Scientific Research Publishing

Open Journal of Applied Sciences, 2021, 11, 254-263
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Evaluation of Potential Extracts Antioxydant (Aqueous, Hydro-Ethanolic and Ethanolic) of an Aquatic Plant from the River Djoue (*Ledermanniella schlechteri*)

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How to cite this paper: Nguie, R., Gouollaly, T., Tamba Sompila, A.W.G., Moussounga, J.E., Dzondo, M.G., Pambou-Tobi, N.P.G., Diakabana, P. and Gampoula, R.H. (2021) Evaluation of Potential Extracts Antioxydant (Aqueous, Hydro-Ethanolic and Ethanolic) of an Aquatic Plant from the River Djoue (*Ledermanniella schlechteri*). *Open Journal of Applied Sciences*, **11**, 254-263.
https://doi.org/10.4236/ojapps.2021.113018

Received: January 12, 2021
Accepted: March 12, 2021
Published: March 15, 2021

Abstract

The aim for this present study was to evaluate the antioxidant potential of aqueous extracts (AE), hydro-ethanolic extracts (HE) and ethanolic extracts (EE) obtained from an aquatic plant (*L. schlechteri)* using a simple and fast method that is the CCM. This method revealed the presence of phenolics and flavonoids at different levels but with higher antioxidant activity in EE compared to AE. Among the two families of antioxidants evaluated, the phenolic compounds were found to be higher on the EE (5.85 mgEAG/MS) followed by the HE (5.06 mgEAG/MS) and less and less important on the AE (3.661 mgEAG/MS). While the less significantly elevated flavonoids showed values of 1.146 mgECa/MS for EE, 0.406 mgECa/MS for HE et 0.181 mgECa/MS for AE. However, the anti-free radical activity was also evaluated. Unlike the antioxidant activity, the ant-free radical activity with a greater IC50 was observed on AE with a rate of 66.66 mg/mL, then less on the hydro-ethanolic and ethanolic extracts, respectively at levels of 26.15 mg/mL et 19.18 mg/mL.

Keywords

Evaluation, Antioxidant Potential, Extracts, Aquatic Plant, *Ledermanniella schlechteri*

1. Introduction

The consumption of vegetables and fruits known as potential sources of antioxidants, is becoming more and more interesting given that human health has become precarious following the appearance of many diseases (cardiovascular, diabetes, cancer) [1]. When the presence of toxic oxygen radicals AOS (active oxygen species) becomes uncontrollable in the body, the weakening of our antioxidant defenses occurs (deficiencies in vitamins and trace elements. A high consumption of fruits and vegetables could have been associated with the decrease in the risk of these diseases in numerous epidemiological studies [2]. Scientific progress has shown that these natural products (vegetables, fruits, etc.) can account for enormous sources of antioxidants making it possible to counteract pathological disorders linked to oxidative stress in the human body. It turns out that this protective effect is based on multiple constituents of these foods (fruits and vegetables) such as fibers, vitamins, minerals and polyphenols [1]. Indeed, total polyphenols are natural compounds widely distributed in the vegetable realm (particularly abundant in fruits, cereals and vegetables); they are increasingly important particularly thanks to their beneficial effects on health [3]. Their role as natural antioxidants is generating a growing interest in preventing and treating cancer, inflammatory and cardiovascular diseases [4].

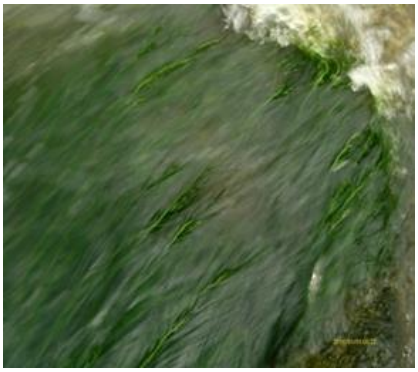
Ledermanniella schlechteri, one of the vegetables that may have antioxidant potential, is an aquatic plant found in the river Djoué, one of the tributaries of the great Congo River and whose flora has several plant resources. This vegetable highly prized by the population of Brazzaville, especially in the southwest, is commonly called *Michiélé*. It belongs to the *Podostémaceaé* family. The vegetable is submerged in water under the rocks of the large tributary of Djoué. This edible plant, little known to the Congolese population, has not yet been the subject of a scientific study.

It is for this reason that we have set ourselves the objective of evaluating the antioxidant potential of three extracts of *Michiélé* plant by evaluating the contents of polyphenols (total phenols and flavonoids) and subsequently evaluating the anti-radical potentialities of these different plant extracts.

