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Spectrophotometric Detection of Beta-Lactamase Inhibition by Plant Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EA, IW, WM and SF were responsible for the conception and the design of the study, wrote the protocol. Authors EA and SF supervised the data collection, the execution of the experiments and wrote the manuscript. Author WM participated to the data management and the literature review. All authors read and approved the final manuscript.

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ABSTRACT

Background: Antibiotic-resistant bacteria have been spreading with increasing frequency over the past several decades. The decreasing effectiveness of therapies in treating bacterial resistance, due to the production of beta-lactamase (EC.3.5.2.6), has led to combining beta-lactamase inhibitors with commonly used beta-lactam antibiotics. Phytochemical compounds produced by plants have varied biological properties such as antioxidant, antimicrobial, antidiabetic, anti-

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inflammatory, anticancer, antihypertensive and others. Polyphenolics, flavonoids, coumarins, alkaloids, tannins, steroids and terpenoids are the main phytochemicals found in plants extracts.
This study aims to assess the inhibitory activity of *Annona senegalensis Pers.*, *Terminalia superba Engl. & Diels*, *Ipomoea batatas Lam.*, and *Psidium guajava L.* against a *Bacillus cereus* beta-lactamase and four beta-lactamases extracted from clinical isolates of *Escherichia coli*.
Methods: Beta-lactamase inhibition was measured using a spectrophotometric assay with benzylpenicillin as the substrate, by a modified acidimetric method. Clavulanic acid was used as the standard beta-lactamase inhibitor. Subsequently, the inhibition of the hydrolysis of other commonly used beta-lactam antibiotics was assessed using a UV spectrophotometric method.
Results: Among the tested extracts, *Ipomoea batatas* showed no effect on the beta-lactamases, whereas *Terminalia superba* and *Annona senegalensis* exhibited inhibitory activity against all the beta-lactamases. *Psidium guajava* inhibited two of the five tested enzymes. The highest percentage of inhibition was observed with *Terminalia superba* on the beta-lactamase of *Bacillus cereus*, reaching 75.73%. Regarding the combination of beta-lactam antibiotics with plant extracts, the combination of *Terminalia superba* and ceftriaxone showed the highest inhibition, with a percentage of 64.90%. The combination of *Annona senegalensis* and ceftazidime was the least effective, with an inhibition rate of only 12.95%.
Conclusion: Two of the tested ethanolic plant extracts showed interesting beta-lactamase inhibitory effects and could contribute to the development of more effective therapies against beta-lactamase-producing bacteria by combining natural inhibitors from plants with existing beta-lactam antibiotics.

Keywords: Beta-lactamase inhibitors; plant extracts; extended spectrum beta-lactamase; Bacillus cereus; Escherichia coli.

1. INTRODUCTION

Antibiotic resistance is a major public health concern worldwide. Soon after the introduction of penicillin, the emergence of resistant microorganisms was observed. Since then, this problem has steadily increased and become a challenge for scientists in industrialized and developing countries. Beta-lactams are the most widely used class of antibiotics due to their broad spectrum of activity and good safety profile (Bush and Bradford 2016). Their widespread use is associated with cases of misuse and administration at levels lower than those recommended in treatment guidelines. This leads to resistance in both Gram-positive and Gram-negative bacteria, primarily through the production of beta-lactamases, and additionally through reduced affinity and susceptibility of Penicillin-Binding Proteins (PBPs) in Gram-positive bacteria (Bush and Bradford 2020, Jubeh et al. 2020). Beta-lactamases are hydrolytic enzymes that catalyze the opening of the beta-lactam ring, resulting in the inactivation of the antibiotic, which can no longer interact with its target, the PBPs (Oelschlaeger 2021).

