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Phytochemical Screening and Antioxidant Activities of Water Lilies Seeds, Neglected and Underused Species in the Delta and Lower Valley of the Senegal River

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Abstract

Studies carried out in the delta and the lower valley have demonstrated, through ethnobotanical surveys, the importance of the species *Nymphaea lotus* L. and *Nymphaea micrantha* Guill. et Perr in the diet. The seeds are used in food and pharmacopoeia with a good appreciation by the indigenous population, especially in the diet of diabetics. However, studies on their biochemical aspects are still very rare in the literature. It is for this reason that this study was conducted to gather useful scientific information to allow a better appreciation of the pharmacological potentialities of these seeds. To do so, seeds were collected in ponds, tributaries of the Senegal River, dried in the shade, and divided into three different batches: a batch of red seeds of *N. lotus*, a batch of black seeds of *N. lotus*, and a batch of seeds of *N. micrantha*. Chemical screening, tannin, flavonoid, and polyphenol assays as well as antioxidant power of the seeds were carried out according to official standard methods. The results obtained in the qualitative analysis showed the presence of a high quantity of tannins, total phenols, flavonoids, and alkaloids, as well as a lower quantity of cardiac glycosides and the absence of anthracene compounds and saponosides in the seeds. The assay showed that Tannins are the highest (38.118 ± 0.021 to 118.132 ± 0.034 mg EAT/g DM) followed by flavonoids (24.057 ± 0.113 to 31.970 ± 0.087 mg EC/g DM) and polyphenols which are the lowest (0.544 ± 0.054 to 1.955 ± 0.120 mg EAG/g DM). In addition, these seeds have a high antioxidant power with high inhibition percentages, ranging from 83.92% ± 1.40% to 87.67% ± 0.16%. The promotion of their consumption could thus have a positive impact on the health of the

populations of the Senegal River valley.

Keywords

Water Lilies, Women of the Senegal River Valley, Food Security, Neglected and Underused Species (NUS)

1. Introduction

Cardiovascular disease is the leading cause of death worldwide with 80% of deaths directly related [1]. Traditional diets, largely based on plants, have been replaced by high-fat, energy-dense diets with a significant amount of meat-based foods [1]. This is one of the main risk factors for heart disease. In view of this, it is necessary to better consider the so-called neglected and underused plant species. These species exist locally and have considerable potential to cover the health care needs of the population [2] [3]. The fruits of Nymphaea lotus and Nymphaea micrantha fall into this group of neglected species and yet are very abundant in the fresh waters of the Senegal River valley. Much attention has been focused on their ornamental character through their domestication [4]. Recently, the therapeutic properties of *N. lotus* leaves have been demonstrated in the treatment of breast cancer in Côte d'Ivoire [5], in the regulation of blood pressure, and the restoration of erectile function [6]. More recent studies in the delta and lower valley of the Senegal River have revealed the use of *N. lotus* and *N. micrantha* seeds in the diet of diabetics [7] and their major nutritional compounds [8]. All these works show the potential of these two species. However, studies on their biochemical aspects, especially in polyphenols and their antioxidant power, are still very rare in the literature. It is for this reason that this study was conducted to gather useful scientific information to improve the valorization of these species.

2. Material and Method

2.1. Biological Material

For the study of biochemical characteristics, three batches of water lily seeds were constituted: a batch of black seeds and a batch of red seeds of *Nymphaea lotus*, and the third batch of seeds of *Nymphaea micrantha.* The first two lots (Lot 1) and (Lot 2) were collected from three tributaries of the Senegal River: "Ndiaye", "Mboudom" and "Ndiandane" (**Figure 1**). The third lot of *N. micrantha* seeds (Lot 3) was collected from two tributaries: "Saint-Louis" and "Ross-bethio" (**Figure 1**).

