



Research Article

***Talinum fruticosum* (L.) Juss. (Talinaceae), an Edible and Medicinal Plant, Enhances the Activity of Antibiotics Against Multidrug-Resistant Gram-Negative Bacteria**

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Abstract

The rapid acquisition of multidrug-resistant (MDR) phenotypes by bacteria creates life-threatening complications in infection control. Accordingly, this study aims to investigate the antibacterial activity of the methanol extract of *Talinum fruticosum* leaves and its interaction effects with antibiotics against MDR Gram-negative bacteria. The broth microdilution method was used to evaluate the antibacterial activity and antibiotic-resistance modulation effects of the extract. The interaction between antibiotics and *T. fruticosum* leaf extract (TFLE) was conducted using the checkerboard assay. Phytochemical screening was assessed using standardized qualitative tests. TFLE displayed low antibacterial activity, with MICs ranging from 512 to 2048 µg/mL against the tested bacteria. Interestingly, TFLE at its subinhibitory concentration (MIC/8) enhanced the efficacy of antibiotics by 2- to 64-fold, particularly tetracycline, doxycycline, imipenem, kanamycin, and ciprofloxacin, against at least one of the examined MDR bacteria. In addition, TFLE displayed a synergistic effect ($\Sigma\text{FIC} < 0.5$) with kanamycin and doxycycline against *K. pneumoniae* K2 and *E. aerogenes* EA298. The phytochemical screening indicated that TFLE contained flavonoids, saponins, tannins, terpenoids, phenols, and anthocyanins. Overall, this study shows that *T. fruticosum* leaf extract could be used in combination with commonly used antibiotics to fight infections involving MDR bacteria. Therefore, further studies are needed to identify in this plant a likely nontoxic antibiotic modulator.

Keywords

Talinum fruticosum, Gram-Negative Bacteria, Multidrug-Resistance, Antibacterial, Synergy

1. Introduction

Multidrug-resistant (MDR) bacteria pose a significant challenge to the global health system, particularly affecting low- and middle-income countries [1]. In fact, the swift acquisition of the MDR phenotypes enables bacteria to evade routinely used antibiotics, hence resulting in life-threatening difficulties in infection management [2, 3]. Antimicrobial

resistance (AMR) is projected to result in 10 million annual deaths and economic losses exceeding 100 trillion dollars by 2050 [4]. Gram-negative bacteria, including Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*) and *Pseudomonas aeruginosa*, are particularly worrisome as they are becoming resistant to

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practically all known antibiotic treatments [5, 6]. This is mainly due to the overexpression of efflux pumps belonging to the Resistance Nodulation Cell Division (RND) family, respectively, the AcrAB-TolC and MexAB-OprM [7, 8]. Tackling this issue appears as a global health challenge.

Several developing countries use medicinal plants as a first response to certain diseases, including bacterial infections [9]. Their characteristics, including bioavailability, target specificity, and microbial metabolism, have demonstrated their capacity to enhance the antibiotic drug discovery pipelines [10, 11]. The discovery of new chemical scaffolds from plants, together with their use in combination with traditional antibiotics, has emerged as a viable strategy for managing infections [12]. Research has shown that plant extracts exhibit remarkable activity against microbes, including Gram-negative MDR bacteria, both alone and in conjunction with antibiotics [8, 13-15].

Talinum fruticosum (L.) Juss, commonly called “waterleaf” in Cameroon, is a plant belonging to the Talinaceae family. It is an edible leafy vegetable with high nutritional and medicinal properties [16]. In traditional medicine, *T. fruticosum* has been used to manage obesity, diabetes, stroke, gastrointestinal disorders, cancer, wounds, and measles [17]. Leaf and root extracts of waterleaf are used to cure asthma, kidney disorders, gout, and rheumatoid arthritis [18]. Moreover, this plant is used to treat diarrhoea [19, 20] and respiratory infections in Nigeria [21]. The plant contains various phytoconstituents belonging to flavonoids and lignans, alkaloids, tannins, saponins, and benzoic acid derivatives, which are known for their wide range of pharmacological effects [17, 21]. Previous studies have demonstrated that *T. fruticosum* has antioxidant [22, 23], anti-inflammatory [18, 23], antidiabetic [24], neuroprotective [25], anticancer [26], and many other pharmacological activities [17]. Furthermore, the antibacterial activity of the silver nanoparticles synthesized using the aqueous leaf extract of this plant was shown by the agar well diffusion method [27, 28]. This study aimed to evaluate the antibacterial activity of the methanol extract of *Talinum fruticosum* leaves and its interaction effects with antibiotics against MDR Gram-negative bacteria.