2. Materials and Methods

2.1. Vegetal Materiel

Michiélé plant was collected in a district in the southwestern part of Brazzaville along the Congo River, particularly under the rocks of its main tributary the Djoué. This aquatic plant was left to dry at room temperature, 25˚C, in the shade for about a week. The dry matter was ground with a device of the IKA-WERKE Gmbh-CO-KG, D-79219 Staufen type, fitted with a sieve with a 0.25 mm granulometry. The plant was identified by the national herbarium located within the grounds of the National Institute of Research in Exact and Natural sciences (l'Institut national de Recherche en Sciences Exactes et Naturelles IRSEN) of the Ministry of Scientific Research and Technological Innovation (**Figure 1**).



(a)

(b)

(c)

Figure 1. (a): Michielah's plant in water; (b): Recovery of Michiélé's plant; (c): Part of Michiélé's plant.

2.2. Methods

2.2.1. Preparation of Extracts

The various extracts produced for the numbering of the total poluphenols and flavonoids were obtained by mixing 30 g of the plant material in 2 × 150 mL of different organic solvents, respectively for the preparation of aqueous extracts (AE), hydro-ethanolic extracts (HE) and ethanolic extracts (EE) in the same proportions 50% (v/v). The mixture was then macerated by stirring for 48 h, then filtered through filter paper. The filtrate obtained was concentrated to dryness at 50˚C at reduced pressure, using N-1 rotatory evaporator (Eyela, Tokyo Rikakikal co., Ltd. Japon) and stored in an oven at 25˚C, the kept cool (+4˚C) awaiting analysis.

2.2.2. Preparation of Dosing Solutions at Different Dilutions

In a series of glass tubes, mixtures of extract and solvent were mixed either 40 mg for the ethanolic extract, 80 mg for the hydroethanolic extract and 160 mg for the extract; then 2 mL of solvent was added (ethanol, water-ethanol and water) in each tube. The solutions were mixed under magnetic stirring for a few moments and the stock solutions were obtained for each extract. From the stock solutions, we also prepared the daughter solutions for each extract by dilution to ½. We prepared a total of 4 (S1 to S6) for each extract from which we then obtain 12 daughter solutions.

2.2.3. Thin Layer Chromatography Method: TLC

The quantitative identification of substances with antioxidant activity was carried out according to the "bioautography" method [5] by thin layer chromatography where the antioxidant activity was revealed by DPPH, according to [6]. The CCM was carried out on a 60 F254 silica gel chromatographic plate on a 20 cm × 20 cm aluminum foil support from Merck.

The CCM was carried out in normal phase on aluminum plates with the solution of ethyl acetate/formic acid/water in the ratio 8/1/1. Le chromatogram obtained was revealed by spraying Nœud's solution (0.5 g of 2 amino diphenyl borinate + 0.5 g of PEG_{400} + 100 mL of ethanol.

The plates were observed under UV-visible and under UV at 366 nm, before and, in some cases, after visualization by the appropriate reagents.

2.2.4. Polyphenol Analysis

The total phenol content of the various extracts of the plant of *L. schlechteri* was determined according to the **Folin-Ciocalteu** method. For this, 0.1 mL of each extract (aqueous, hydro-ethanolic and alcoholic) was used; to this mixture was added 0.9 mL of distilled water followed by 0.9 mL of the 1 N Folin-Ciocalteu method. Immediately 0.2 mL of the sodium carbonate solution was added (Na_2CO_3 à 20%). The resulting mixture was incubated at room temperature of 25˚C for about 40 minutes protected from light.

The absorbance was measured with a spectrophotometer at 725 nm against a solution of methanol used as a blank. The results obtained were expressed in mg gallic acid equivalent per gram of dry matter (EGA/g Ms).

2.2.5. Total Flavonoïds Analysis

The total flavonoid content of the various extracts of *L. schlechteri* was obtained using Aluminum Trichoride ($AlCl_3$) [7]. In a 100 mL flask were successively introduced 250 µL of each extract (aqueous, hydro-ethanolic and alcoholic). 1 mL of distilled water was combined with this solution, 7.5 µL of a solution of sodium nitrate ($NaNO_2$ at 5%); the mixture was allowed to stand for 5 minutes. Then 75 µl of aluminum trichloride ($AlCl_3$ at 10%) was added. After 6 minutes, 500 µL of sodium hydroxide NaOH with a concentration of 1 N and 2.5 mL of distilled water were added successively to the mixture.

