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The *In vitro* Screening for Acetylcholinesterase Inhibition by Extracts from *Sesbania pachycarpa* DC & *Sesbania rostrata* Bremek. & Oberm. (FABACEAE), Used in Traditional Medicine in Burkina Faso

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Abstract: The aim of this study is to evaluate the potential of extracts from *Sesbania pachycarpa* DC and *Sesbania rostrata* Bremek. & Oberm. to inhibit the acetylcholinesterase involved in Alzheimer's disease. Aqueous extracts, methanolic extracts and hydro-acetonic extracts of leaves, stem, seeds, pod and root from *Sesbania pachycarpa* DC and *Sesbanaia rostrata* Bremek. & Oberm. were tested for their ability to inhibit acetylcholinesterase. Inhibition of acetylcholinesterase was tested by the method described in the Material and mehtods. Some extracts had an acetylcholinesterase inhibitory activity greater than or equal to 50%. These are, for *Sesbania pachycarpa* DC: aqueous extracts of stem (50.33%), aqueous extracts of pod (51.18%), methanolic extracts of stem (65.34%), methanolic extracts of pod (58.81%), hydroacetone extracts of stem (79, 06%); with extracts of *Sesbania rostrata* Bremek. & Oberm: methanolic stem extracts gave 68.98% inhibition of acetylcholine esterase.

Key words: Acetylcholine Esterase · Inhibition · Extracts · S*esbania pachycarpa* · *Sesbanaia rostrata*

INTRODUCTION

Inhibition of acetylcholine esterase is considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease. Many plants and identified molecules are a potential source of acetylcholine inhibitors, Lopez *et al.* and Ingkaninam *et al.* and Risa *et al.* and Mukherjee *et al.* [1-4].

Acetylcholine and dopamine are the most well-known neuromediators that allow the coordination of movements. Their absence causes respectively Alzheimer's disease and Parkinson's disease. At the synapses, acetylcholine is rapidly inactivated and degraded by an enzyme, acetylcholine esterase. The reaction of acetylcholine esterase on acetylcholine provides acetyl and choline, [5]. However, the decrease of acetylcholine (by the enzyme, acetylcholine esterase), causes a decrease in the memory observed in Alzheimer's disease.

No treatment today cures Alzheimer's disease, nor even stops its evolution. There are inhibitors of acetylcholine esterase. A variety of enzymes called cholinesterases rapidly inactivate acetylcholine, so acetylcholine cannot be used in drug form. The strategy is to inhibit these enzymes, especially acetylcholine esterase. Inhibitors used are structural analogues of acetylcholine, Courtney *et al.* [6].

Knowledge of the neurotransmitter disturbances in Alzheimer's disease has led to the development of drugs with symptomatic effects, Blennow *et al*. [7]. The drugs approved for the Alzheimer disease therapy act by counteracting the acetylcholine deficit that is the try to enhance the acetylcholine level in the brain, Heinrich and Teoh [8].

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We study two species of *Sesbania* (FABACEAE) used in traditional medicine in Burkina Faso: *Sesbania pachycarpa* DC*, Sesbania rostrata* Bremek & Obern*.* Several extracts tested showed levels of inhibition of acetyl cholinesterase important to have observed special attention.

MATERIALS AND METHODS

This research was conducted at the University Ouaga I, Pr Joseph KI-ZERBO, (Burkina Faso), UFR/SVT, department of Biochemistry-Microbiology, in the Laboratory of Applied Chemistry and Biochemistry, specializes in medicinal plants. The studies were conducted from March to July 2017.

Biological Materials: Leaves, stems, granulate pods and roots of *Sesbania pachycarpa* DC*, Sesbania. rostrata* Bremek & Obern. were collected at Burkina Faso. The vegetable specie was identified by the botanists of the University Ouaga 1 Pr Joseph KI-ZERBO. Parts of plants were dried during ten days at the laboratory at a temperature of surroundings 30°C, pulverized and preserved in plastic.