2.2. Research of the Groups of Chemical Compounds

2.2.1. Chemical Screening

1) Flavonoids

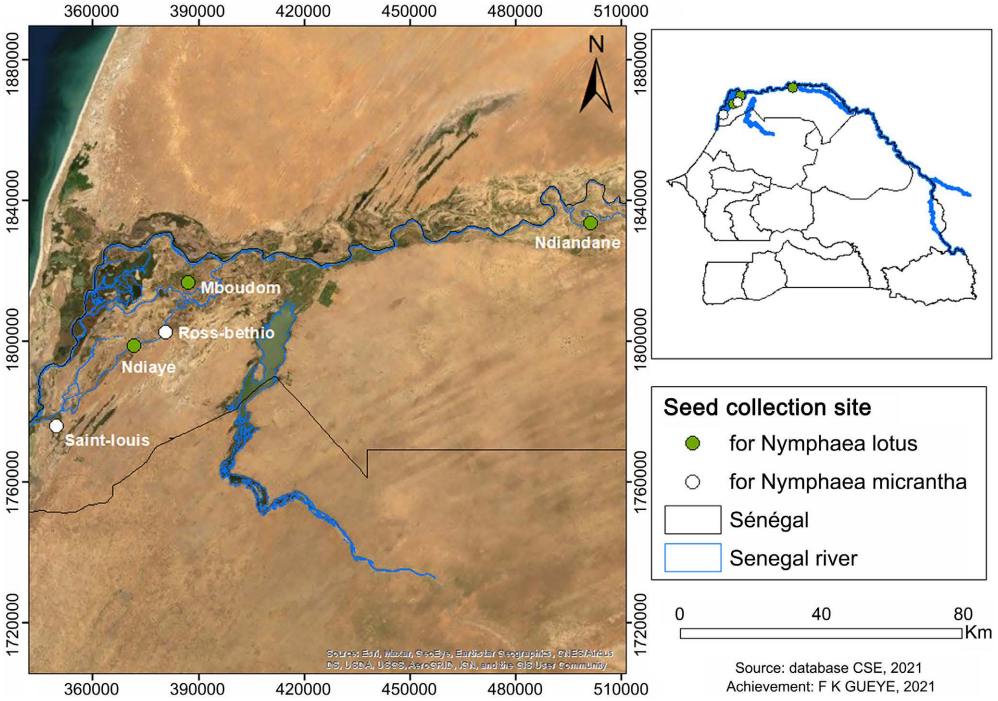

Figure 1. Collection place of the analyzed seeds.

Flavonoids were screened by the cyanidin reaction. Two (2) ml of each extract was evaporated and the residue was taken up in 5 ml of 2 times diluted hydrochloric alcohol. On addition of 2 to 3 chips of magnesium, there is a heat release and then a pink-orange or purplish coloration. The addition of three drops of isoamyl alcohol intensified this coloration which confirmed the presence of flavonoids [9].

2) Alkaloids

The tests are carried out by precipitation reactions with Dragendorff's reagent. 10 g of dry plant powder is introduced into an Erlenmeyer flask, to which 50 ml of H_2SO_4 diluted to 1/10 with distilled water is added. This mixture was stirred and macerated for 24 h. Then, in 1 ml of the filtrate, 5 drops of Dragendorff's reagent are added. The appearance of an orange precipitate reveals the presence of alkaloids [9].

3) Tannins

The presence of tannins is demonstrated by adding to 1 ml of each extract, 1 ml of water and 1 to 2 drops of Fecl3 solution diluted to 1%. The appearance of a dark green or blue-green coloration indicates the presence of tannins. The appearance of a dark green coloration indicates the presence of hydrolyzable tannins. The appearance of a blue-green color indicates the presence of condensed tannins [10].

4) Saponins

In a series of 10 test tubes numbered from 1 to 10, introduce respectively 1, 2, 3, …, 10 ml of the solution to be analyzed prepared by decoction in aqueous medium, hydroalcoholic or by infusion. Adjust the volume of each tube to 10 ml

with distilled water. Shake each tube lengthwise for 15 seconds at a rate of 2 shakes per second. Allow to stand for 15 min and measure the height of the foam produced in each tube.

The foam index (I) is calculated by the following formula:

$$I = 1000/N$$

N is the number of the tube where the foam height is equal to 1 cm [10].

5) Anthracene Derivatives

Anthracene derivatives were identified by the following procedure: Free Anthracenics were detected naked from one of the tubes, 0.5 ml is added NH_4OH at 25%. The fluorescence is observed under U.V at 366 nm [11].

Cardiac glycosides: Two ml of chloroform is added to 1 ml of the extract, the appearance of a reddish-brown coloration after the addition of H_2SO_4 indicates the presence of cardiac glycosides [11].

2.2.2. Quantitative Analysis of Polyphenols, Tannins and Flavonoids

The contents of total phenols were evaluated according to the method of Georgé *et al.* (2005) [12] while flavonoids are evaluated by the colorimetric method described by Kim *et al.* (2003) [13] and tannins by the method described by Joslyn (1970) [14]. The results are expressed as milligram of gallic acid equivalent per gram of dry matter (mgEAG/gMS) for phenols, milligram of catechin equivalent per gram of dry matter (mgEC/gMS) for tannins and milligram of quercetin equivalent per gram of dry matter (mg EQ/g MS) for flavonoids.