2. Materials and Methods

2.1. Plant Material and Extraction

Leaves of *Talinum fruticosum* were harvested in January 2023 in Dschang, Menoua Division, West Region of Cameroon. The plant was identified at the National Herbarium of Cameroon in Yaoundé under the reference number 630774/HNC. These leaves were cleaned, dried away from sunlight, and subsequently crushed. The obtained powder was soaked in 95% methanol at room temperature for 48 hours, with continuous stirring. Following this, the mixture was filtered through Whatman filter paper No. 1, and the filtrate was concentrated at 65°C under reduced pressure using a

rotary evaporator (BÜCHI R-200). The crude extract was then collected, dried in an oven at 40°C, and stored at 4°C for future use.

2.2. Preliminary Phytochemical Screening

T. fruticosum leaf extract (TFLE) was subjected to a phytochemical screening to identify key secondary metabolites such as alkaloids, anthocyanins, flavonoids, phenols, saponins, tannins, and terpenoids. This screening was conducted using well-established phytochemical tests [29, 30]. The tests primarily relied on observing changes in colour or the formation of a precipitate.

2.3. Chemicals for Antibacterial Assays

Nine frequently used antibiotics, comprising chloramphenicol, tetracycline, doxycycline, kanamycin, ciprofloxacin, streptomycin, vancomycin, ceftriaxone, imipenem, and ampicillin (Sigma-Aldrich, St. Quentin Fallavier, France) were employed. p-Iodonitrotetrazolium chloride (INT) 0.2% (Loba Chemie, Mumbai, India) was the bacterial growth indicator. Dimethyl sulfoxide (BDH Chemicals Ltd, Poole, England) was used to dissolve the crude extract.

2.4. Microbial Strains and Culture Conditions

The Gram-negative bacteria tested included both reference strains and clinical isolates of *Escherichia coli* (ATCC 10536, AG100, and AG102), *Klebsiella pneumoniae* (ATCC 11296, KP2, and KP55), *Enterobacter aerogenes* (EA3, EA298, and EA27), and *Pseudomonas aeruginosa* (ATCC 27853, PA01, and PA124). Their antibiotic resistance features were previously reported [31, 32] and can also be found as supporting information (Table S1, Supplementary material). The microbial culture and antibacterial assays were conducted using Mueller-Hinton agar and Mueller-Hinton broth (Accumix, India), respectively.

2.5. Determination of Minimal Inhibitory and Minimal Bactericidal Concentrations

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of TFLE were determined by the microdilution method using INT calorimetric assays [33, 34]. Briefly, the tested samples were first dissolved in DMSO/MHB. The resulting solution was then added to MHB and serially diluted twice (in a 96-well microplate), followed by the addition of 100 µL of inoculum (2×10^6 CFU/mL) to each well. The final concentrations of extracts and chloramphenicol (reference antibacterial) ranged from 2048 to 16 µg/mL and from 64 to 0.5 µg/mL, respectively. Wells containing DMSO 2.5%+MHB and those with MHB alone were utilized as negative controls, while

those containing bacterial inoculum served as a growth control. The MIC for each sample was defined as the lowest concentration that completely inhibited bacterial growth, indicated by the absence of pink color in the wells. In contrast, the MBC was determined as the lowest concentration of the samples that showed no coloration after an additional 48 h of incubation and the addition of INT [14, 34]. The antibacterial efficacy of TFLE was interpreted according to the latest MIC cut-off values. For Enterobacteriaceae, they were defined as follows: outstanding activity ($\text{MIC} \leq 8 \mu\text{g/mL}$), excellent activity ($8 < \text{MIC} \leq 64 \mu\text{g/mL}$), very good activity ($64 < \text{MIC} \leq 128 \mu\text{g/mL}$), good activity ($128 < \text{MIC} \leq 256 \mu\text{g/mL}$), average activity ($256 < \text{MIC} \leq 512 \mu\text{g/mL}$), weak activity ($512 < \text{MIC} \leq 1024 \mu\text{g/mL}$), and not active ($\text{MIC} > 1024 \mu\text{g/mL}$) [35]. For *Pseudomonas aeruginosa*, they were defined as follows: outstanding activity ($\text{MIC} \leq 32 \mu\text{g/mL}$), excellent activity ($32 < \text{MIC} \leq 128 \mu\text{g/mL}$), very good activity ($128 < \text{MIC} \leq 256 \mu\text{g/mL}$), good activity ($256 < \text{MIC} \leq 512 \mu\text{g/mL}$), average activity ($512 < \text{MIC} \leq 1024 \mu\text{g/mL}$), weak activity or not active ($\text{MIC} > 1024 \mu\text{g/mL}$) [36]. Each assay was done in duplicate and repeated three times.