Preparation of Extracts and Chemical Screenings: Aqueous, methanolic and hydro-acetone extractions were made as described by Ouattara *et al.* [9, 10].

Thin Layer Chromatography (TLC): Thin layer chromatography for phenolic acid and flavonoid was realized by Wagner and Bladts [11] and Medié-Sarié *et al*. [12] methods by using plates (silica gel 60F254, KIESEL GEL, 10 cm x 10 cm) which spotted by standards and samples. The system of migration used is ethyl acetate/formic acid/acetic acid glacial/ water (7/1.1/1.1/2).

Determination of Total Phenolics: Spectrophotometrical method described by Lamien-Meda *et al*. [13] was used to quantify polyphenol in extract. Briefly, 100 µL of extract, 500 µL of reagent of Folin-Ciocalteu (0.2N) were mixed and incubated during 5 minutes, following by adding 400 µL of aqueous sodium carbonate solution (75g/l). After dark incubation the absorbencies were read at 760 nm. The gallic acid is used as standard for the establishment of the curve ($y = 0.0095x$, with $R^2 = 0.99$). The results were expressed as standard for the establishment of the curve ($y = 0.0095x$, with $R^2 = 0.99$).

Acetylcholinesterase Inhibition: The procedure described by Lopez *et al*. [1], inspired by the method of Ellman *et al*. [14] has been slightly modified. Acetylcholinesterase hydrolysis the substrate ATCI (AcetylThioCholine Iodide) in non-colored, the thiocholine and acetate. The thiocholine in the presence of DTNB (5, 5 '-Dithiobis-2-nitrobenzoic acid) gives a yellow product, 5-thio-2-nitrobenzoate, which allows to follow the kinetics spectrophotometer at 405nm.

The reaction mixture consisting of buffer 200µl, 100µl of extract (1mg/ml) and 100µl of enzyme (0.22U/ml) was incubated at room temperature for 5 min. Then 500µl of DTNB (3 mM) and 100µl of ATCI are added and the kinetics is followed at 405 nm in a spectrophotometer for 3 minutes. The percentages of inhibition were calculated relative to a negative control (without inhibitor) according to Equation 2. The reference inhibitor, galanthamine, is used.

Statistical Analysis: Data were averages of three results ± standard deviations (SD) by using Microsoft Excel. Analyses of variance (ANOVA), the Tukey HSD Test were carried out using XLSTAT 7.1 and $p < 0.05$ values were considered statistically significant. For correlation studies, Pearson's correlation test was used and $p < 0.05$ values were considered statistically significant.

RESULTS AND DISCUSSION

Inhibition of Acetyl Cholinesterase: The rate of inhibition of acetylcholinesterase is shown in Table 1 and Table 2, respectively for extracts from *Sesbania pachycarpa* DC or *Sesbania rostrata* Bremek. & Oberm. The leaves, stems, seeds, pods, roots of these plants have been extracted by water (decoction), methanol and hydro-acetone.

Some extracts showed a remarkable inhibitory activity greater than 50% inhibition at a concentration of 1 mg / ml. Some extracts had an acetylcholinesterase inhibitory activity greater than or equal to 50%. These are, for *Sesbania pachycarpa* DC: aqueous extracts of stem (50.33%), aqueous extracts of pod (51.18%), methanolic extracts of stem (65.34%), methanolic extracts of pod (58.81%), hydroacetone extracts of stem (79, 06%); with extracts of *Sesbania rostrata* Bremek. & Oberm: methanolic stem extracts gave 68.98% inhibition of acetylcholinesterase.

Extracts that inhibited acetylcholineterase therefore contain compounds responsible for the observed inhibitory activity.