2.3. Evaluation of the Antioxidant Activity of Seeds

The antioxidant activity was evaluated with 2,2 diphenyl-1-pycrilhydrazyl (DPPH) following the method described by Oliveira *et al.* (2009) [15].

2.4. Statistical Analysis

All assays were performed in duplicate and statistical treatments were performed with R software. Statistical differences were considered significant with a probability less than 0.05 ($P < 0.05$).

3. Results and Discussion

3.1. Qualitative Phytochemical Screening of the Seed Extract

The screening reveals three major elements that are common to all analyzed water lily seeds, showing that they have an identical phytochemical profile (**Table 1**).

The two groups of tannins (hydrolyzable tannins and non-hydrolyzable condensed tannins), alkaloids and flavonoids are present in large quantities in all three types of seeds.

Cardiotonic heterosides are present but in lesser quantities.

Saponosides and anthracene compounds are not identified in the samples analyzed.

Table 1. Phytochemical groups in water lily seeds.

	Flav.	Tannins		Sap	A. D.	C. G.	Alc.
		Hydr.	Cond.				
Red seeds of *N. lotus*	++	++	++	−	−	+	++
Black seeds of *N. lotus*	++	++	++	−	−	+	++
N. micrantha	++	++	++	−	−	+	++

Flav: flavonoids; Hydr: hydrolysable; Cond: condensed; Sap: saponins; Alc: alkaloids; A.D: Anthracene derivatives; C.G: Cardiac glycosides; ++: Very positive reaction (large quantity); +: Moderately positive reaction (small quantity); − Negative reaction (no reaction).

The presence of tannins, alkaloids, flavonoids as well as cardiotonic compounds would partly explain the use of seeds of *N. lotus* and *N. micrantha* species in the diet of diabetics in the delta and lower valley of the Senegal River [16]. These elements were also reported in the species *Nymphaea lotus* by Nicodème *et al.* (2016) [16].

These phytochemicals have many health benefits. Indeed, tannins have an astringent effect and manifest properties of vitamin P. They strengthen blood vessels and contribute to the accumulation of vitamin C in the body [5]. Some alkaloids enhance cardiac activity, excite the central nervous system, symptomatic nerves and stimulate blood circulation [17]. Flavonoids are well known as antioxidants [18] [19]. They are also anti-ulcer, anti-tumor, anti-spasmodic, anti-secretory and anti-diarrheal [20], anti-allergic, anti-inflammatory, hypotensive and protect against cancer and cataract [21]. They also have aphrodisiac properties [22]. Polyphenols have a potassium retention effect and therefore have a diuretic activity [23]. Cardiac glycosides have a fundamental therapeutic action which is the increase of cardiac output [11]. The presence of all these elements supports the hypothesis that the use of seeds would be good in the diet of diabetics. The extracts from *N. lotus* would act in the regulation of blood pressure [6]. Thus, this additional information would encourage efforts to promote the widespread use of water lily seeds, at least in endemic areas.

3.2. Quantitative Analyses of Phytochemicals

Quantitative estimation of some phytochemicals present in the methanolic extract of *N. lotus* and *N. micrantha* shows according to **Table 2**, that tannins are the highest (38.12 ± 0.021 to 118.13 ± 0.034 EAT/g DM) followed by flavonoids (24.06 ± 0.113 to 31.97 ± 0.087 mg EC/g DM) and total polyphenols are the lowest (0.54 ± 0.054 to 1.95 ± 0.120 mg EAG/g DM).

Tannin determination revealed statistically variable amounts among seed types (p value < 0.05%) (**Table 2**). Black and red *N. lotus* seeds had the highest values (118.132 ± 0.034 mg EAT/g DM and 89.904 ± 0.037 mg EAT/1 g DM, respectively). These results agree with the studies of Mourtala *et al.* (2019) [24] who found a content of 90.00 ± 0.50 mg/g in the methanolic extract of *N. lotus*

Table 2. Determination of polyphenols in seeds of species of the genus Nymphaea.

seed Type /Polyphenol	Tannins (mg EAT/g DM)	total polyphenols (mg EAG/g DM)	Flavonoids (mg EC/g DM)
N. micrantha	38.12 ± 0.021[c]	0.544 ± 0.054[a]	27.03 ± 0.074[b]
N. lotus (Black seeds)	118.13 ± 0.034[a]	1.95 ± 0.120[a]	24.057 ± 0.113[c]
N.lotus (Red seeds)	89.904 ± 0.037[b]	1.398 ± 0.082[a]	31.970 ± 0.087[a]
Mean value	**82.051 ± 1.82**	**1.299 ± 0.08**	**27.684 ± 079**
p-values	0.00208	0.1386	0.00476

a, b, c: In the same column, means followed by a different letter are significantly different at the p < 5% threshold (Newman and Keuls method). DM: Dry Matter.

