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Antibacterial activity of methanol bark extract from *Acacia dudgeoni* craib. ex holl (Mimosaceae) on growth of cefotaxime resistant *Escherichia coli*

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ABSTRACT

The development of bacterial resistance to available antibiotics is a worldwide problem and recent researches focused on the development of new strategies to combat this bacterial resistance. The current study was designed to evaluate the inhibitory property of the methanol bark extract from *Acacia dudgeoni* on cefotaxime resistant growth of *Escherichia coli.* The capacity of extract to inhibit the *E. coli* growth was evaluated *in vitro* by using agar diffusion assay. The free radical scavenging property of extract was assessed by using the standard DPPH assay. Furthermore, flavonoids and phenolic contents of extract were determined spectrophotometrically. Extract at concentration of 50 µg/mL showed an interesting inhibitory activity on *E. coli* strain 376 growth that is resistant to the antibiotic cefotaxime. Moreover, extract exhibited a good DPPH scavenging activity with IC_{50} value ranged from 16.52 ± 1.04 µg/mL. The flavonoid and phenolic contents of extract were 7.31 ± 0.2 mg EQ/g of extract and 14.25 ± 1.25 mg EAG / g extract respectively. The methanol bark extract from *A. dudgeoni* is a potent source of bioactive compounds to combat bacterial resistance.

Keywords: *Acacia dudgeoni*, cefotaximase, Bacterial resistance, *Escherichia coli*

1. INTRODUCTION

Bacterial resistance is a worldwide crisis and is responsible for more than 70% of deaths caused by pathogenic microorganisms [1]. According to WHO [2], bacterial resistance causes more than 560 000 annual deaths corresponding to the 1/5th of the 2.7 million annual neonatal deaths in the world. The use of antibiotic was the main and exclusive option for the treatment of infectious diseases [2]. However, the development of antibiotic resistance generates serious consequences for antibiotic therapy and severely complicates treatment options. The bacterial resistance mechanisms include the alteration of the membrane receptors, the decrease of the membrane permeability reducing the penetration of the antibiotic into the cytosol and the increase of the bacterial enzymes concentration that become more effective for antibiotics inactivation[3]. β-lactamases are enzymes produced by certain bacteria and are responsible for the development of antibiotic resistance [4]. β-lactams are the main class of antibiotics frequently used in modern medicine because of their low toxicity and their effectiveness to common human pathogens [4]. Unfortunately, most bacteria with β- lactamases had developed resistance to the available β-lactam antibiotics [5].In front of this complex situation, new strategies must be developed to resolve this startling situation. Recent researches have been developed to attenuate bacterial resistance by inhibiting bacterial

enzymes activity or production [6, 7]. In Burkina Faso, medicinal plants plays a crucial role in the ethno-medicine and represents an alternation solution for the treatment of infectious diseases [8]. *A. dudgeon* is frequently used in the folklore medicine in Burkina Faso to treat several diseases. The roots are used against snake bites while pods are used in the treatment of tract respiratory diseases. The decoction of the barks are used in the treatment of diarrhea and childhood dysentery[8]. However, the properties of the bark to inhibit cefotaximase produced by *E. coli* are less documented.

This present investigation was designed to evaluate the capacity of the methanol bark extract from *A. dudgeoni* to inhibit the growth of the β-lactamine resistant *E. coli.* Furthermore, the antioxidant potent of extract as well as its flavonoid and phenolic contents were investigated *in vitro*.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals were from analytical grade. Gallic acid, quercetin, dimethyl sulfoxide (DMSO), folin-ciocalteu, sodium carbonate, aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Muller Hinton agar were purchased from Sigma–Aldrich (St. Louis, USA), cefotaxime was purchased from Reyoung Pharmaceutical Co., Ltd (Shandong, China).. Methanol was purchased from Prolabo (Paris, France)

2.2 Bacterial strains

The bacterial strains used for the study were Gram-negative bacteria that had at least third-generation cephalosporin (C3G) resistance. These strains were isolated in the stools of hospitalized or non-hospitalized children aged less than two years between July 2010 and March 2012 at the University Hospital center Pediatric Charles De Gaulle (Ouagadougou, Burkina Faso). The origin and the characteristics of the different strains are indicated in the Table 1.