A decrease in the search for new antibiotics has been observed over the past few decades, in contrast to the growing need for drugs to treat multidrug-resistant bacteria (Hutchings 2019, Miethke et al. 2021). Authors have pointed out that the multiresistant nature of pathogens, the various resistance mechanisms, and financial constraints make the development of new antibiotics extremely difficult (Jubeh et al. 2021, Miethke et al. 2021). Since the late 1980s, beta-lactamase inhibitors have been successfully used with current beta-lactams to counter the threat of beta-lactamase-producing bacteria. Augmentin, a well-known brand name containing a combination of amoxicillin and clavulanate, is one of the best examples of this approach. However, soon after introducing beta-lactamase inhibitors, inhibitor-resistant TEM beta-lactamases (IRT) emerged, prompting the search for new inhibitors, including those derived from plants. Among beta-lactamases, penicillinases hydrolyze beta-lactam antibiotics of the first and second generations, while enzymes called Extended Spectrum Beta-Lactamases (ESBLs) inactivate third-generation and higher beta-lactam antibiotics "(Bush and Bradford 2020, Oelschlaeger 2021).

Natural products that have historically served as antimicrobials are regaining increased interest due to the threats of antimicrobial resistance (Moloney 2016). Therefore, beta-lactamase inhibitors of plant origin represent a promising approach to reducing antibiotic resistance. They can be co-administered with existing beta-

lactams, thereby broadening therapeutic possibilities. Studies conducted in our laboratory, as well as those by other researchers, have demonstrated the antibacterial activities of the plants investigated in this study (Ani et al. 2023, Koggie et al. 2022). This work aims to evaluate the beta-lactamase inhibitory potential of ethanolic extracts from *Annona senegalensis*, *Ipomoea batatas*, *Psidium guajava*, and *Terminalia superba*, and their association with commonly used beta-lactam antibiotics.

2. MATERIALS AND METHODS

2.1 Bacterial Strains

Four beta-lactamases (two penicillinases and two ESBLs) producing Escherichia coli clinical isolates, collected during previous studies in our laboratory were selected as Gram-negative bacteria. Cultures of these bacterial strains, preserved at -80°C in tryptic soy broth (Biomerieux, France) with 20% glycerol, were used for beta-lactamase extraction. The beta-lactamase from *Bacillus cereus* (HiMedia, India) was used to represent Gram-positive strains. The antibiotic susceptibility profiles of the E. coli strains used are summarized in Table 1.

2.2 Plant Material

The leaves of *Annona senegalensis* (A. *senegalensis), Terminalia superba* (*T. superba*), *Ipomoea batatas* (*I. batatas),* and the stem bark of *Psidium guajava* (*P. guajava*) were collected for this study. They were harvested in Abomey-Calavi, southern Benin. Samples were identified at the Benin National Herbarium, University of Abomey-Calavi, where voucher specimens of each plant were deposited with the following numbers: *A.* senegalensis - YH626/HNB, *T.* superba - YH623/HNB, *I. batatas* - YH624/HNB, and *P. guajava* - YH625/HNB.

2.3 Preparation of Plants Extracts

To 100 g of each powdered plant material, 500 mL of ethanol was added. The mixture was incubated with shaking at 37°C for 72 hours. The macerate was filtered first through hydrophilic cotton, and then through Whatman filter paper. The supernatant was evaporated using a rotary evaporator (IKA HB10S40, Germany) under reduced pressure. The resulting extracts were collected in vials and stored at 4°C.

2.4 Preparation of Crude Beta-Lactamase Extracts

Sonication was combined with a chemical method to disrupt the cell membrane and collect the beta-lactamase. The lysis buffer containing 5 mmol/L EDTA and 0.6% Triton X-100 in 10 mmol/L phosphate buffer at pH 8, was prepared based on the method of Hadi *et al*. with minor modifications (Hadi and Ali 2022).

For the overnight culture, a single colony of *E. coli* was transferred into 5 mL of Luria-Bertani (LB) liquid medium. The suspension was homogenized and incubated under shaking conditions at 37°C. A quantity of 1 mL of the overnight culture was used to inoculate 300 mL of fresh LB liquid medium. The culture was then incubated on a shaker (250 rpm) at 37°C for 4 hours to allow bacterial growth. Subsequently, the bacterial cells were harvested by centrifugation at 4000 rpm for 15 minutes at 4°C and washed twice with 10 mmol/L Na_2HPO_4/KH_2PO_4 (pH 8.0). The lysis buffer was added, and the suspension was incubated on ice for 30 minutes. The bacterial cells were disrupted by sonication for 1 minute, and the cell debris was removed by centrifugation at 7000 g for 20 minutes. Aliquots of the supernatant, containing the crude beta-lactamase, were stored at -20°C.