rhizome. This bioactive compound is known to have potential antiviral activity [25] as well as potential prophylactic and therapeutic effect against cancer cells [26]. The high amounts found in this study would justify the antibacterial effects [27] and cytotoxic effects on cancer cells reported in *N. lotus* species by Ashidi *et al.* (2010) [28].

Total polyphenols are in almost similar amounts in the three types of seeds, with values ranging from 0, 54 ± 0.054 to 1, 95 ± 0.120 mg EAG/g DM. Compared to the results obtained with dates, considered rich in phenolic compounds (566 mg/100g) [29] and those of grape seeds (750 to 4040 mg/100g) [30], it is easy to say that the seeds of *Nymphaea sp.* are quite rich in total polyphenols. Polyphenols are considered a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups [31].

A high amount of flavonoid was found in all three types of seeds tested but varying considerably among seed types (**Table 2**). The highest value was found in red *N. lotus* seeds (31.970 ± 0.087 mg EC/g DM) while black *N. lotus* seeds had the lowest value (24.057 ± 0.113 mg EC/g DM). *N. micrantha* seeds had a content of 27.026 ± 0.074 mg EC/g DM. However, these values are lower than those from the work of Murtala *et al.* (2009) [24] who found a flavonoid content of 80.0 mg EC/g in the rhizome of *N. lotus*. This difference could be due to the difference in the organ tested. Flavonoids have many useful properties. They generally serve as flavoring ingredients in plants [24] and are also expressed in plants in response to microbial infection, suggesting their antimicrobial activity [32]. Flavonoids are also antioxidants such as those in tea that are believed to reduce oxidation of low-density lipoproteins and lower blood cholesterol and triglyceride levels [33].

3.3. Antioxidant Activity of Selective Extracts

The methanolic extracts show a notable antioxidant potential, regardless of the type of seed with a non-significant variation (**Table 3**). All the inhibition percentages are high ranging from 83.92% ± 1.40% to 87.67% ± 0.16%. However, black *N. lotus* seeds have significantly the highest antioxidant activity compared

Table 3. Antioxidant activity of seeds of *Nymphea sp.*

Seed type	Unit	Seeds of *N. micrantha*	Black seeds of *N. lotus*	Red seeds of *N. lotus*	p-value
Activity Antioxidant	(%)	83.92 ± 1.40**a**	**87.67** ± 0.16**a**	**86.63** ± 0.88**a**	0.21967

to the other two seed types. These differences appear to be correlated with genotypic and phenotypic differences [34]. The synergistic action of tannins, total phenols, flavonoids found would explain the antioxidant power of plant extracts [35]. Previous work by Aliyu *et al.* (2018) on the in vitro antioxidant potential of oils extracted from the seeds of *Nymphaea lotus* and *Nymphaea pubescens* has prompted their use in the treatment of diseases caused by oxidative stress.

4. Conclusion

This study is a contribution to the knowledge of neglected and underused species in order to better understand their various virtues. The recognition and pharmacological interest of *N. lotus* seeds and *N. micrantha* seeds are well established. It was demonstrated in this study, through chemical screening, the presence of total phenols, tannins, flavonoids, alkaloids, and cardiotonic compounds in the seeds of both species studied. The results obtained make it possible to deduce the presence of a high quantity of polyphenols, thus confirming their pharmacological qualities. Moreover, these seeds have a strong antioxidant power due to the synergistic action of tannins, total phenols, and flavonoids found in high quantities. However, in situ biological tests in the delta and lower valley of the Senegal River should make it possible to evaluate the "health" impact of these seeds on the population, as well as the domestication of *Nymphaea.*

Authors' Contributions

GUEYE Fatou Kine is the main author of the article, she published part of her already defended thesis; Niang Lahat helped with the biochic analyses and thoroughly corrected the content, Gueye Modou FALL and DIENG Birane helped with the data collection and analyses; AYESSOU Nicola Cyrilles and MBAYE Mame Samba supervised the work and did the total revision of the article, they also accepted to be responsible for all aspects of the work; NOBA Kandioura is the head of the laboratory that carried out this work and read and validated the aspects developed.

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Conflicts of Interest

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

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