Table 1: Inhibition of extracts from *Sesbania pachycarpa* DC on Acetylcholinesterase (n = 3)

Species	Organ	Extract	Inhibition of acetyl cholinesterase (%)
S. pachycarpa	Leaves	Aqueous	35.12 ± 01.05
S. pachycarpa	Stems	Aqueous	50.33 ± 01, 80
S. pachycarpa	Granulates	Aqueous	51.18 ± 00.65
S. pachycarpa	Pods	Aqueous	03.01 ± 01.45
S. pachycarpa	Roots	Aqueous	27.15 ± 01.12
S. pachycarpa	Leaves	Methanolic	04.49 ± 01.50
S. pachycarpa	Stems	Methanolic	65.34 ± 00.15
S. pachycarpa	Granulates	Methanolic	25.45 ± 07.23
S. pachycarpa	Pods	Methanolic	58.81 ± 10.07
S. pachycarpa	Roots	Methanolic	35.40 ± 03.45
S. pachycarpa	Leaves	Acetonic	79.06 ± 05.39
S. pachycarpa	Stems	Acetonic	49, 53 ± 00, 50
S. pachycarpa	Granulates	Acetonic	23, 15 ± 02, 35
S. pachycarpa	Pods	Acetonic	47, 56 ± 05, 41
S. pachycarpa	Roots	Acetonic	37, 51 ± 01, 15

Table 2: Inhibition of extracts from *Sesbania rostrata* Bremek. & Oberm. on Acetylcholinesterase (n = 3)

Species	Organ	Extract	Inhibition of acetyl cholinesterase (%)
S. rostrata	Leaves	Aqueous	09.90 ± 01.80
S. rostrata	Stems	Aqueous	03.78 ± 00, 55
S. rostrata	Granulates	Aqueous	35.39 ± 06. 81
S. rostrata	Pods	Aqueous	09.18 ± 00.86
S. rostrata	Roots	Aqueous	21.15 ± 01.17
S. rostrata	Leaves	Methanolic	12, 74 ± 01, 18
S. rostrata	Stems	Methanolic	68, 90 ± 02, 59
S. rostrata	Granulates	Methanolic	21, 74 ± 04, 97
S. rostrata	Pods	Methanolic	13, 32 ± 00, 94
S. rostrata	Roots	Methanolic	17, 12 ± 01, 07
S. rostrata	Leaves	Acetonic	25, 70 ± 03, 55
S. rostrata	Stems	Acetonic	30, 75 ± 03, 82
S. rostrata	Granulates	Acetonic	31, 50 ± 01, 40
S. rostrata	Pods	Acetonic	20, 33 ± 05, 17
S. rostrata	Roots	Acetonic	18, 15 ± 01, 60

The extracts contain alkaloids and flavonoids including rutin and quercetin, by layer chromatography and phytochemicals studies, [9], [10]. Researchers have demonstrated the role of these molecules in the fight against the disease of Alzheimer disease. The role of alkaloids as inhibitors of acetylcholinesterase has been demonstrated in the work of Blennow *et al*. [7], Kherjee *et al*. [15], Zhang *et al*. [16] have shown that quercetin and/or rutine would target ROS (*Reactive oxygen species*), metal ions, xanthine oxidase, protein tau, monoamine oxidase. All these targets are involved in Alzheimer disease.

Results of total phenolics compounds, tannins and flavonoids are published in our previous studies, Ouattara *et al.* [9, 10]. We found a correlation between the anti-radical activity and antioxidant levels of polyphenols. There were also a correlation between the flavonoids and the inhibitory activity of the inhibitory activity of acetylcholinesterase. Lamien-Meda *et al*. [13] were found a correlation between antioxidant activity and the levels of polyphenols.

Studies have shown a relationship between oxidative stress and Alzheimer's disease, Gibson and Huang [17].

However, there are a negative correlation between tannins and the inhibitory activity observed.

CONCLUSION

Research on plants that have an inhibitory effect of acetyl cholinesterase has great interest since the discoveries in this area will develop a drug with some efficacy in the fight against the Alzheimer disease and other diseases related to disorders nerve. Our studies have yielded an extract which has a potential inhibitor of acetylcholinesterase and we will conduct studies to identify the molecules responsible for this activity.

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