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Original Article

Total phenols, total flavonoids content; antioxidant and antifungal activities of ethanolic and aqueous extracts of *Eleutherine bulbosa* (Iridaceae)

C.J.Morabandza[1], M. G. Okiemy-Akieli[2], E. Okiemy[1], T. Andzi-Barhé[3], P.R.Ongoka[4]

[1] Laboratory of Biochemistry and Pharmacology, Health Sciences Faculty, Marien Ngouabi University, P.O. Box 69, Brazzaville, CONGO
[2] Departments of Natural Sciences, ENS, Marien Ngouabi University, P.O. Box 69 Brazzaville, CONGO
[3] Natural substances and organics synthesis Laboratory, Faculty of Sciences USTM, P.O. Box 941, Franceville Gabon
[4] Departments of Exact Sciences, ENS, Marien Ngouabi University, P.O. Box 69 Brazzaville, CONGO

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ABSTRACT

Ethanolic and aqueous extract of the bulb of *Eleuterine bulbosa* have been analyzed for their content in total phenols, total flavonoids; antioxidant and antifungal activities. The average value of total phenols was 27.12 ± 0.62 and 9.36 ± 0.55 mg. Eq.G.A /g d.M for ethanolic and aqueous extract respectively and the one of total flavonoids, 17.97 ± 0.07 and 6.15 ± 015 mg. Eq.Rt /g.d.M. In vitro antioxidant activity by DPPH method revealed the I C_{50} of 0.595 ± 0.004 and 1.251 ± 0.083 mg/ml respectively for ethanolic and aqueous extract, against 0.123 ± 0.005 mg/ml for Rutin (control). Antifungal activity carried out by diffusion in solid medium method showed the inhibition of *Candida albicans* growth by the two extracts. The IMC was 3.75 for ethanolic extract and 7.5 mg/ml for aqueous extract. The FMC was 30 mg/ml for ethanolic. These results may explain the abundant uses of this plant as a nutritional and medicinal plant.

Keywords: *Eleuterine bulbosa*, phenols, flavonoids, antioxidant, *Candida albicans*

INTRODUCTION

Eleuterine bulbosa (Miller) Urban is the herbaceous belonging to the Iridaceae family. It's can reach 30 cm height with leaves resembling to a young palm tree and contain the bulb under stock, the small spangled white flowers [1, 2]. Commonly called "Bikùa-bia-mbuaki" (red onion) in Congo, the bulb of *E. bulbosa* is used in food as onion, vegetable and tomato which gives a red color to food. It is used in traditional medicine as, vermifuge, abortive agent, against intestinal disorders, acute dermatoses with etchings and coetaneous eruptions, painful and irregular menstruations, haemostatic agent, antifungal, antibacterial treating various infections and also as anti inflammatory [1]. Other studies have revealed some chemical constituents and the pharmacological activities of the Rhizome of the bulb [2, 3]. Many of these quoted pathologies coming from oxidative stress which is currently accused in the release and the progression of several diseases. Many studies have revealed that phenolic compounds are considered as antioxidant and had capacity to neutralized free radicals responsible of oxidative stress [4, 5]. They also have been reported to possess antimicrobial activity [6]**.** Considering the critical point reached these last decades by the resistances to antibiotics and, urgent necessity to find new compounds to complete the present number of anti-infectious agents [7], the use of vegetable phenolic compounds accessible to all could constitute a way of solution. That is the present interest research of the uses of antimicrobial virtues of several plant species accessible to all [8, 9]. Thus, the goal of this work is to evaluate the content in total phenols, total flavonoids; antioxidant and antifungal activities of ethanolic and aqueous extract of the bulb of *E. bulbosa*.

MATERIAL AND METHODS

Plant material: The bulb of *E. bulbosa* was collected from a private garden at Brazzaville (Congo) in November 2014 during the rainy

***Corresponding Author Address:** Dr.C.J. MORABANDZA Laboratory of Biochemistry and Pharmacology, Faculty of the Sciences of the Health University Marien Ngouabi, Brazzaville – Congo; E-mail: cyrmoras@yahoo.fr*

season. The plant was identified by Camille Kami in the Botanic Laboratory Center of Study of Vegetal Resources (CERVE).

Preparation of extracts: The collected bulbs were previously washed, cut off using a knife; air dried during 10 days at 25°C in laboratory and grounded into powder, thanks to a mortar. 20 g of powder was subjected to magnetic maceration extraction in 200 ml of solvent (distilled water or ethanol) respectively during 48 hours. The macerates were filtered three (3) times to the absorbent cotton and the filtrate concentrated at 50°C in a steam room. The extracts tightly closed and kept at 4^0C in the refrigerator for every test.