2.6. Modulation Assay

To evaluate the antibiotic-resistance modulation effects of TFLE, the MICs of antibiotics were determined alone and in the presence of plant extracts using the liquid microdilution method as previously described [37]. Briefly, after a serial dilution of antibiotics, the plant extract at its sub-inhibitory concentration ($\text{MIC}/8$) was introduced to each well. Thereafter, the inoculum was added to the wells, and the MICs were assessed as previously described. The modulation factor (MF) was determined as the ratio of the MIC of the antibiotic alone to that of the antibiotic in the presence of the extract. $\text{MF} \geq 2$ was set as the threshold for the biological significance of antibiotic potentiation effects [38]. All experiments were conducted in triplicate.

2.7. Checkerboard Essay

The interactions involving antibiotics and TFLE were examined using the checkerboard broth microdilution technique as previously described [39]. Briefly, the antibiotic was diluted along the x-axis, while the extract was diluted along the y-axis. Thereafter, inoculum ($2 \times 10^6 \text{ CFU/mL}$ of bacteria) was introduced into the wells, and the plates were incubated for 18 hours at 37°C , followed by MIC detection as described above. The fractional inhibitory concentration index

(ΣFIC) was determined by the formula: $\Sigma\text{FIC} = \text{FIC of extract} + \text{FIC of antibiotic}$, where $\text{FIC of extract} = \text{MIC of extract in combination} / \text{MIC of extract alone}$ and $\text{FIC of antibiotic} = \text{MIC of antibiotic in combination} / \text{MIC of antibiotic alone}$. The results were interpreted as: synergy ($\Sigma\text{FIC} \leq 0.5$); additivity ($0.5 < \Sigma\text{FIC} \leq 1$); indifference ($\Sigma\text{FIC} > 1$ to 2); and antagonism ($\Sigma\text{FIC} > 2$) [40]. All experiments were done in triplicate.

3. Results

3.1. Qualitative Chemical Composition of TFLE

T. fruticosum leaf extract was subjected to phytochemical screening to identify the main classes of secondary metabolites present. It was found that, except alkaloids, this extract contained all the other classes of secondary metabolites sought in this study, namely flavonoids, saponins, tannins, terpenoids, phenols, and anthocyanins (Table 1).

Table 1. Qualitative chemical composition of *T. fruticosum* leaf extract.

Phytochemical classes	<i>T. fruticosum</i> leaf extract
Terpenoids	+
Saponins	+
Alkaloids	-
Phenols	+
Flavonoids	+
Tannins	+
Anthocyanins	+

(+): present; (-): absent.

3.2. In vitro Antibacterial Activity

The antibacterial activity of TFLE was assessed by determining its MIC and MBC against Gram-negative bacteria, including multi-resistant clinical isolates. The results showed that MIC of TFLE ranged from 512 to 2048 $\mu\text{g/mL}$ and that for chloramphenicol (reference drug) from 4 to 256 $\mu\text{g/mL}$ (Table 2).

Table 2. MIC and MBC (in µg/mL) of *T. fruticulosum* leaf extract and chloramphenicol against Gram-negative bacteria.

Bacteria	<i>T. fruticulosum</i>		Chloramphenicol	
	MIC	MBC	MIC	MBC
<i>E. coli</i>				
ATCC 10536	1024	2048	4	16
AG100	—	—	8	16
AG102	512	—	64	>256
<i>K. pneumoniae</i>				
ATCC 11296	—	—	4	32
KP2	2048	—	16	256
KP55	—	—	64	>256
<i>E. aerogenes</i>				
EA3	—	—	64	>256
EA27	2048	—	8	64
EA298	—	—	64	256
<i>P. aeruginosa</i>				
ATCC 27853	2048	—	8	64
PA01	—	—	8	32
PA124	—	—	32	>256

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; —: >2048 µg/mL.

3.3. Antibiotic-Resistance Modulation Effects

The antibiotics were tested in the presence of TFLE at its sub-inhibitory MIC/8 concentration against seven (07) MDR isolates, including *E. coli* (AG100 and AG102), *P. aeruginosa* (PA121 and PA124), *K. pneumoniae* (K2 and KP55), and *E. aerogenes* EA298. The results showed that TFLE (MIC/8)

enhanced 2- to 64-fold the activity of the tested antibiotics against at least one of the MDR bacteria. It enhanced the activity of tetracycline on 85.71% (6/7); doxycycline and imipenem on 71.43% (5/7), and kanamycin and ciprofloxacin on 57.14% (4/7) of the tested isolates. Furthermore, the highest modulation factors (MF) were obtained with kanamycin and doxycycline ($4 \leq MF \leq 64$) (Table 3).