2.5 Detection of Benzylpenicillin Hydrolysis and Beta-Lactamase Inhibition by Plant Extracts

The hydrolysis of β-lactam was determined by the acidimetric method using benzylpenicillin as the substrate (Ravi et al. 2018). The hydrolysis of benzylpenicillin (penicillin G) leads to the formation of penicilloate, which results in the acidification of the reaction medium, accompanied by a color change of the phenol red indicator. The gradual disappearance of the fuchsia color of phenol red, following the formation of penicilloic acid, was monitored at 578 nm.

To prepare the substrate reagent, which was the indicator solution, 1,000,000 units of benzylpenicillin were dissolved in 1 mL of phosphate buffer. A few drops of 1N NaOH solution were added to adjust the pH to 8. The mixture turned fuchsia after the addition of 300 µL of 0.3% phenol red.

For the detection of benzylpenicillin hydrolysis, 150 µL of the prepared indicator solution was added to 200 µL of the crude beta-lactamase (penicillinase or ESBL) diluted in 800 µL of phosphate buffer, pH 8. To measure beta-lactamase inhibition, 200 µL of the crude enzyme extract was added to 760 µL of phosphate buffer solution, pH 8, and 40 µL of plant extract at a concentration of 100 mg/mL. The mixture was then incubated for one hour at 37°C to allow the potential inhibitors to act on the beta-lactamase. After one hour, 150 µL of the prepared indicator solution with benzylpenicillin substrate was added. The kinetics of the reaction were recorded at 578 nm for five minutes using a Kenza Max spectrophotometer (Biolabo, 02160 Maizy, France).

Table 1. Antibiotic susceptibility profile of the *E. coli* strains used

	TEM strains		SHV strains	
Antibiotics	**ESBL 01**	**Peni 01**	**ESBL 02**	**Peni 02**
Amoxicillin	R	R	R	R
Amoxicillin + clavulanic acid	R	R	R	I
Cefalotin	R	I	S	S
Ceftriaxone	R	S	S	S
Aztreonam	R	S	R	I
Cefotaxime	R	S	R	S
Imipenem	S	S	S	S
Doxycycline	R	R	R	S
Netilmicin	R	S	R	R
Gentamicin	R	S	R	S
Ciprofloxacin	S	S	R	R
Ofloxacin	S	S	R	I
Nalidixic acid	S	S	R	R
Sulfamethoxazole /Trimethoprim	R	R	R	R

Peni: Penicillinase; ESBL: extended-spectrum beta-lactamase; R: resistant; I: intermediate; S: sensitive

Clavulanic acid (1.20 mg/ml) was used as the reference molecule and considered as the positive control. The inhibition of beta-lactamase was expressed as the variation in optical density. The percentage of inhibition of the beta-lactamases was calculated as follows:

$$\frac{\Delta DO\ of\ sample\ without\ plant\ extract - \Delta\ OD\ of\ the\ sample\ with\ plant\ extract}{\Delta DO\ of\ sample\ without\ plant\ extract}$$

2.6 Determination of the Synergistic Effect of Plant Extracts and Beta-Lactams

These tests were performed to determine the effects of plant extracts on the hydrolysis of third-generation beta-lactam antibiotics, ceftriaxone and ceftazidime, by crude beta-lactamase extracts. For this purpose, extracts of *Annona senegalensis* and *Terminalia superba*, which displayed good inhibitory effects in the previous inhibition tests, were used to inhibit the two ESBLs of the TEM and SHV types, whose antibiotic susceptibility profiles are indicated in Table 1. Beta-lactamase inhibition was measured according to the method described by Chandar *et al*. with minor modifications (Chandar et al. 2017).

