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Chapter

Determination of In Vitro Antiprotease, Antimicrobial, and Antibiofilm Activities of *Beta vulgaris* var. cicla against Multidrug-Resistant Strains of *Pseudomonas aeruginosa*

Hayet Edziri, Rim Nasri, Marwa Hamdi and Maha Mastouri

Abstract

Antibiotic resistance of *Pseudomonas aeruginosa* causes many infectious diseases and it is agreat. So, the aim of the present work was to assess the antibacterial, antibiofilm activity of *Beta vulgaris* extracts against resistance bacteria *P. aeruginosa* that were clinically isolated and tested for their antiprotease potential. Result showed that methanol extract exhibited important antiprotease activity against Trypsin, Savinase, and digestive proteases of blue crab with percentage of inhibition of 94.66, 91.39, and 86.41%, respectively. It showed also important antibiofilm activities against multidrug-resistant *P. aeruginosa* with inhibition values upper than 80% with a concentration of 4MIC. Our investigation delivered that *Beta vulgaris* might be possible source of natural antienzymatic, antimicrobial, and antibiofilm agents.

Keywords: *Beta vulgaris*, antibacterial, antibiofilm, antiprotease, multidrug-resistant *P. aeruginosa*

1. Introduction

Many studies have demonstrated that vegetables play a significant role in human nutrition. They reduced risk of many chronic diseases, like diabetes, cardiovascular illnesses, and cancers [1–3] and Alzheimer's diseases [4, 5]. These advantageous properties of vegetables are due to the bioactive compositions known for their important antioxidant activities [6, 7].

Microbial contamination and the resistance of pathogenic bacteria to antibiotics are considered as major problems of public health [1, 8, 9].

Pseudomonas aeruginosa are bacteria that cause nosocomial infections. They are able to be resistant to a great number of antibiotics such as Carbapenems like imipenem [10, 11].

Beta vulgaris L. belongs to the *Amaranthaceae* family. Its juice had important biological properties like as antimicrobial, hemostatic, and anticancer [12, 13]. *Beta vulgaris* is categorized among the best vegetables with important antioxidant activity; in addition, many researches have showed that *Beta vulgaris* extracts had other important activities (anti-inflammatory, antiallergenic, antithrombotic, and anticoagulant) [14, 15].

The objective of this chapter was to investigate the antiprotease, antimicrobial, and antibiofilm activities of *Beta vulgaris var. cicla* against multidrug-resistant strains of *Pseudomonas aeruginosa*.

2. Materials and methods

2.1 Plant material

The fresh beetroots were bought from a market in Sousse (Tunisia) and the roots were identified and a voucher specimen was placed in our laboratory at the Faculty of Pharmacy (Monastir).

2.2 Preparation of aqueous extract of *Beta vulgaris*

About 200 ml of distilled water was added to 50 g of *Beta vulgaris* pieces. Then they were allowed to boil for 30 min. The extract was filtered using a Whatman paper. The filtrate was kept at −25°C.

2.3 Preparation of methanolic extract of *Beta vulgaris*

Beetroots were washed and sliced into small pieces and then 200 ml of methanol was added to 100-g root in brawn bottle for 3 days at room temperature, filtered through Whatman filter paper, and dried with rotavapor. Then the extract was kept at 4°C.

2.4 Total polyphenol content

The total phenolic content was tested by Folin-Ciocalteu method (Edziri et al.) [16]. The total polyphenols content is expressed as mg gallic acid equivalents (GAEs) per g of extract.

2.5 Total flavonoid content

The flavonoids content was tested by the method of Othmana et al. [17]. The result is expressed in mg quercetin equivalents (QEs) per g of extract.

2.6 Total tannin contents

Total tannin content in *Beta vulgaris* extracts was tested by using Folin-Denis reagent [18].

2.7 Total carotenoid content

Total carotenoids content of *Beta vulgaris* was determined by the ARNON method [19].

2.8 Antiprotease activity

The impact of *Beta vulgaris* extracts, at a concentration of 250 μg/ml, on several proteases' activity was evaluated. So, enzymes were pre-incubated with each extract for 30 min at 30°C. Then, the residual enzyme activity was evaluated according to the method of Georgé et al. [20] using casein as a substrate at the optimal pH and temperature for each enzyme: Purafect (pH 10.0; 50°C), Savinase (pH 10.0; 60°C), (pH 8.5; 50°C) and enzyme of blue crab (pH 8.0; 60°C), trypsin and chymotrypsin (pH 8.0; 37°C). The activity of the enzyme assayed in the absence of inhibitors was taken as 100%.