Table 1: Origin and characteristics of *E. coli* strains

Num. strains	Children sex	Services	β-lactamase enzymes		
			Cefotaximase	sulfhydryl variable	TEM-1 enzyme
376	Female	Infectious diseases	Positive	Negative	Positive
1184	Female	Infant	Positive	Positive	Positive
672	Female	Surgery	Positive	Negative	Negative
796	Female	Infant	Positive	Negative	Positive
679	Male	Big Child	Positive	Negative	Negative
1188	Male	Infectious disease	Positive	Negative	Negative

2.3 Plant material and extraction

The barks of *Acacia dudgeoni* were purchased from the herbalists in the Naabi-yaar market of Ouagadougou (Burkina Faso) in May 2017. The barks were powdered and 25gof the powder were extracted in methanol by maceration (24h, 25°C, continuous stirring). Extract was filtrated, concentrated to dryness in a vacuum evaporator and stored at 4°C for further investigations.

2.4 Antibacterial activity of extract

The capacity of extract to inhibit the growth of cefotaxime resistant *E coli* was performed as describe previously [9].

To determine the concentration of cefotaxime that does not affect the bacteria growth, *E. coli* strain was incubated for 24h with different concentrations of cefotaxime (12.5µg/mL, 25µg/mL and 50µg/mL) in petri plates containing Mueller Hinton medium, and the bacteria growth was observed. Furthermore, to determine the potentiality of extract to inhibit the growth of *E. coli* cefotaxime resistant, bacteria strain was incubated both with extract at different concentrations and a non-bactericidal concentration of cefotaxime, and the bacterial growth was compared to cefotaxime treatment alone.

2.5 Phytochemical screening

The phenolic content of the methanol bark extract from *A. dudgeoni* was determined followed by the method described previously [10]. Twenty five (25) microliter of extract (0.1 mg / mL)was mixed with 12.5 µL of the FCR solution (0.2 N). The mixture was incubated for 5 min at room temperature, and 100 µL of sodium carbonate (75 g / L) were added. After incubation in the dark for 1h, the absorbance was measured at 760 nm. Gallic acid (0-100 µg/mL) was used to plot a standard curve (y = 201x -21, 22; R^2>0.99; p<0.0001) and data were expressed in mg Equivalent Gallic Acid /g (mg EGA/g) of extract.

The flavonoid content of extract was determined by using the standard colorimetric method as described by Compaoré *et al.* [10]. For the experiment, 100 µL of extract (10 mg/mL in methanol) were mixed with the same volume of aluminum chloride (2% in methanol). The blank containing extract without aluminum chloride was also maintained. After incubation for 10 min in the dark at room temperature; absorbance was measured at 415 nm. Quercetin (0-100 µg/mL) was used to generate a standard curve (y = 39.8x - 3.5; R^2 = 0.99; p<0.0001) and the results were expressed in mg Equivalent Quercetin / g (mg EQ / g)of extract.

2.6 Anti-radical activity

The antioxidant activity of the methanol bark extract from *A. dudgeoni* was evaluated by using the DPPH method as described by Rouamba *et al.*[11]. Briefly, 100 µL of sample at different concentrations were

mixed with 200 µL of DPPH (20 mg / L in methanol). After 15 minutes of incubation in the dark, the absorbance was measured at 517 nm using a 96-microplate reader. Inhibition percentage of DPPH was calculated for each tested concentration and concentration of extract that scavenge 50% of DPPH radical (IC_{50}) was determined using the logarithm regression ($y=15.74\ln x+113.9$; $R^2=0.81$; $p<0.0001$).

2.7 Statistical analysis

Experiments were performed in triplicate and data were presented as mean ± SD. The graphs were drawn by using Microsoft Excel software and statistical analyzes were carried out using Graph Pad Prism 5 software. Let test was used to compare the statistical difference between extract and standard compound antioxidant activity. *P* value <0.05 was considered as being significant.