The reaction mixture consisted of 30 µL of crude beta-lactamase extract, to which plant extract was added at a concentration of 5 mg/mL. The mixture was incubated for 1 hour at 37°C. At the end of the incubation, 20 µL of ceftriaxone or ceftazidime was added, and the reaction medium was monitored with a spectrophotometer at 254 nm. Clavulanic acid served as the reference molecule. In the absence of plant extracts, the concentration of the antibiotic may decrease over time. The synergistic effect between the tested antibiotic and plant extracts would result in a reduction in the hydrolysis of the antibiotic by beta-lactamase. The variation in optical density was compared with that of the control (without plant extracts) to determine the inhibition rate, which was calculated every five minutes of incubation over a period of thirty minutes. The percentage of inhibition was determined as follows:

$$\frac{\Delta DO\ of\ sample\ without\ plant\ extract - \Delta\ OD\ of\ the\ sample\ with\ plant\ extract}{\Delta DO\ of\ sample\ without\ plant\ extract}$$

2.7 Statistical Analysis

Statistical analysis of the data was performed using Microsoft Excel 2016. The Tukey test was used as a post-hoc analysis to compare the inhibition percentages. Quantitative variables are presented as average percentages. A *P*-value of ≤ 0.05 was considered statistically significant.

3. RESULTS

3.1 Detection of Benzylpenicillin Hydrolysis by the Four Crude Beta-Lactamase Extracts

Fig. 1 shows the kinetics of benzylpenicillin hydrolysis by the four crude beta-lactamase extracts, two of the TEM type (graph at left) and two of the SHV type (graph at the right side). The plots for penicillinases (indicated as S+TEM penicillinase and S+SHV penicillinase) and cephalosporinases (indicated as S+TEM ESBL and S+SHV ESBL) showed decreasing kinetics. The data from the reaction medium with the substrate reagent indicated that no spontaneous hydrolysis of benzylpenicillin occurred during the reaction monitoring period.

3.2 Beta-Lactamase Inhibition by Plant Extracts

According to the results summarized in Table 2, the plant extracts showed varying effects on the five tested beta-lactamases. *Annona senegalensis* and *Terminalia superba* exhibited inhibitory effects on all the tested beta-lactamases. A reduction in the hydrolytic activity of two beta-lactamases was observed with *Psidium guajava*, while *Ipomoea batatas* showed no inhibitory effect. The highest inhibition rate (76.08%) was observed with clavulanic acid on *Escherichia coli* TEM penicillinase, while the lowest (12.04%) was recorded for *Annona senegalensis* on *Bacillus cereus* beta-lactamase.

When comparing the four crude extracts of *E. coli* beta-lactamases, the inhibition of penicillinase was generally higher than that of ESBLs, except the very low inhibition rate of SHV penicillinase by *Terminalia superba*. Clavulanic acid exhibited inhibition rates that were sometimes stronger and other times weaker than those of the plant extracts tested.

3.3 Inhibition of Ceftriaxone and Ceftazidime Hydrolysis by Plant Extracts

The results of the capacity of *Terminalia superba* and *Annona senegalensis* extracts to inhibit the hydrolytic activity of one TEM-type and one SHV-type ESBLs, are reported in Table 3. The search for synergistic action between plant extract beta-lactamase inhibitors and third-generation cephalosporins was conducted by determining the degree of inhibition of ceftriaxone and ceftazidime hydrolysis by the extracts of *Terminalia superba* and *Annona senegalensis*. Both extracts inhibited the hydrolysis of benzylpenicillin by all five tested beta-lactamases in the previous experiment (Table 2). Overall, an increase in the percentage of inhibition of ceftriaxone and ceftazidime hydrolysis was observed, depending on the incubation time of the plant extract with the enzyme.

The highest inhibition percentage was exhibited by the *Terminalia superba* extract against the hydrolysis of ceftriaxone by the TEM ESBL, although it remained lower than that of clavulanic acid.

Table 2. Inhibition of 4 crude extracts of *Escherichia coli* beta-lactamases and beta-lactamase of *Bacillus cereus*

Beta-lactamases	Percentage of inhibition (%)				
	Plant extracts				Control
	A. sen	***I. bat***	***P. gua***	***T. sup***	**Clavulanic acid**
TEM Penicillinase	60.13^{a}	0^{b}	50.47^{c}	68.44^{d}	76.08^{e}
TEM ESBL	36.46^{a}	0^{b}	0^{b}	74.4^{c}	59.92^{d}
SHV Penicillinase	52.63^{a}	0^{b}	0^{b}	13.91^{d}	50^{e}
SHV ESBL	35.8^{a}	0^{b}	0^{b}	44.89^{c}	72.99^{d}
Beta-lactamase of *Bacillus c.*	43.69^{a}	0^{c}	12.04^{d}	75.73^{b}	56.32^{e}

Values are expressed as mean (n = 3). Bacillus c.: Bacillus cereus; A. Sen: Anonna senegalensis; I.bat: Ipomoea batatas; P.gua: Psidium guajava; T.sup: Terminalia superba. Values in the line followed by a different letter superscript (a-e) are significantly different (P = 0.05) and values having the same superscript are not statistically significant.