2.9 Antibacterial activity of *Beta vulgaris* extracts

2.9.1 Microdilution assay

Minimum inhibitory concentration (MIC) values were determined by a microdilution method as indicated by Edziri et al. [21]. The MIC was defined as the lowest concentration that inhibits the development of bacteria, after 24 h of incubation [22].

2.9.2 Antimicrobial activity

2.9.2.1 Micro-well determination of MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for all bacteria tested in this work by a microdilution method as described by [23]. The MIC was distinct as the lowest concentration of the extract to inhibit the development of the bacteria.

2.9.3 Inhibition of biofilm formation

The biofilm inhibition was tested against five multidrug-resistant strains of *Pseudomonas aeruginosa*, by microdilution technique according to Edziri et al. [21].

3. Results and discussion

3.1 Phytochemical screening

The proportions of the phenols, flavonoids, tannins, and carotenoids contents are summarized in **Table 1**. Methanolic extract of *Beta vulgaris* was the richest with phenols, flavonoids, tannins, and carotenoids as shown in **Table 1**.

3.2 Antiprotease, antibacterial and antibiofilm activities

The antiprotease activity of various *Beta vulgaris* extracts was tested at a concentration of 250 μg/ml. **Table 2** demonstrates that all extracts were able to decrease the protease activities by about 51.53–94.66%, suggesting that they were rich sources of the protease inhibitors. In fact, results revealed that the preincubation of Trypsin, Savinase, and digestive proteases of blue crab with methanol extract caused the loss of 94.66, 91.39, and 86.41% of their activity, respectively; however, a decrease of about 89.31 and 91.39% was observed after incubation of this extract with Purafect and chymotrypsin, respectively.

It is interesting to note that the proteolytic activities of Savinase® and Purafect®, commercial microbial proteases, were mostly inhibited by methanol extract by about 91.39 and 89.31%, respectively. In addition, it was efficient to reduce 94.66% of digestive trypsin activity. In addition aqueous extracts exhibit good antiprotease activity.

According to **Table 3**, the values of MIC for two extracts against multidrug-resistant *Pseudomonas aeruginosa* varied between 50 and 100 mg/ml, without difference between the two extracts of *Beta vulgaris*. Furthermore, the MBC values were of 250 mg/ml. The observed activity of *Beta vulgaris* roots may be attributed to the higher content of polyphenols, flavonoids, and tannin, which are known for their important antimicrobial activity.

The two extracts showed important antibiofilm activity (**Figures 1** and **2**). Furthermore, methanolic extract exhibited the greatest antibiofilm property against all resistant strains of PA with inhibition values greater than 80% at the concentration of 4MIC. In addition, aqueous extract inhibited the biofilm formation of PA greater than 50% with 2MIC. We can observe that there is not any difference between the tested strains of PA. Also methanol extract of *Beta*

	Aq	M
Total polyphenols (mg GAE/ g) extract	99.47 ± 0.45	134.55 ± 0.6
Total flavonoids (mg EQ/g) extract	1.29 ± 0.50	4.34 ± 0.02
Total tanin content(mg TA/g)	6.15 ± 1.4	7.5 ± 0.5
Carotenoids (mg/100 g FW)	2.1 ± 1.2	2.97 ± 0.4

GAE: gallic acid equivalent, CE: catchin equivalent, TA: tannic acid, Aq: aqueous extract, M: methanol extract.

Table 1.
Phytochemical analysis of Beta vulgaris *var. cicla*.

Enzymes	M	Aq
Purafect	89.31 ± 1.13	60.70 ± 0.96
Savinase	91.39 ± 1.38	81.07 ± 0.43
Chymotrypsin	65.28 ± 0.47	51.53 ± 0.51
Trypsin	94.66 ± 0.32	87.42 ± 1.55
Digestive proteases of blue crab	86,41 ± 0.34	70.11 ± 0.61

Values are mean ± SD of three replicate analyses, Aq: aqueous extract, M: methanol extract.

Table 2.
Antiprotease activity of Beta vulgaris *L.*

Strains extracts	P.S1		P.S2		P.S3		P.S4		P.S5	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Aq	100	250	100	250	100	250	100	250	100	250
M	50	250	100	250	100	250	100	250	100	250

Aq: aqueous extract, M: methanol extract, MIC and MBC are in mg/ml.