Table 3. Antibiotic-resistance modulation effects of *Talinum fruticosum* leaf extract against selected MDR bacteria.

Antibiotic	TFLE	Tested MDR bacteria, MIC (µg /mL), and modulation factor (in brackets)							Modulation effect (%)
		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>E. aerogenes</i>	
		AG100	AG102	PA121	PA124	K2	KP55	EA298	
Kanamycin	0	<2	128	<2	8	>256	4	>256	57.14
	MIC/8	<2 (na)	2 (64)	<2 (na)	2 (4)	64 (>4)	4 (1)	32 (>4)	
Ciprofloxacin	0	16	<0.25	0.5	>32	2	0.25	2	57.14
	MIC/8	4 (4)	<0.25 (na)	0.25 (2)	16 (>2)	2 (1)	0.25 (1)	0.25 (4)	
Streptomycin	0	32	4	128	32	4	<2	16	

Antibiotic	TFLE	Tested MDR bacteria, MIC ($\mu\text{g}/\text{mL}$), and modulation factor (in brackets)							Modulation effect (%)
		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>E. aerogenes</i>	
		AG100	AG102	PA121	PA124	K2	KP55	EA298	
Tetracycline	MIC/8	8 (4)	4 (1)	64 (2)	16 (2)	4 (1)	<2 (na)	16 (1)	42.86
	0	32	64	128	32	128	256	128	
	MIC/8	8 (4)	32 (2)	64 (2)	16 (2)	64 (2)	256 (1)	16 (8)	85.71
Doxycyclin	0	128	16	128	64	64	128	64	
	MIC/8	64 (2)	4 (4)	128 (1)	2 (32)	8 (8)	128 (1)	4 (8)	71.43
	0	>256	>256	>256	256	>256	>256	>256	
Ampicillin	MIC/8	128 (>2)	128 (>2)	256 (>1)	16 (16)	>256 (na)	>256 (na)	>256 (na)	42.86
	0	8	64	64	64	128	64	64	
	MIC/8	4 (2)	64 (1)	64 (1)	32 (2)	32 (4)	256 (0.25)	64 (1)	42.86
Imipenem	0	64	64	32	32	32	64	32	
	MIC/8	16 (4)	32 (2)	128 (0.25)	8 (4)	16 (2)	32 (2)	64 (0.5)	71.43
	0	64	>256	>256	256	>256	128	>256	
Vancomycin	MIC/8	8 (8)	>256 (na)	>256 (na)	128 (2)	256 (>1)	64 (2)	256 (>1)	42.86

TFLE: *Talinum fruticosum* leaf extract; MIC: Minimum inhibitory concentration; na: not applicable; Bold values represent modulation factors ≥ 2 .

3.4. Interaction Effects Between Antibiotics and TFLE

The results of the interactions between antibiotics and TFLE against selected MDR bacteria showed that TFLE

displayed synergy effects ($\Sigma\text{FIC} < 0.5$) with kanamycin and doxycycline against *K. pneumoniae* K2 and *E. aerogenes* EA298. Moreover, additive effects ($0.5 < \Sigma\text{FIC} \leq 1$) were observed for the combination of TFLE with these antibiotics against *E. coli* AG102. No antagonist was observed for the tested combination (Table 4).

Table 4. Interaction effects between antibiotics and TFLE against selected MDR bacteria.

Antibiotic	Bacteria	Fractional inhibitory concentration		ΣFIC	Interpretation
		Antibiotic	Extract		
Kanamycin	<i>K. pneumoniae</i> K2	0.25	0.125	0.32	Synergy
	<i>E. aerogenes</i> EA298	0.25	0.06	0.31	Synergy
	<i>E. coli</i> AG102	0.5	0.03	0.53	Additivity
Doxycyclin	<i>K. pneumoniae</i> K2	0, 03	0.25	0.28	Synergy
	<i>E. aerogenes</i> EA298	0.01	0.12	0.13	Synergy
	<i>E. coli</i> AG102	0.03	0.5	0.53	Additivity

ΣFIC : Fractional Inhibitory concentration index.