3. RESULTS AND DISCUSSION

3.1 Antibacterial activity

To determine the concentration of cefotaxime that doesn't affect the bacterial growth, bacteria strains were incubated with different concentrations of cefotaxime and bacterial sensitivity to the antibiotic was recorded (table 2). According to data, bacteria strains 796, 1188 and 1184 were resistance to cefotaxime at concentration 12.5 µg/mL while bacteria strains 376, 672 and 679 were resistant to all tested concentrations of cefotaxime. For further investigations, a non-bactericidal concentration of cefotaxime for each bacteria strain was used.

Table 2: Strains sensitivity to cefotaxime

E. coli	Cefotaxime concentration (µg / mL)		
	12.5	25	50
796	Resistant	Sensitive	Sensitive
1188	Resistant	Sensitive	Sensitive
1184	Resistant	Sensitive	Sensitive
376	Resistant	Resistant	Resistant
672	Resistant	Resistant	Resistant
679	Resistant	Resistant	Resistant

To assess the potentiality of extract to inhibit the growth of *E. coli* cefotaxime resistant, bacteria strains were incubated both with the extract at different concentrations and the non-bactericidal concentration of cefotaxime, and the sensitivity of bacteria strains to the cefotaxime was analyzed (table 3). Extract at concentration of 25µg/mL did not inhibit the growth of all bacteria strains. However, extract at 50µg/mL inhibited the growth of the bacteria strain 376 suggesting that extract inhibited the cefotaximase produced by this bacteria strain and hereby restored the effectiveness of the antibiotic cefotaxime.

Table 3: Inhibitory activity of the extract on *E. coli* growth

Bacteria strains + Cefotaxime (CTX)	*Acacia dudgeoni* extract (µg / mL)	
	25	50
E. coli 796 + CTX 12.5 µg/mL	Non active	Non active
E. coli 1188 + CTX 12.5 µg/mL	Non active	Non active
E. coli 1184 + CTX 12.5 µg/mL	Non active	Non active
E. coli 376 + CTX 50 µg/mL	Non active	Active
E. coli 672 + CTX 50 µg/mL	Non active	Non active
E. coli 679 + CTX 50 µg/mL	Non active	Non active

The sensitivity of the bacteria strain 376 to the cefotaxime prior to the extract addition was confirmed in MH agar (Photo 1). Extract treatment did not affected negatively on the bacteria growth. The incubation of bacteria strain with cefotaxime alone showed a little inhibitory effect of the antibiotic. However, the concomitant incubation of bacteria with extract and cefotaxime showed a great inhibition of bacteria growth suggesting that extract restored the effectiveness of the antibiotic by inhibiting the cefotaximase produced by the bacteria.

3.2 Phytochemical screening and antioxidant activity

The capacity of extract to scavenge the radical DPPH was evaluated and its flavonoid and phenolic contents were determined. . The phenolic and flavonoid contents of extract were ranged from 14.25 ± 1.25mgGAE/g and 7.31 ± 0.20mgQE/g respectively. Flavonoids contents is less than two-fold lower than phenolic content suggesting that the methanol bark extract from *A. dudgeoni* is rich in flavonoids content. Moreover, though the antioxidant potent of extract was less than quercetin, extract showed an interesting DPPH scavenging activity with IC_{50} value less than 20µg/mL. These finding suggested that the methanol bark extract from *A. dudgeoni* is a potent source of antioxidant phytomolecules.

The present investigation demonstrated that *A. dudgeoni* bark is a potent source of antibacterial phytomolecules. Previous studies showed that *E. coli* is

resistant to many antibiotics including cefuroxime, cefotaxime and ceftriaxone [12]. Moreover, *E. coli* was reported to be resistant to cefotaxime [13]. The main mechanism of this antibiotic resistance is the enzymatic hydrolysis of the antibiotic by the bacteria. In this study, the effectiveness of the antibiotic cefotaxime was enhanced by extract addition suggesting that extract exerted some inhibitory effect on the enzyme cefotaximase. This finding is an accord with previous studies which demonstrated that the methanol bark extract from *Garcinia lucida* shows a great inhibitory activity on beta-lactamase P99 at a concentration of 12 µg / mL [14]. Furthermore, the methanol fruit extract from *Bridellia micrantha* exhibited a good inhibitory activity on beta-lactamase OXA-10 at a concentration of 30µg / mL while ethyl acetate aerial part extract from *Justicia subsessilis* Oliv restored the effectiveness of many β-lactams antibiotics[15].

