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Evaluation of Xanthine Oxidase Inhibition by Extracts from *Sesbania pachycarpa* DC and *Sesbania rostrata* Bremek. & Oberm

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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 xanthine oxidase, which plays a positive role in metabolic reactions and defense of the body, but an enzyme involved in several pathologies of the body (generation of free radicals involved in oxidative stress), in the inflammatory reaction and other pathologies. The inhibition of xanthine oxidase in the extracts was evaluated. Several types of extracts were tested: aqueous extracts, methanolic extracts and hydro-acetonic extracts of leaves, stem, seeds, pod and root for both plant species. Some extracts had an xanthine oxidase inhibitory activity greater than or equal to 50%. These were, for *Sesbania pachycarpa* DC: aqueous extracts of granulates (57.79 %), aqueous extracts of pods (51.77%), methanolic extracts of stems (64.40%); these were, for extracts from *Sesbania rostrata* Bremek. & Oberm: aqueous extracts for granules (58.28%) and aqueous extracts for pods (55.72%).

Key words: Xanthine Oxidase · Inhibition · Extracts · *Sesbania pachycarpa* · *Sesbanaia rostrata*

INTRODUCTION

Xanthine oxidase (XO) is one of two forms of xanthine oxidoreductase (XOR) that is widely distributed in living beings. Each of the two forms prefers an electron acceptor different from that of the other form. Xanthine dehydrogenase (XDH) prefers the reduction of NAD^+ (or $NADP^+$) and xanthine oxidase prefers O_2 reduction, Harrison [1]. The conversion of XDH into XO is possible and is done by different ways. The XOR gene is highly expressed in the liver, the small intestine where it has high activity, Wright *et al.* [2] and the mammary gland, Linder, Rapola and Raivio [3].

Xanthine oxidase is a biological source of free radicals from oxidation and causing tissue damage and involved in many pathological processes as arteriosclerosis, cancer, Wu and Ng [4], Havlik *et al.* [5]. During inflammation, XOR is activated by TNF-α and C5a, which cause the conversion of XDH to XO, Friedl *et al.* [6] which generates the production of ROS or "Reactive oxygen species", that is, reactive oxygen derivatives, Burdon and Gill [7]. XOR-produced ROS can also react with cell membrane compounds, such as arachidonic acid, to produce chemo-attracting neutrophil lipids, thereby enhancing the inflammatory response, Perez *et al.* [8]. In rheumatoid arthritis, XOR amplifies synovial inflammation leading to bone erosion and spread of the disease, Blake *et al.* [9].

Some works have shown the role showed the role of inhibition of this enzyme for the fight against gout and hepatitis, Song *et al.* [10] or other pathologies, Wortmann [11].

We study two species of *Sesbania* (FABACEAE) are used to treat schistosomiasis in traditional medicine in Burkina Faso: *Sesbania pachycarpa* DC*, Sesbania rostrata* Bremek & Obern*.* Some extracts tested showed levels of inhibition of xanthine oxidase that is important to have observed special attention.

MATERIALS AND METHODS

This research was conducted at the University of Ouagadougou (Burkina Faso), UFR/SVT, department of Biochemistry-Microbiology, in the Laboratory of Applied Chemistry and Biochemistry, specializes in medicinal plants.

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Biological Materials: Leaves, stems and granulates pods and roots of *Sesbania pachycarpa* DC*, Sesbania rostrata* Bremek. & Oberm. were collected at Burkina Faso. 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 are made as described by Ouattara *et al.* [12, 13].

Determination of Total Phenolics: Spectrophotometrical method described by Lamien-Meda *et al.* [14], 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 5mn, 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).

Antioxidant Activity by DPPH and FRAP Assays: For the DPPH assay, determination of the antioxidant activity of the extracts was realized by DPPH method of Lamien-Meda *et al.* [14] with light modifications. Briefly 250 µL of variation concentration extract in methanol and 500 µL of the solution of DPPH (20mg/l) were incubated during 10 min. The absorbance was read at 517 nm and the percentage of inhibition calculated in order to determine the concentration that was able to inhibit 50% graphically.

For the FRAP assay, the iron (III) reduction ability of extract was performed according to Lamien-Meda *et al*. [14]. Briefly, 0.5 mL of each extract (1 mg/mL) was mixed with 1.25 mL of phosphate buffer and 1.25 mL of aqueous potassium hexacyanoferrate solution (1%). After 30 min incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 2000 × rpm for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared $FeCl_3$ solution (0.125 mL, 0.1%). Absorbencies were read at 700 nm and ascorbic acid was used to produce the calibration curve (Y= 0.008x-0.0081; R^2 = 0.99). The iron (III) reducing activity determination was determined in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of extract.

Inhibition of Xanthine Oxidase: The inhibition of xanthine oxidase in the extracts was evaluated using the procedure described by Owen and Timothy [15] with some modifications. The reaction mixture was formed by 150ìl of phosphate buffer (0.066 M, pH 7.5), 50 L of extract solution (1mg/ml), 50 L of enzyme solution (0.28U/ml) After incubation 3 min at room temperature (25°C), the reaction was initiated by addition of 250 L of substrate (xanthine, 0.15mm) and the kinetics was measured at 295 nm for 2 min against a blank without enzyme. The initial rates were used to calculate the percentages of inhibition compared to a negative control (without extract). Allopurinol was used as reference inhibitor. We used the following equation to evaluate de inhibition percentage.