Total phenol: The content in total phenol was evaluated according to Folin-Ciocalteu method [10]. A volume of 0.1 ml of extract was mixed to 0.9 ml of solvent (distilled water or ethanol). 0.9 ml of the reagent of Folin-Ciocalteu (1N) prepared freshly and 0.2 ml of Na_2CO_3 (20%) solution were added simultaneously. The obtained solution was hatched to ambient temperature during 40 min safe from light. The absorbance was measured with the spectrophotometer at 725 nm against ethanol used like control. A curve of standardization was achieved previously before the analysis with Gallic acid in the same conditions that the samples were analyzed. The obtained results are expressed in milligrams equivalent Gallic acid by gram of dry matter (m. EqG.A/g d.M).

Total flavonoids: Total flavonoids have been determined using colorimetric method [10]. 250µl of extracts were mixed with 1ml of distilled water and 75µl NaNO2 (5%) solution. 75µl of AlCl3 (10%) were added 5 min after. 6 min later, 500µl of NaOH (1N) and 2.5ml of distilled water are added successively to the solution. The whole was agitated with the help of a vortex and the absorbance was measured at 510 nm. A curve of standardization was achieved previously with a standards solution of different concentration of Rutin. The comparison of the optic density observed to the one obtained by a stallion of known concentration Rutin permits to value the total content in flavonoids. The results expressed in milligrams are equivalent of Rutin by gram of dry matter (m. Eq.Rt/g.d.m).

Antioxidant activity: Antioxidant power was quantitatively evaluated using 1.1-diphenyl-2-picrylhydrazyle (DPPH) method. 100 µl of ethanolic and aqueous extracts of decreasing concentrations (10; 5; 2.5; 1.25; 0.625 mg/ml) are mixed with 2 ml of DPPH solution, prepared by dissolution of 10 mg of DPPH in 250 ml of ethanol. In the same way a negative control was achieved with 100 µl of ethanol and distilled water with ethanolic solution of DPPH. Absorbance reading was made at 517 nm against control with a spectrophotometer (UV-Visible) after 30 min of incubation in obscurity and ambient temperature [10]. The positive control was represented by Rutin, whose absorbance was measured in the same conditions. The percentage of inhibition was given by the following relation:

$$\% I = [(D.O_{control} - D.O_{EI}) / D.O_{control}] \times 100$$

$D.O_{control}$ = control Optic density

$D.O_{EI}$ = extract/inhibitory optic density

The concentration that inhibits 50 % of DPPH (C.I50) has been determined by proportion.
All measures have been achieved in triplicate.

Antifungal activity: Antifungal activity was achieved with *C. albicans* by diffusion on solid medium technical [11, 8] with a specific medium culture. Decreasing concentrations of 30; 15; 7.5 and 3.75 mg/ml of aqueous and ethanol extract, was prepared corresponding to the dilutions of 1, 1/2, 1/4 and 1/8 either a geometric continuation of reason 2 from the concentrate extract.

Preparation of medium: Sabouraud medium is a specific medium for *C. albicans* growth. 16.26 g of powder are dissolved in 250 ml of distilled water, heated then in a ball at 121°C during 15 minutes until complete dissolution. The solution was recovered in an erlenmeyer and sterilized to the autoclave, flowed then in three boxes of kneaded at the rate of 20 ml per box.

Preparation of inoculums: A colony of *C. albicans* obtain from National public Health Laboratory of Congo-Brazzaville was taken with a shackle then introduced in 5ml distilled water. After agitation, one gets a suspension that is kept during 24 hours in the steam room at 37°C to permit the growth of the germ.

Antibiogram and reading of the results: The cupules are prepared previously so that, on the boxes of kneaded sowed, one achieves some wells with the tip unraveled of the pipette pastor of 5 mm of diameter. 1ml of inoculums prepared is poured in the boxes of kneaded and preciously spread on the surface of medium and is hatched at 37°C to the steam room during 20 min. l00 µl of extracts are introduced according to the concentration (30; 15; 7.5 and 3.75 mg/ml) in the wells with the help of micropipette followed standard antibiotic used in the same conditions. Distilled water and ethanol are used like control. After diffusion of extracts, the boxes of kneaded containing the solution are hatched at 37°C to the steam room during 24 hours.

Reading achieves itself by the measure in millimeter (mm) of inhibition diameter. The experiences were done in triplicate. The sensitivity of the germ towards extracts is classified according to the inhibition diameter halos [12]. Ø < 8mm: non sensitive strain; 9 < Ø <14 mm: sensitive strain; 15 < Ø < 19 mm: very sensitive strain and Ø > 20mm: extremely sensitive. The inhibitory minimal concentrations (IMC) and fungicidal minimal concentration (FMC) were evalued by comparing inhibition diameters of the extracts (3.75; 7.5; 15 and 30 mg/ml) to the one of the control and in relation with dilutions.

Statistical analysis: The results of the set of the tests achieved are expressed with the standard mistake on average more or less on the average (M ± E.S.M) with the software Statistica 7.1.