4. Discussion

MDR bacteria have emerged as a significant global health issue [3, 41]. Consequently, it is imperative to find alternatives for the successful management of infections caused by MDR bacteria. Plants have diverse chemical structures and mechanisms of action, which may be valuable in developing new treatments against resistant infections. Many phytochemicals have demonstrated their potential as antimicrobial agents or modulators of conventional antibiotics [35, 42]. So, this study aimed at investigating the antibacterial activity of the methanol extract of *Talinum fruticosum* leaves and its interaction effects with antibiotics against MDR Gram-negative bacteria. The satisfying medicinal properties of herbal medicinal plants, such as their antimicrobial properties, are due to their secondary metabolites, such as phenols, terpenoids, alkaloids, flavonoids, monoterpenes, etc. [43]. In this study, we found that the tested extract contained these metabolite classes except alkaloids. A previous study identified alkaloids such as theophylline and ellipticine in this plant [44]. This absence may be due to the low sensitivity of the qualitative test used, which is not able to detect very low amounts of metabolites.

Based on the MIC cut-off values established by Kuete [35] for Enterobacteriaceae, and by Tankeo et al. [36] for *P. aeruginosa*, the TFLE was not active on the tested gram-negative bacteria except *E. coli* AG102 and ATCC10536, on which it presented average ($256 < \text{MIC} \leq 512 \mu\text{g/mL}$) and weak activities ($512 < \text{MIC} \leq 1024 \mu\text{g/mL}$), respectively. The resistance feature of the studied bacterial strains or a possible low quantity of bioactive ingredients in the extract could explain the lack or poor activity of this extract. In fact, it is commonly known that a plant extract's activity is influenced by the amounts of bioactive components it contains as well as any potential interactions with other constituents [45]. To the best of our knowledge, no study has been carried out on the extract of *T. fruticosum* against MDR gram-negative bacteria. Therefore, Ojo et al. [27] revealed low antimicrobial activity of silver nanoparticles from *T. fruticosum* against *C. albicans*, *E. coli*, and *S. aureus*, with zones of inhibition of 2.35 ± 0.64 mm for *S. aureus*, 2.55 ± 0.64 mm for *E. coli*, and 1.65 ± 0.07 mm for *Candida albicans*. Similarly, the silver nanoparticles synthesized using its aqueous leaf extract showed antimicrobial properties against some Gram-positive (*S. aureus* and *Bacillus subtilis*), Gram-negative (*Escherichia coli*, *Salmonella typhi*) bacteria, as well as the fungus *Candida albicans* [28]. This is in accordance with the findings obtained in this work.

Reversing resistance to counter antimicrobial resistance in the WHO's critical priority list of most dangerous pathogens appears today as one of the strategies to solve the problem of bacteria's resistance [41, 46]. It was demonstrated that various phytochemicals can function as adjuvants by enhancing the effectiveness of antibacterials [35, 42, 47]. In this study, it was

found that *T. fruticosum* leaf extract enhanced the activity of the tested antibiotics, especially tetracycline, doxycycline, imipenem, kanamycin, and ciprofloxacin against the tested MDR bacteria. Knowing the MDR feature of the tested bacteria, this finding suggests that some compounds present in this extract might act as inhibitors by blocking the resistance mechanisms, such as efflux pumps [49]. Several studies have shown that flavonoids [48] and triterpenes [49, 50] could potentiate the activity of antibiotics against resistant bacteria. So, this potentiating activity could be attributed to the presence of flavonoids and terpenoids found in the tested extract. Additionally, this extract showed synergy with doxycycline and kanamycin. This suggests that its phytochemicals may act on different bacterial targets than the tested antibiotics. So, the possible modes of action of TFLE may include alteration in pH, DNA fragmentation, inhibition of bacterial gene expression, ion binding, free radical formation, etc. [51].

5. Conclusion

Globally, this study suggested that *T. fruticosum* leaf methanol extract in combination with antibiotics could lead to the development of new treatment options for infections caused by MDR bacteria. Future research regarding the identification of a prospective nontoxic antibiotic modulator should be done. As *T. fruticosum* is currently used in many parts of the world as an edible plant, we can encourage its consumption as a complementary medicine in patients suffering from infections due to MDR bacteria. Therefore, the toxicity and *in vivo* studies of this extract should be envisaged.

Abbreviations

AMR	Antimicrobial Resistance
DMSO	Dimethyl Sulfoxide
FIC	Fractional Inhibitory Concentration
INT	p-Iodonitrotetrazolium Chloride
MBC	Minimum Bactericidal Concentration
MDR	Multidrug-Resistant
MF	Modulation Factor
MHB	Mueller-Hinton Broth
MIC	Minimum Inhibitory Concentration
RND	Resistance Nodulation Cell Division
TFLE	<i>Talinum fruticosum</i> Leaf Extract

Supplementary Material

The supplementary material can be accessed at <https://doi.org/10.11648/j.jdmp.20261201.12>

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Data Availability Statement

The data supporting the findings of this study are available within the article.

Conflicts of Interest

The authors report no conflicts of interest.

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