

Evaluation of the Alpha-Glucosidase Inhibitory Activity of Endophytic Bacteria Extracts Isolated from *Ludwigia octovalvis* (Jacq.) P. H. Raven (Onagraceae)

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Abstract

The study aimed at evaluating the alpha-glucosidase inhibitory potential of extracts of endophytic bacteria isolated from *Ludwigia octovalvis* (Jacq.) P. H. Raven (Onagraceae). Isolation of endophytic bacteria was done on supplemented and non-supplemented nutrient agar. The extracts of these endophytes were obtained after fermentation in Mueller-Hinton Broth (MHB). The inhibitory effect on the alpha-glucosidase enzyme of the extracts of endophytic bacteria was determined in the presence of starch and sucrose at 6 mg/mL at 37°C and by measuring the absorbance at 517 nm. Nineteen endophytic bacteria were isolated from the leaves, stems, roots, flowers, fruits and twigs of *L. octovalvis*. The extracts obtained from these endophytic bacteria all showed an alpha-glucosidase inhibitory effect. The S4155 extract showed less than 50% enzyme inhibitory activity with an IC₅₀ of 163.98 µg/mL. Endophyte bacteria associated with *L. octovalvis* provided a source of bioactive compounds that can prevent or reduce the prevalence of diabetes.

Keywords: *Ludwigia octovalvis*, endophytic bacteria, inhibitory activity, alpha-glucosidase.

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INTRODUCTION

Diabetes is one of the main killers in the world, which constitutes a major public health problem and despite prevention efforts, the pandemic continues and the number of people affected is growing [1]. This increase is due to population growth, the aging of the population, urbanization, sedentarization and the development of obesity in populations. Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or the body does not properly use the insulin it produces. Insulin is a hormone that regulates blood sugar concentration [2]. One of the frequent effects of uncontrolled diabetes, which over time leads to serious damage to the organ system, is hyperglycemia, which occurs when the β -pancreatic cells no longer manage to secrete enough insulin. It causes glucotoxicity and alters their function, which can ultimately lead to an irreversible diabetic state [3]. One

of the therapeutic approaches consists of inhibiting digestive enzymes (α amylase and α glucosidase) after taking a meal, hydrolyzing complex dietary carbohydrates into simple sugars, thus delaying their absorption in the small intestine and consequently hyperglycemia [4]. Many plants are traditionally used to treat diabetes, these plants have the ability to inhibit the action of α amylase and α glucosidase enzymes in the digestive tract, this is particularly the case of *Ludwigia octovalvis* (Onagraceae). This species is an herbaceous plant widespread in tropical and subtropical regions. Previous studies have shown that it has many properties in regulating the glycemic index and diabetes [5-7]. Microorganisms associated with plants, in particular endophytic bacteria and fungi, have been shown to offer bioactive compounds with high therapeutic potential. Endophytes are microorganisms that colonize the internal tissues of plants without immediate and

apparent effects for the host plant [8]. Endophytes receive nutrition and protection from the host plant and in return they produce the secondary metabolites (auxins) that promote the growth of the host [9]. Endophytes in addition to being a source of bioactive compounds, they would have low toxicity and a minor impact on the environment [10]. The objective of this study is to highlight the inhibitory effect of alpha-glucosidase of extracts of endophytic bacteria isolated from *L. octovalvis*.

MATERIALS AND METHODS

1. Harvesting plant material

The whole plant of *Ludwigia octovalvis* was harvested fresh from the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. Harvesting targeted plants showing no visible pathological signs. The sample was placed in a sterile plastic bag to be transported to the laboratory kept at a temperature of 4°C for 24 h [11, 12]. A sample was identified at the National Herbarium of Yaoundé in comparison with the reference number HNC-48396.

2. Isolation and Purification of Endophytic Fungal Strains

The various organs (leaves, flowers, fruits, twigs, stems and roots) of *Ludwigia octovalvis* were separated and washed under running water to remove dust and debris. In a sterile hood, the plant organs were cut into fragments of approximately 1 cm x 1 cm using a sterile blade. The explants are disinfected in 70° ethanol for 1 min, followed by immersion in a 1% sodium hypochlorite (NaOCl) solution. The fragments thus sterilized are rinsed with sterile distilled water for 1 min, then dried on sterilized filter paper [13]. In order to validate the effectiveness of surface sterilization, the waters from the last rinses were inoculated onto the “nutrient Agar” medium and stored for a period of one week following the protocol described by Schulz *et al.*, [14]. Surface sterilization was validated because no bacterial growth was observed. The sterilized fragments of the different organs of the plant were then dried on the blotting paper previously sterilized in the autoclave near the flame. The dried fragments were cultured in Petri dishes on Nutrient Agar Agar previously prepared according to the manufacturer's instructions with the addition of Ketoconazole to inhibit the growth of fungal endophytes. Dishes were sealed and incubated at room temperature and observed daily for 7 days. The first bacteria to appear in the medium after 24 hours were subcultured onto Nutrient Agar solid medium without the addition of antifungal.