The absorbance was measured with a UV-visible spectrophotometer at 413 nm and the results were expressed as mg catechin equivalent per gram of dry mater (ECa/g Ms).

2.2.6. Evaluation of the Anti-Radical Activity of the Different Extracts (Method Using DPPH)

The evaluation of the anti-free radical activity was carried out using 5 mL of the solution of 1.1-diphenyl-2-picrylhydrazyl (DPPH at 10 mg in 250 mL of ethanol) and 100 µL of each extract diluted at concentration raging from 1.25 to 20 or even 40 mg/mL, all mixed in EDTA type glass tubes. After 30 minutes of incubation in the dark, the anti-free radical activity was measured in a spectrophoto-

meter at 517 nm in the dark [8]. The percentage of inhibition was calculated by the following relationship

Avec $D.O_{Blanc}$: 0.727, Avec $D.O_{Blanc}$: 0.788

$$I = \frac{D.O_{blanc} - D.O_{extrait}}{D.O_{blanc}} \times 100 \quad (1)$$

The IC50 parameter (50% inhibitory concentration) is defined as the concentration of the substrate which causes the loss of 50% of the activity of DPPH. The antioxidant power is determined so that an amount of the extract of a specific concentration neutralizes 50% of the DPPH radical. In order to compare the extracts with each other, this index is obtained either by deduction from the curves of the variation in the percentage of inhibition I% or calculated graphically by the formula for the regression of the percentages of the inhibition as a function of different concentrations of the extracts, tested using the Origine Pro 8 software. The value of the anti-free radical activity, such that y = 50%, corresponds to the IC50 inhibitory concentration of the extract studied [8] [9] [10]. It should be remembered that the lower the value of IC50, the greater the antioxidant activity of the extract [11]. The results expressed in IC50 were deducted from the data presented of the variation in the percentage inhibition I% depending on the concentration of each extract. It should be remembered that the lower the value of IC50, the greater the antioxidant activity of the extracts [11].

3. Results and Discussion

3.1. Thin-Layer Chromatography

The chromatographic profile of the hydro-ethanolic extract and the four (04) reference compounds (Quercetin, Rutin, Acide Caffeic Acid and Chlorogenic Acid) obtained after exposure of the plate to the UV-lamp (**Figure 2**) show a succession of the spots materializing the presence of polyphenolic compounds.

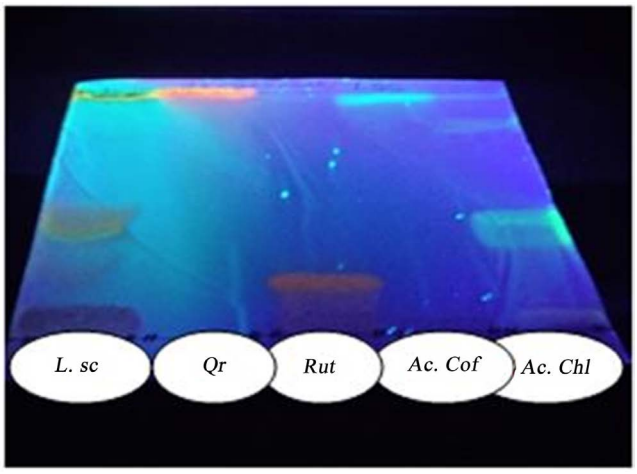


Figure 2. Chromatographic profiles of the hydro-ethanolic extract and some reference compounds.

Thin layer chromatographic analysis of the hydro-ethanolic extract of *L. schlechteri* showed the presence of a few chemical families. Specific developers were used; the staining of the spots was associated with the presence of a chemical family. On this CCM plate revealed at the node and observed at UV (365 nm) revealed the blue, bluish white spots which recall the presence of polyphenols, on the other hand, the white, pink, orange and green spots are characteristic of flavonoids. CCM analysis of *L. schlechteri* extracts in **Figure 2** revealed the presence of flavonoids, polyphenols. On the CCM plate obtained, the appearance of yellow, yellow-orange fluorescences correspondingto the reference compounds Quercetine (*Qr*) and caffeic Acid (AcCaf) is observed. In the middle, yellow and green fluorescences corresponding to the reference compound Chlorogenic Acid (Ac.Chl); and a little downwards an orange fluorescence for the reference compound Rutin (*Rut*). According to [12] [13], the color fluorescences observed on the various reference compounds (*Qr*, *Rut*, *Ac.Caf*, *Ac.Chl*) are characteristic of flavonoids and polyphenols.