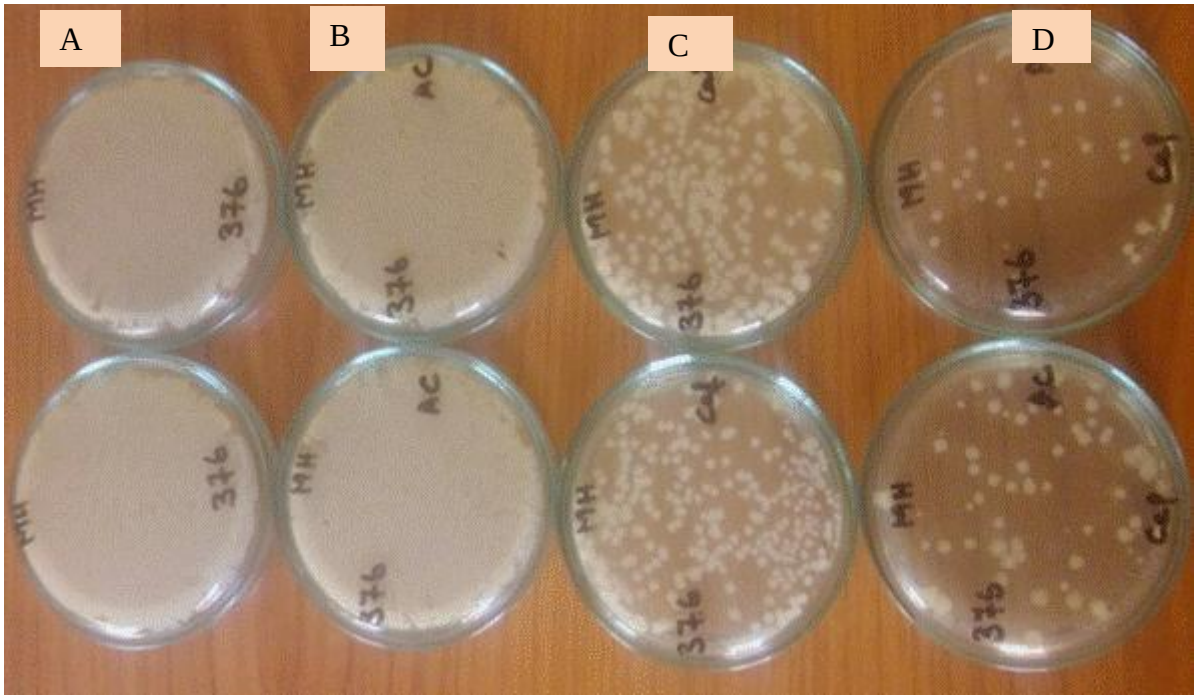

Figure 1: Inhibition activity of extract on cefotaximase produced by *E. coli* strain 376
A: Bacteria alone; B: Bacteria + extract; C: Bacteria + cefotaxime; D: Bacteria + extract + céfotaxime.

Previous studies have established a relationship between plant antioxidant activities and the bactericidal properties on *E. coli* [16, 17]. The methanol bark extract from *A. dudgeoni* showed a good anti-radical activity that could justify its antibacterial potentiality. Previous study demonstrated that the contribution of plant flavonoids to the antibacterial activity on *E. coli* was 0.88 [18]. Extract of *A. dudgeoni* showed in this study resulted in highest content of total flavonoid that may be in partly responsible to its bacterial activity on *E. coli.*

4. CONCLUSION

This study highlighted the potentiality of the methanol bark extract from *A. dudgeoni* to inhibit the cefotaximases produced by *E. coli.* This antibacterial potent of extract may be due to its phenolic and flavonoids contents. *A. dudgeoni* is a potent source of bioactive compound to combat bacterial resistance.

Conflict of interest

All authors declare that no conflict of interest exist.

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