The purification of the strains was done by successive subcultures of the bacteria isolated on Nutrient Agar until obtaining pure strains characterized by the uniform morphotypes in the Petri dishes [15]. All microbiological manipulations are carried out under aseptic conditions under a fume hood using a Bensen nozzle.

3. Mass culture (fermentation) and extraction of metabolites from endophytic bacteria

The inoculum of each bacterial sample was prepared by diluting a bacterial load in a volume of Muller Hilton Broth (MHB) liquid medium (15 ml). Each inoculum was divided into 4 batches in the flasks for fermentation at different times (1, 2, 3 and 4 weeks, i.e. T1 T2 T3 T4) at room temperature and with daily stirring. Samples of isolated strains were stored in a glycerol solution (15%) at -20°C. At the end of each fermentation cycle, methanol (v/v) was added to each flask, after homogenization followed by incubation at room temperature without shaking for 72 hours. In each fermentation tube, DCM (v/v) was added, a settling formed separating the medium into two phases: phase 1 containing the fermentation medium and methanol and phase 2 containing the extract of endophytes dissolved in DCM. The separatory funnel made it possible to separate the fermentation medium from the extract dissolved in the DCM. The phase obtained was dried under ventilation until the dry extract of each isolated bacterial strain was obtained [16, 17]. The dry extracts were then dissolved in DMSO to obtain a stock solution of 10 mg/mL.

4. Extraction of the enzyme alpha-glucosidase

Crude enzyme extract containing alpha-glucosidase (glucosidase concentrate) was isolated from the small intestine mucosa of healthy rats fasted for 16 h. The rats were sacrificed by cervical distortion and underwent surgical procedures according to the protocol described by Guan *et al.*, 2006 [18]. The small intestine was removed and the mucosa rinsed thoroughly with an ice-cold phosphate buffer solution (pH 7). The intestine was then introduced into another phosphate buffer solution (pH 7) where it was opened and its mucosa scraped with a glass slide. The solution obtained was centrifuged at 3000 revolutions for 10 minutes and the supernatant obtained representing the enzymatic solution which was stored at 4° C [19]. The tests were carried out on two substrates starch and sucrose. A developer, glucose oxidase peroxidase (GOD-POD) was used to verify enzyme activity.

5. Evaluation of alpha-glucosidase inhibitory activity

The alpha-glucosidase inhibitory effect of extracts of endophytic bacteria from *Ludwigia octovalvis* was carried out following the protocol described by Kim *et al.*, 2005 with some modifications [20]. In 04 microplates of 96 wells, 30 µL of extract of the alpha-glucosidase enzyme were introduced into each well, the microplates were then incubated in an oven at 37°C for 30 min to create the maximum reaction conditions enzymes. 30 µL of the various endophyte extracts at 200 µg/mL were added followed by incubation in an oven at 37°C for 10 min. The microplates were separated into 2 groups of 2 plates each. In one group, 60 µL of the 6 mg/ml starch substrate and in the other 60 µL of the 6 mg/ml sucrose

substrate were then introduced into the various wells. These were incubated in an oven at 37°C for 1 hour. The microplates were introduced into boiling water for 3 min, thus causing denaturation of the enzymes to stop the enzymatic reaction. The microplates were then incubated in the presence of GOD-POD developer (120 µL/well) in an oven at 37°C for 10 min, the absorbance was read on a spectrophotometer at 510 nm. The inhibition activity was calculated by the equation described by Françoise Marc *et al.*, [21]:

$$\text{Inhibition (\%)} = \frac{(\text{Abs}_O - \text{Abs}_E)}{\text{Abs}_O} \times 100\%$$

Abs_O = control absorbance
Abs_E = extract absorbance

From the calibration curve, the concentration of the extract resulting in a 50% inhibition of the

enzymatic activity (IC₅₀) was determined by linear interpolation on Xlstat Excel.

RESULTS AND DISCUSSION

1. Bacterial strains isolated from *Ludwigia octovalvis* organs

A total of 396 fragments of the different organs of *Ludwigia octovalvis* were examined. After purification and on the basis of morphological characteristics, 101 endophytic bacterial strains were isolated from *L. octovalvis*, i.e. 60 strains in the culture medium supplemented with the extract. After successive subcultures of the isolated bacteria, 60 strains were isolated and grouped into 19 pure strains characterized by uniform morphotypes (Table I).