3.2. Phenols and Flavonoids Content

The contant of total phenols and flavonoids in the various extracts of *Ledermanniella schlechteri* (**Figure 3**) was determined using separately colorimetric methods (Folin-Ciolcateaux and aluminum Trichlorure). The quantitative analysis of polyphenols gives content values of 5.85, 5.06 and 3.66 mgEAG/MS respectively for the ethanolic, hydro-ethanolic and aqueous extracts and those of the flavonoids in the same order of 1.15, 0.41 and 0.18 mgECa/MS. All the extracts are found to be rich in polyphenols and have low flavonoids. This difference in content between the different compounds can be explained by the fact that total polyphenols include flavonoids and other compounds. It is also observed that the alcoholic extracts are quantitatively richer in these phenolic compounds. According to the literature, alcoholic extracts are the richest in phenolic compounds, their high level of ethanolic and hydro-ethanolic extracts in our plant leads to the conclusion that alcohol remains the best solvent to extract these compounds. This affinity is supported by several studies [14]. This is

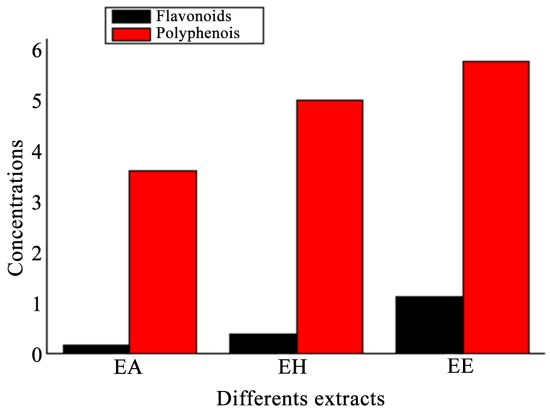


Figure 3. Dosage of total polyphenols and flavonoids in aqueous, hydro-éthanolic and ethanolic extracts.

due to the ability of alcohol to inhibit the action of polyphenol oxidase which causes the oxidation of polyphenols in plant tissues [15].

The quantitative analysis of total polyphenols and flavonoids shows that the ethanolic (EE) and hydro-ethanolic (HE) extracts are quantitatively richer in total polyphenols and flavonoids than in the aqueous extract (EA). The polyphenol content in the extracts are respectively 5.85 mgECa/MS for the ethanolic extract (EE), 5.06 mgECa/MS for the hydro-ethanolic extract (HE) and 3.66 mgECa/MS for the aqueous extract (AE) against 1.15 mgECa/MS for the flavonoids for the ethanolic extract (EE), 0.0406 mgECa/MS for the hydro-ethanolic extract (HE) and 0.18 mgECa/MS for the aqueous extract (AE). It is found that all the extracts of *L. schlechteri* are rich in polyphenols and have low levels of flavonoids. These differences in content between different compounds can be explained by the facts that total polyphenols include flavonoids and other compounds. It is also noted that the alcoholic extracts are quantitatively richer in phenolic compounds that is to say that this solvent extracts polyphenols better compared to other mixtures. The literature reports that it is in alcoholic extracts that we find more phenolic compounds [14]. The high level of these compounds in the ethanolic and hydro-ethanolic extracts leads us to conclude that alcohol remains the solvent of choice for extracting these compounds. This affinity is supported by [14]. This is due to the ability of alcohole to inhibit the action of polyphenols oxidase which causes the oxidation of polyphenols in plant tissues [15].

It can also be noted that the stationary phase used (polyamide 6-Fluka) made it possible to enrich these extracts with polyphenolic compounds. The high levels of total polyphenols and flavonoids obtained in the present study could be justified by the very clear evidence observed by thin layer chromatography (CCM) and the presence of these metabolites reported by several authors in the plant [16].

3.3. Anti-Free Radical Activity of the Different Extracts

3.3.1. Percent Inhibition of the DPPH Radical

The results of the anti-free radical activity of the various extracts on DPPH are shown in the series of **Tables 1-3**. The series of **Tables 1-3** shows at a low concentration of 1.25 mg/ml, the ethanolic (EE), hydro-ethanolic (HE) and aqueous (AE) extracts show percentages of reduction of DPPH, respectively 10.73%, 5.09% and 6.46% but at high concentrations from 20 mg/ml, we noted in the same order 52.13%, 38.24% and 15%. It is noted that the values of the anti-radica; activity increase according to the concentration in the extracts.