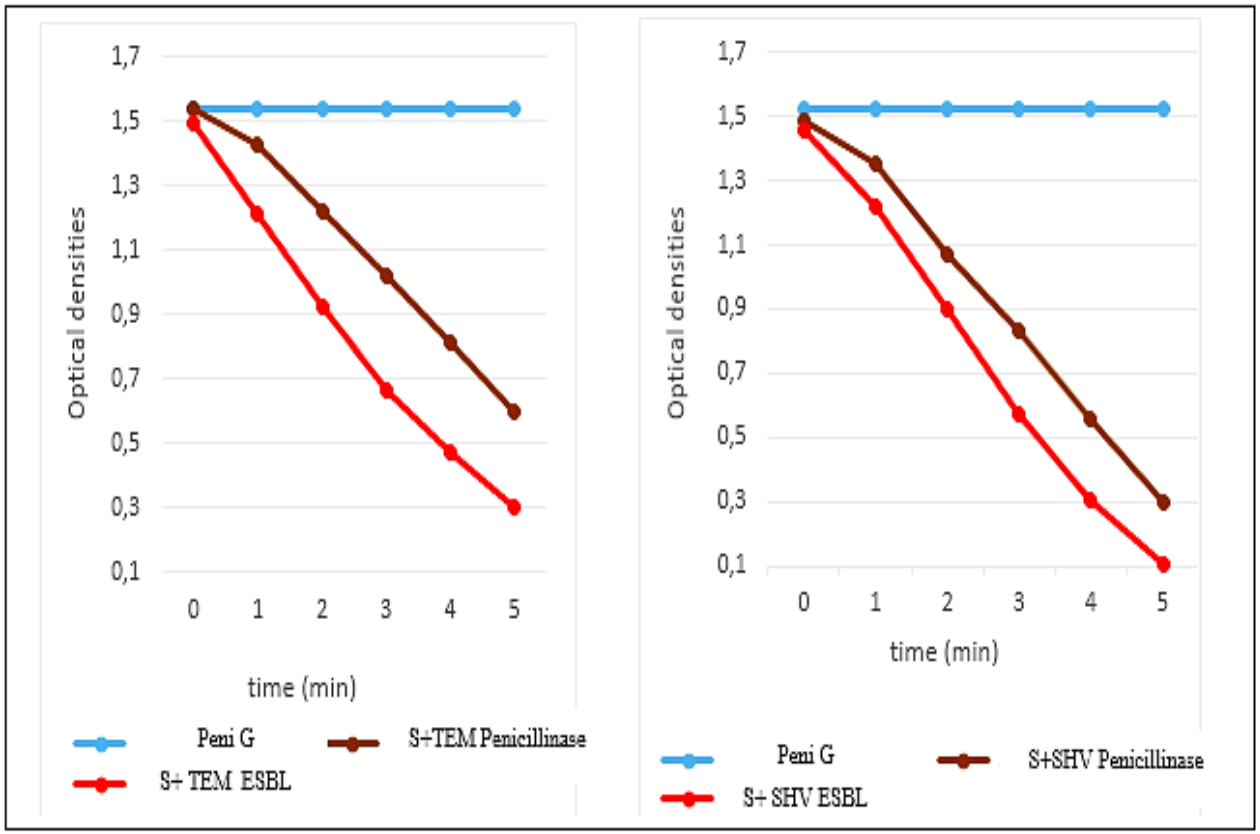

Fig. 1. Hydrolysis of benzylpenicillin by the crude extracts of *E. coli* beta-lactamases

Peni G: the reaction medium with the substrate benzylpenicillin and phenol red; S+TEM penicillinase: reaction medium with the substrate benzylpenicillin, phenol red and TEM penicillinase. S+TEM ESBL is the reaction medium containing the substrate benzylpenicillin with TEM–ESBL crude extract. Similarly, two reaction mediums with SHV penicillinase and SHV ESBL were analysed.