RESULTS AND DISCUSSION

This work was initiated in order to evaluate the content in total phenol, flavonoids; antioxidant and antifungal activities of the bulb of *E. bulbosa*. The results of extraction show that ethanolic extract presents the most important yield comparatively to aqueous extract (table1). Quantitative analyzes by UV-visible spectrophotometer after establishment of standard curves (R^2 = 0,994) with Gallic acid (control) revealed that ethanolic extract contain three times more total phenols than aqueous extract: 27.12 ± 0.62 against 9.36 ± 0.55 mg Eq.G.A/g d.M. The same tendency was observed with the content in total flavonoids (Rutin as control, R^2 = 0,987): 17.97 ± 0.07 and 6.15 ± 015 mg. Eq. Rt/g.d.M respectively for ethanolic and aqueous extract. This fact certainly explains itself by the polarity of solvents. Indeed ethanol is more polar than distilled water.

Phenols are recognized as compounds endowed antioxidant capacity [13]. Several studies proved that aqueous, ethanolic and hydroethanolic extracts were the seat of phenolic compounds sensors of the free radicals [14, 15]. These observations explain the quantitative evaluation of vitro antioxidant activity. Table 3, revealed that the bulbs of *E. bulbosa* have interesting antioxidant potentialities according to the used solvent. The result shows that ethanolic extract presents more important antioxidant activity and is closer to the control (Rutin) than aqueous extract. Indeed, the I C_{50} values are 0.595 ± 0.004 and 1.251 ± 0.083 mg/ml respectively for ethanolic and aqueous extract compared to the Rutin 0.123 ± 0.005 mg/ml. Our results are in agreement with the total phenols and total flavonoids analysis and would also explain the important use of this plant in the treatment of some pathology. It's also agreed to those of the cancer inhibiting activity of the breath realized with the same species by other authors [3]. The content in phenols of these extracts would explain certainly the antioxidant activity observed. These results open an alternative of research in the sense that the phenolic compounds have been reported to possess various biological activities against several pathologies. These last decades, the resistance to antibiotics reached critical point, especially in hospital environment [7]. We particularly focused our attention on *C. albicans* which nowadays has enormous resistance. Table 4 present antifungal activities of ethanolic and aqueous extract. It appears that the two extracts and the antibiotic inhibit significantly the growth of *C. albicans*. Indeed, with ethanolic extract, *C. albicans* is more sensitive with an IMC of 3.75 mg/ml than with aqueous extract 7.5 mg/ml. In the same way, ethanolic extract is fungicidal at 30 mg/ml, whereas it is higher than this concentration with the aqueous extract (no determinate value). Antifungal activity and the tests of sensitivity achieved show that ethanolic extract of *E. bulbosa* is more active on the studied strain than aqueous extract and competes with Fluconazol* while comparing inhibitory diameters and the action specter. A critical analysis of inhibition parameters (IMC) and fungicidal (FMC) makes it possible to quantify, confirm, compare and, characterize the nature of the activity revealed by the extract on the tested strain. The report of IMC and FMC of ethanolic extract is lower than 1, the value is 0.12 for *C. albicans* thus confirming the fungicidal effect of ethanolic extract. The results obtained join those of quantitative proportioning which showed a yield, content of phenols, flavonoids and out of higher antioxidant with the ethanolic extract than with the aqueous extract. This activity could be explained by the content in phenolic compounds.

Conclusion

From our investigation, we came to conclude that *E. bulbosa* contains considerable amount of total phenols and flavonoids and exhibits good antioxidant and antifungal activities. These results provide scientific evidence to explain the abundant uses in traditional medicine and make it possible to classify this specie among the antioxidant plants. These activities might be due to the actions of evaluated bioactive compounds; which opens another significant pharmacological prospects.

Table 1: Yield (%) of extraction of *E. bulbosa* with different solvents

Extracts	Yield (%)
Ethanolic	10,10
Aqueous	7,01

Table 2: Content in Total phenols and total flavonoids

Compounds	Ethanolic extract	Aqueous extract
Total phénol	27.12 ± 0.62 (mg Eq.G.A/g d.M)	9.36 ± 0.55 (mg Eq.G.A/g d.M)
Total flavonoids	17.97 ± 0.07 mg. Eq. Rt/g.d.M	6.15 ± 015 mg. Eq. Rt/g.d.M

Table 3: Antioxidants activity ($I.C_{50}$ mg/ml) of the bulb of *E. bulbosa*

Extracts	Inhibitory Concentration ($I.C_{50}$ mg/ml)
Ethanolic	0,595 ± 0,004
Aqueous	1,251 ± 0,083
Rutin	0,123 ± 0,005

Table 4: Antifungal activity of ethanolic and aqueous extract of *E. bulbosa* at different concentrations

Products	Diameters (mm)	
Fluconazol*	26.00 ± 0.00	26.00 ± 0.00
Ethanol	1	-
Distilled water	-	0
E. bulbosa (mg/ml)	Ethanolic extract	Aqueous extract
30	26.76 ± 0.66	17.53 ± 0.55
15	20.70 ± 0.60	13.16 ± 0.15
7.5	13.30 ± 0.26	9.03 ± 0.15
3.75	9.36 ± 0.55	5.33 ± 0.25

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