Table 1: Endophytic bacteria from *Ludwigia octovalvis* purified by organ

<i>Ludwigia octovalvis</i> Organs	Medium with no supplement	Supplemented medium	Total
Leaves	1	3	4
Fruits	1	1	2
Flowers	2	1	3
Twigs	1	2	3
Roots	3	1	4
Stem	2	1	3
Total	10	9	19

Morphological analysis shows that many bacterial strains isolated are identical and that in reality only 19 different strains have been isolated. The endophytes present in plant tissues are not all cultivable. The number of endophyte strains that develop during initial isolation cannot actually be grown under laboratory conditions, this is due to the presence of residual plant-specific compounds and metabolites that are still present during isolation but which are not present during a possible reculture [22, 23]. Dominant fast-growing bacterial strains can also invade slow-growing endophytic strains, so that the latter do not have the opportunity to grow [23]. Medium supplementation has been studied to improve the number and diversity of endophytes as well as growth capacity when subcultured after isolation [24]. The nutrient medium affects both the number and diversity of endophytes that can be isolated from a specific plant tissue and it determines the ultimate cultivability of certain endophytic bacterial strains. It is possible that some bacterial strains are limited in their growth by exposure to agar, supplementation of the medium was intended to optimize the isolation processes and subsequently cultivate these bacteria.

3.3. Alpha-glucosidase inhibitory potential of extracts of endophytic bacteria associated with *L. octovalvis* organs

In order to study the inhibitory effect of extracts of the endophytic bacteria of *L. octovalvis*, an

in vitro screening on the α-glucosidase inhibition test was carried out in duplicate using two substrates (starch and sucrose). All extracts exhibited alpha-glucosidase inhibitory activity at 200 µg/ml. The extract of the endophyte bacteria isolated from the fruits of the plant from the second week of fermentation in supplemented medium (S4155) gave an alpha-glucosidase inhibitory activity greater than 50%. Inhibition of the digestive enzyme activity of α-glucosidases, as well as the delay of digestion and absorption of carbohydrates by the small intestinal tract are considered to be one of the main treatment options for T2D. By inhibiting these key enzymes, minimal amounts of glucose would be absorbed into the bloodstream, so plasma glucose will not rise after a meal [25]. The percentages of enzymatic inhibition are higher in the test using starch as substrate than that using sucrose as substrate in our study. This could mean that the extracts of the endophytic bacteria of *L. octovalvis* would have a greater affinity for the hydrolytic sites of the enzymes debranching amylo α 1-6 glucosidase (a glycoside hydrolase which hydrolyzes α 1-6 -D- bonds starch) and α 1-4 glucosidase or maltase (which hydrolyzes the maltose released by the amylose coming from the starch during its digestion) of the starch only for the hydrolytic sites of the sucrose which is the β 1 -2 fructosidase (sucrase) [26]. The calibration curve of the S4155 extract was produced and the concentration necessary to inhibit 50% (IC₅₀) of the enzyme obtained by linear extrapolation on Xlstat Excel is 163.98 µg/mL (Figure 1).

Table 2: Percentage of alpha-glucosidase inhibition of bacterial endophyte extracts of *L. octovalvis* in the presence of starch

Endophytes			Inhibition (%)			
N°	Code	Medium	S1	S2	S3	S4
1	4002	SM	39.23	36.89	33.11	44.05
		NSM	38.21	37.71	36.74	43.14
2	4003	SM	37.52	38.32	22.52	35.49
		NSM	33.11	36.74	40.59	47.10
3	4008	SM	43.53	37.89	37.00	42.80
		NSM	42.49	48.70	42.30	36.89
4	4109	SM	38.62	40.76	37.71	34.54
		NSM	34.13	30.30	34.69	31.73
5	4155	SM	30.30	50.15	32.53	31.12
		NSM	37.67	36.40	41.50	38.32
6	4202	SM	31.12	24.48	47.96	40.53
		NSM	37.33	32.76	42.80	37.89
7	4261	SM	32.76	46.06	44.22	40.53
		NSM	36.40	27.51	38.32	34.13
8	4262	SM	36.74	34.02	37.69	40.66
		NSM	36.74	36.74	33.56	36.53
9	4304	SM	37.00	31.12	37.00	31.12
		NSM	43.29	37.00	42.15	44.85
10	4305	SM	37.71	27.94	37.71	42.58
		NSM	37.71	39.23	24.33	30.30
11	4307	SM	40.53	32.44	40.53	42.34
		NSM	40.53	37.52	23.64	46.12
12	4357	SM	33.56	49.62	33.56	44.65
		NSM	33.56	37.89	40.63	24.48
13	4402	SM	47.98	49.61	40.59	40.66
		NSM	33.56	36.74	43.96	30.28
14	4451	SM	36.40	32.40	37.71	37.00
		NSM	28.20	48.18	27.01	36.40
15	4452	SM	47.12	33.11	36.53	42.15
		NSM	40.76	36.74	37.00	37.52
16	4456	SM	32.92	40.53	37.00	36.74
		NSM	33.11	22.52	37.71	37.00
17	4458	SM	42.15	32.92	33.56	38.32
		NSM	38.32	34.49	37.71	39.23
18	4552	SM	37.00	36.53	37.71	36.89
		NSM	36.89	37.00	42.15	40.53
19	4556	SM	38.62	30.30	31.12	32.76
		NSM	36.74	37.00	37.71	37.89