Table 3. Percentage of inhibition of beta-lactamase activity of two *E. coli* ESBLs

Reaction medium	Percentage of inhibition (%)					
	5 min	10 min	15 min	20 min	25 min	30 min
TEM ESBL + An. s + CAZ	1.24	3.97	6.72	10.3	12.7	12.95
TEM ESBL + Ter. s + CAZ	5.91	7.45	9.15	10.61	13.04	14.82
SHV ESBL + An. s + CRO	2.51	6.37	10.61	14.47	18.22	23.48
TEM ESBL + Ter. s + CRO	54.2	56	58.8	61.6	63.5	64.9
TEM ESBL + CA + CAZ	28.26	40.59	52.28	60.20	70.58	88.02
SHV ESBL + CA + CRO	25.12	32.52	42.4	56.78	60.23	76.23

CA (clavulanic acid), ESBL (extended spectrum beta-lactamase), An. s (Annona senegalensis), Ter. s (Terminalia superba), CRO (Ceftriaxone), CAZ (ceftazidime).

4. DISCUSSION

Ethanolic extracts of four antibacterial plants were evaluated for their inhibitory activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Bacillus cereus*) beta-lactamases. The production of penicillinase and ESBLs was investigated using the acidimetric method. The chromogenic method with nitrocefin, the iodometric method using starch, and the acidimetric method are among the most common, simple, and affordable biochemical techniques for detecting beta-lactamase activity (Ravi et al. 2018). In this study, the acidimetric method was adapted for spectrophotometric detection.

Terminalia superba extract exerted the highest inhibition rate toward *Bacillus cereus* beta-lactamase, even higher than that of clavulanate. *Terminalia superba and Annona senegalensis* inhibited the hydrolysis of benzylpenicillin by all five beta-lactamases, including those extracted from *Escherichia coli* clinical isolates. This result demonstrates the presence of beta-lactamase inhibitors in the plant extracts of *Terminalia superba, Annona senegalensis, and Psidium guajava.* Similar findings were reported by Arora in a study on two other *Terminalia* species. The author showed the inhibition of beta-lactamase activity from *E. coli* clinical isolates by *Terminalia chebula and Terminalia bellerica* (Arora and Nandi 2017).

Inhibition tests of two cephalosporinases with the beta-lactams ceftazidime and cefotaxime, using UV spectrophotometry, complement and strengthen the findings from other qualitative and quantitative tests for the presence of beta-lactamase inhibitor active ingredients in plant extracts with antibacterial activity. Spectrophotometric methods on enzyme extracts offer greater precision than qualitative biochemical methods on bacterial suspensions, as they can detect beta-lactamase inhibition even when hydrolysis is minimal or when the plant extract color tends toward red or brown. The UV spectrophotometry method is especially useful for plant extracts with high activity, as they exhibit significant inhibition at very low concentrations. Additionally, UV spectrophotometry requires a lower concentration of the plant extract compared to the other three methods tested, due to the plant extracts' absorption in the ultraviolet range.

In this study, the lack of inhibition of beta-lactamases by the plant extracts may be due to the presence of beta-lactamases resistant to so-called IRT inhibitors. These beta-lactamases, through gene mutations, generate complex mutants (CMT enzymes) that exhibit resistance to cephalosporins, thereby affecting the affinity between the beta-lactam substrate and the beta-lactamase enzyme inhibitor (Canton 2008).

The detection of clavulanic acid in the extract of *Rumex vesicarius* against the beta-lactamase of *Pseudomonas aeruginosa* was demonstrated by (Al Sahli et al. 2011, Yang et al. 2010) showed that the extract of *Fissistigma cavaleriei* possesses inhibitory activity against beta-lactamase. (Al-Hayanni and El-Shora 2021) investigated various extracts from medicinal plants as inhibitors of beta-lactamase activity and found that beta-lactamase from *Staphylococcus sciuri* and *Klebsiella pneumoniae* were inhibited by extracts from *Eucalyptus camaldulensis* and *Schinus terebinthifolius*.