$I (\%) = (V_0 \text{ control} - \text{sample } V_0) \times 100/V_0 \text{ control}$

V_0 is the control enzyme activity in the absence of the extract solution

V_0 sample enzyme activity in the presence of the extract solution

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

The rate of inhibition of xanthine oxidase is shown in Table 1 and Table 2, respectively for extracts from *Sesbania pachycarpa* DC and *Sesbania rostrata* Bremek. & Oberm.

We studied the inhibition of XO, because the species studied are shown in traditional medicines in the fight urinary schistosomiasis and some inflammatory diseases.

The extract it which exceeded the rate of inhibition of 50% was for:

- *Sesbania pachycarpa* DC: aqueous extracts of granulates (57.79 %), aqueous extracts of pods (51.77%), methanolic extracts of stems (64.40%);
- *Sesbania rostrata* Bremek. & Oberm: aqueous extracts for granules (58.28%) and aqueous extracts for pods (55.72%).

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

Species	Organe	Extract	Inhibition of Xanthine oxydase (%)
S. pachycarpa	Leaves	Aqueous	38.12 ± 00.93
S. pachycarpa	Stems	Aqueous	33.27 ± 02.8
S. pachycarpa	Granulates	Aqueous	57.79 ± 00.97
S. pachycarpa	Pods	Aqueous	51.77 ± 02.16
S. pachycarpa	Roots	Aqueous	34.51 ± 01, 37
S. pachycarpa	Leaves	Aqueous	37.44 ± 00.99
S. pachycarpa	Stems	Aqueous	54, 40 ± 00, 61
S. pachycarpa	Granulates	Aqueous	13, 71 ± 03, 30
S. pachycarpa	Pods	Aqueous	05, 57 ± 01, 17
S. pachycarpa	Roots	Aqueous	19, 41 ± 03, 42
S. pachycarpa	Leaves	Methanolic	37, 30 ± 02, 78
S. pachycarpa	Stems	Methanolic	11, 38 ± 00, 65
S. pachycarpa	Granulates	Methanolic	22, 32 ± 05, 32
S. pachycarpa	Pods	Methanolic	48, 31 ± 07, 31
S. pachycarpa	Roots	Acetonic	41, 30 ± 07, 81

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

Species	Organe	Extract	Inhibition of Xanthine oxydase (%)
S. rostrata	Leaves	Aqueous	1.20 ± 00, 51
S. rostrata	Stems	Aqueous	13.21 ± 04. 32
S. rostrata	Granulates	Aqueous	58.28 ± 00.86
S. rostrata	Pods	Aqueous	55.72 ± 12.22
S. rostrata	Roots	Aqueous	43, 51 ± 03, 62
S. rostrata	Leaves	Methanolic	15, 44 ± 03, 55
S. rostrata	Stems	Methanolic	33, 93 ± 00, 28
S. rostrata	Granulates	Methanolic	30, 70 ± 01, 23
S. rostrata	Pods	Methanolic	03, 80 ± 01, 01
S. rostrata	Roots	Methanolic	12, 31 ± 05, 42
S. rostrata	Leaves	Acetonic	01, 78 ± 00, 81
S. rostrata	Stems	Acetonic	03, 74 ± 00, 60
S. rostrata	Granulates	Acetonic	17, 45 ± 02, 31
S. rostrata	Pods	Acetonic	19, 62 ± 03, 12
S. rostrata	Roots	Acetonic	22, 37 ± 01, 51

The other extracts did not give significant inhibition of xanthine oxidase. Most of the extracts had no inhibitory effect of xanthine oxidase. We are currently doing tests to identify the components that exist in the extracts which have an inhibitory activity of the enzyme.

The search for xanthine oxidase inhibitors does not mean that the enzyme does not play beneficial roles for the body. The complexity of the search for inhibitors for xanthine oxidase is based on the fact that this enzyme is involved in beneficial reactions and in diseases of the body, Perez *et al.* [8], Blake *et al.* [9], Song *et al.* [10]. Also, xanthine oxidase is one of the two convertible forms of xanthine oxidoreductase involved in several metabolic reactions. Therefore, by acting on xanthine oxidase, several metabolic pathways may be negatively affected, either positively or negatively and we will only mention the main reactions that may be modified.

The studies of Wortmann [11] have shown the role of inhibitors of this enzyme in the fight against gout, hepatitis. Allopurinol is given as a treatment for gout by administration with diet and drugs, Ouattara *et al.* [12]. Flavonoids are natural inhibitors of XOR isolated from plants. Studies have shown that flavonoids competitively inhibit XOR, Da Silva *et al.* [16]. Studies on the inhibition of XO by flavonoids have theoretically demonstrated the existence of structural similarities between the flavonoid ring A and the purinic nucleus of hypoxanthine and xanthine, Rastelli *et al*. [17].

From our previous results on the level of phenolic compounds and antioxidant activity studies, Blake *et al.* [9], Song *et al.* [10], we evaluated the correlation between the different parameters. Some of our extracts showed a small correlation ($R^2 = 0.74$) between flavonoid levels and inhibition of xanthine oxidase (results no presented here).

CONCLUSION

We identified extracts having inhibitory activity of xanthine oxidase. This enzyme is involved in the drop and release of free radicals during the oxidation of oxygen during metabolism. The use of these species of Sesbania in inflammatory diseases and urinary tract in traditional medicine could be justified by their role of inhibition of xanthine oxidase and antioxidant.

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