SM: supplemented medium; NSM: non supplemented medium

Table 3: Percentage of alpha-glucosidase inhibition of bacterial endophyte extracts of *L. octovalvis* in the presence of sucrose

Endophytes			Inhibition (%)			
N°	Code	Medium	S1	S2	S3	S4
1	4002	SM	36.44	32.44	30.07	31.46
		NSM	36.15	31.46	30.68	32.18
2	4003	SM	32.45	27.86	33.03	31.97
		NSM	24.52	30.70	35.92	33.64
3	4008	SM	31.35	32.95	31.65	32.08
		NSM	33.64	33.68	30.52	31.36
4	4109	SM	26.06	32.33	34.09	24.61
		NSM	34.09	24.61	32.45	34.34
5	4155	SM	28.77	34.80	37.67	32.44

Endophytes			Inhibition (%)			
N°	Code	Medium	S1	S2	S3	S4
		NSM	32.44	33.80	33.68	28.18
6	4202	SM	31.90	31.73	36.16	34.54
		NSM	30.68	33.03	34.44	31.60
7	4261	SM	26.41	27.43	34.02	25.74
		NSM	25.74	33.58	30.07	33.58
8	4262	SM	27.07	30.48	32.41	32.44
		NSM	32.44	31.94	32.36	31.36
9	4304	SM	30.70	31.57	35.59	32.18
		NSM	32.18	29.72	34.21	32.31
10	4305	SM	31.65	37.24	34.30	33.68
		NSM	32.36	36.61	24.79	28.99
11	4307	SM	34.09	29.15	27.62	34.54
		NSM	31.42	34.00	28.47	34.37
12	4357	SM	34.54	33.61	31.93	33.66
		NSM	34.61	36.27	34.02	29.40
13	4402	SM	32.41	35.83	31.73	30.70
		NSM	31.73	30.70	33.77	30.70
14	4451	SM	34.02	22.89	31.65	29.40
		NSM	29.40	30.44	29.62	32.08
15	4452	SM	32.74	30.52	31.60	31.93
		NSM	34.30	28.99	31.57	29.95
16	4456	SM	28.77	34.54	30.70	31.65
		NSM	31.90	32.71	30.44	24.41
17	4458	SM	34.61	24.41	32.25	32.33
		NSM	32.44	28.37	31.73	34.61
18	4552	SM	32.41	34.09	34.02	35.17
		NSM	34.02	35.17	32.03	31.20
19	4556	SM	24.79	28.47	27.43	31.89
		NSM	28.37	30.07	30.48	32.95

SM: supplemented medium; NSM: non supplemented medium

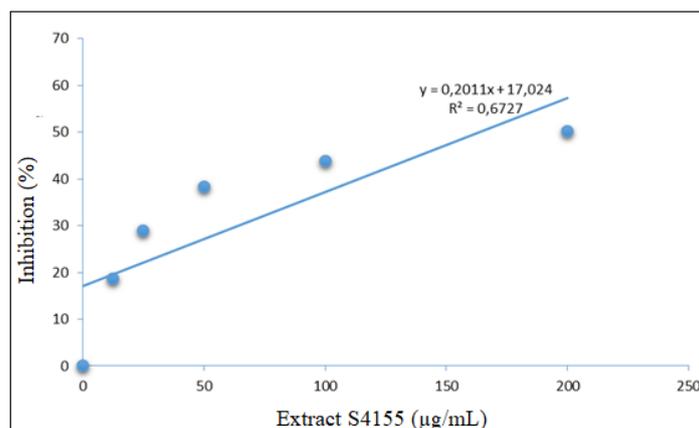


Figure 1: Calibration curve of extract S4155

CONCLUSION

The study aimed at evaluating the alpha-glucosidase inhibitory potential of extracts of endophyte bacteria associated with *Ludwigia octovalvis*. Nineteen endophytic bacterial strains were isolated from different organs of *L. octovalvis* in two different culture media. All the extracts tested showed an alpha-glucosidase inhibitory activity, the S4155 extract showed an inhibitory activity greater than 50%. This study shows

that the extracts of the endophytic bacteria of *L. octovalvis* are a source of compounds to be explored for the fight against diabetes, in particular hyperglycemia.

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