Table 1. Anti-free radical activity of the ethanolic extract of *Ledermanniella schlechteri*.

Ethanolic Extract (EE)					
Concentration (mg/ml)	1.25	2.50	5	10	20
Optical density (D.O)	0.65	0.64	0.60	0.49	0.35
Percent inhibition (%)	**10.73**	**11.55**	**17.05**	**33.01**	**52.13**

3.3.2. 50% Inhibitory Concentrations

The evaluation of the inhibitory concentration at 50% of the different extracts (Figure 4) gave 19.18 mg/mL of the ethanolic extract (EE), 26.15 mg/mL of the hydro-ethanolic extract (HE) and 66.66 mg/mL of the aqueous extract (AE). Based on these results, it was found that the IC50s of the ethanolic (EE) and hydro-ethanolic (HE) extracts are lower compared to that of the aqueous extract (AE). These low values of the 50% inhibitory concentrations (IC50) of the ethanolic and hydro-ethanolic extracts show that they are endowed with a greater antioxidant power than that of the aqueous extract and this explains why alcohol remains the best solvent for extraction for this study. This strong inhibition of free radicals from ethanolic and hydro-ethanolic extracts (Figure 4) could be justified by their high concentrations of phenolic compounds which are known to be powerful compounds having a reducing power of the free radicals [17] [18].

We can also note that anti-free radical activity is the opposite of anti-oxidant activity. In fact, polyphenolic compounds are reputed to be powerful compounds having a reducing power of free radicals [18].

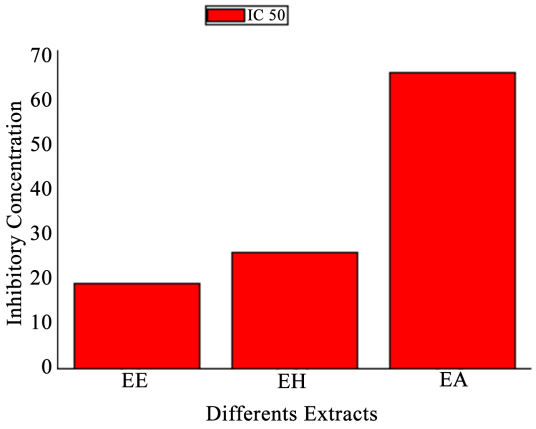


Figure 4. Evaluation of the anti-free radical activity in the different extracts.

Table 2. Anti-free radical activity of the hydro-ethanolic extract of *Ledermanniella schlechteri*.

Hydro-Ethanolic Extract (HE)						
Concentration (mg/ml)	1.25	2.50	5	10	20	40
Optical density (D.O)	0.69	0.68	0.65	0.61	0.45	0.17
Percent inhibition (%)	**5.09**	**6.33**	**11.00**	**15.68**	**38.24**	**64.48**

Table 3. Anti-free radical activity on the aqueous extract of *Ledermanniella schlechteri*.

Aqueous Extract (AE)							
Concentration (mg/ml)	1.25	2.50	5	10	20	40	80
Optical density (D.O)	0.68	0.68	0.67	0.64	0.62	0.50	0.29
Percent inhibition (%)	**6.46**	**7.15**	**7.84**	**12.65**	**15**	**30**	**60**

4. Conclusion

The target for this study was met. Evaluation of the antioxidant activity of extracts from this plant by TLC and by the method using DPPH revealed total polyphenols as well as flavonoids. The determination of polyphenols and total flavonoids on the three extracts EE, HE and EA showed that the ethanolic extract (EE) of the *Ledermanella schelchterie* plant has a high level of polyphenols (5.85 mgEAG/MS) and flavonoids (1.15 mgEAG/MS) totals compare to the other two HE and EA. On the other two extracts, we also note a high content of polyphenonols (5.06 mgEAG/MS) and less of flavonoids (0.46 mgEAG/MS). In general, this plant is rich in polyphenols but also in flavonoids. In addition, they are potentially rich in anti-radical compounds. These results allow us to say that this plant, which is already consumed by the Congolese population, must be popularized to draw a profile of the antioxidant potential that it abounds.

Acknowledgements

We thank all the managers and colleagues of the laboratories where we carried out our work, may they find here our deepest gratitude.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Nomenclature

EGA: Gallic Acid Equivalent
ECa: Catechin Equivalant
L.sc: *Ledermanniella schlechteri*
Qr: Quercetin
Rut: Rutin
Ac.Caf: Caffeic Acid
Ac.Chl: Chlorogenic Acid