules such as polyphenols and flavonoids present in leaf extracts exhibited antimicrobial activity on drug-resistant bacteria. The *T. indica* extract showed antibacterial effect against Gram-positive and Gram-negative bacteria, while *M. esculenta* had activity only on *P. aureginosa* meropenem resistant. Therefore, results suggest that both extracts are an affordable antioxidants source and have antimicrobial effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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