Table 3.
Antipseudomonal activity of Beta vulgaris *extracts.*

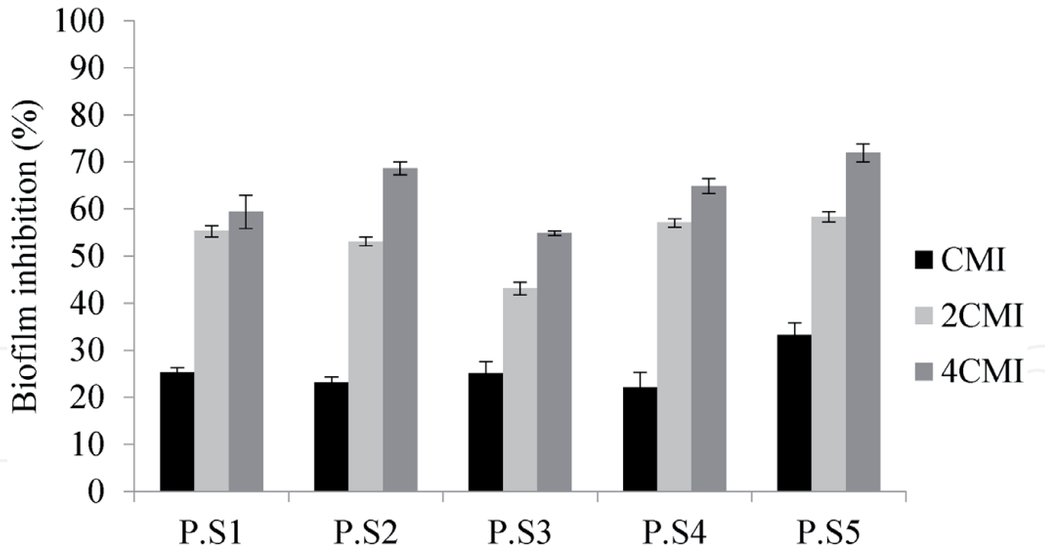

Figure 1.
Antibiofilm activity of Beta vulgaris *aqueous extracts.*

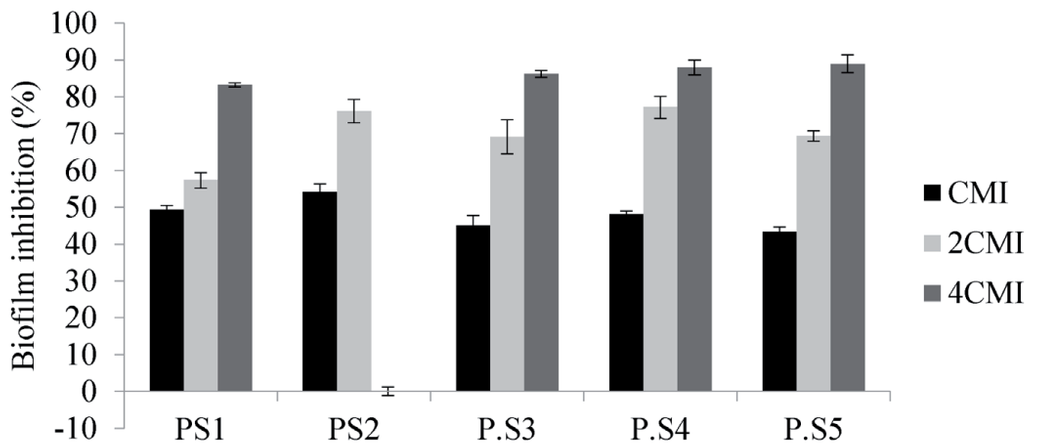

Figure 2.
Antibiofilm activity of Beta vulgaris *methanolic extracts.*

vulgaris displayed a respectable antibiofilm property beside P.S5 with percentages of inhibition of 88.9% at a concentration of 4MIC. The present search was investigated for the first time on the antibiofilm capacity of *Bea vulgaris* against multidrug-resistant strains of *Pseudomonas aeruginosa.* On the other hand, the antibiofilm action is mostly due to the great amount of phenolic content, such as that of flavonoids and tannin known for their good biological activities [24, 25].

4. Conclusion

From this study, we can see that *Beta vulgaris* showed good antiprotease, antibacterial, and antibiofilm activities against different resistant *Pseudomonas aeruginosa* strains. This study also showed that the utilization of this vegetable can lead to the inhibition of bacterial growth.

Furthermore, this vegetable can be used as a source of natural antienzymatic, antimicrobial, and antibiofilm agents. Research is in progress to identify and isolate the bioactive molecules and to test them in vivo.

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