The results revealed synergy between common antibiotics and the inhibitory potential found in the organs of two plants with antibacterial activity. These findings showed an interaction between the beta-lactamase inhibitors present in each of the two plants tested and ceftazidime, a third-generation beta-lactam antibiotic. Previous studies have demonstrated the inhibitory effects of these plants on the growth of clinical strains, which may partly explain their effectiveness.

Inhibition of beta-lactamases by the inhibitors in plant extracts restored the activity of beta-lactam antibiotics against the growth of the strains. However, the effectiveness of these plants depends not only on the content of beta-lactamase inhibitors but also on the type of beta-lactamases produced by the clinical strains tested. Additionally, other unidentified antibiotics may also be present in these plant extracts.

There have been numerous reports showing that not only isolated pure natural products but also plant extracts exhibit strong synergistic effects with antibiotics (Li et al. 2023). The active constituents of *Scutellaria baicalensis*, when combined with penicillin, demonstrated potent synergistic activity against penicillinase-producing *MRSA* in vitro (Qian et al. 2015) Alkaloid compounds from *Cienfuegosia digitata* combined with β-lactams also exhibited strong activity against *MRSA*, likely through the inhibition of β-lactamase (Konaté et al. 2012).

Flavan-3-ols, epicatechin gallate, guttiferone-A, 4-butanylanisole, épigallocatechin gallate, galangin are natural compounds that have been shown to inhibit β-lactamase activity, thereby preventing the hydrolysis of β-lactam antibiotics (Boussoualim et al. 2017, Khameneh et al. 2021). Several authors have reported the presence of tannins and flavonoids in the plants used in this study (Anago 2009, Biswas et al. 2013, Diallo et al. 2022, Gbogbo et al. 2013, Angaman et al. 2020). Among the bioactive molecules in medicinal plants, β-lactamase inhibition properties are often linked to alkaloids, tannins, flavonoids, and polyphenols (Boussoualim et al. 2017). Houchi's work demonstrated the inhibition of *Pseudomonas aeruginosa* beta-lactamase by morin hydrate, myricetin, and rutin trihydrate, three commercially available synthetic flavonoids (Houchi 2014). All the plants used in this study contain flavonoids, which may be linked to the observed beta-lactamase inhibition by the plant extracts tested. The ethanolic extracts of *Annona senegalensis* leaves, *Terminalia superba* leaves, and *Psidium guajava* bark, which are rich in polyphenolic compounds, are likely responsible for the beta-lactamase inhibitory activity noted. However, *Ipomoea batatas*, which also contains these phytochemical compounds, did not exhibit any beta-lactamase inhibitory activity in either the strain tests or the enzyme extractions. It should be emphasized, however, that the small number of five enzymes tested does not allow for a definitive exclusion of a potential beta-lactamase inhibitory effect from this plant.

More than a thousand phytochemicals have been discovered to date. Major phytochemicals founded in plants are carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides (Kumar et al. 2023). Recent works based on in vitro and computational studies revealed quercetin, taxifolin, myricetin, luteolin, and miquelianin as potential inhibitors against SHV1, TEM1, KPC2 and CTX-M-27 (Kongkham et al. 2022). Beta-lactam inhibitors continue to be a current topic of research because they allow use of existing beta-lactam antibiotics by combination with these.

5. CONCLUSION

The present study demonstrated that three plant extracts exhibited potent beta-lactamase inhibitory activity, with two plant extracts showing efficacy against ESBLs from *Escherichia coli* and three extracts targeting penicillinase from *Bacillus cereus*. A synergy of action between common antibiotics and the beta-lactamase inhibitors present in two of the plants was revealed. The results indicated an interaction between the beta-lactamase inhibitors in these plants and ceftazidime, a third-generation beta-lactam antibiotic. The observed effects suggest the potential for combination therapy involving antibiotics and plant extracts to combat beta-lactamase-mediated resistance. These plants could contribute to the management of infectious diseases. However, further studies are needed to isolate the active compounds responsible for this activity. Given that the introduction of new beta-lactams is often followed by the emergence of new beta-lactamases, the discovery of novel beta-lactamase inhibitors remains essential.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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