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Biological activities and phytochemical analysis of extracts *Afrostyrax lepidophyllus* Mildbr. seeds

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ABSTRACT

Oxidative stress, one of the causes of certain diseases, prompts the search for new antioxidant molecules to reduce or limit the damage caused by it. In this study, the antioxidant, anti-inflammatory and anti-xanthine oxidase activity of the extracts (cyclohexane, dichloromethane, ethyl acetate and methanol) of the *Afrostyrax lepidophillus* seeds used in the human diet and the Central African Pharmacopoeia Been measured. Total pholyphenols, flavonoids, tannins and anthocyanins were also quantified in this work .The IC50 (inhibitory concentration which reduces free radicals by 50%) of the antioxidant activity of their extracts are respectively of the order of 3890,5±4,3, 3313,8±3,5, 1872,8±1,0 et 248,4±0,1 mg/L. The phytochemical analysis of the various extracts (cyclohexane, dichloromethane, ethyl acetate and methanol) made it possible to quantify the polyphenol content of 13,1±0,4, 30,5±0,5, 51,8±1,6 et 33,2±4,0 mg eq AG/g Dry matter (MS). The tannin content is of the order of 2,35±0,3, 10,68±0,1, 7,78±0,2, mg eq Cat/g MS, That of anthocyanins is 0.79 ± 0.04, 0.65 ± 0.02, 1.65 ± 0.07 and 0.18 ± 0.03 mg eq C3GE / g MS. The analysis of the different correlations between the chemical families and the antioxidant activity showed that there is a weak correlation between the biological activity and the phenolic compounds.

Keywords: Biological activity, Phytochemistry, Extracts, *Afrostyrax lepidophyllus.*

INTRODUCTION

The Central African Republic has a great floristic biodiversity to which is added a secular tradition of traditional pharmacopoeia. Several local populations use these plant or animal resources to treat certain pathologies [1]. Among these diseases, we can cite diseases related to oxidative stress. Indeed, oxidative stress is considered as a factor responsible for several diseases [2]. Oxidative stress (II) causes, with age, the production of free radicals which are the cause of diseases such as diabetes, cancer Alzheimer's disease and cardiovascular diseases [3]. Given the role that these radicals play in the generation of diseases and despite many modern medicines that exist today, diseases related to oxidative stress remains a serious public health problem. The use of plants for the research of new molecules with antioxidant properties is an important issue.

Afrostyrax lepidophyllus is a plant of the huaceae family and is commonly found in Equatorial and Tropical Africa [4]. This plant is used in Congo as an antiseptic and in traditional medicine for the treatment of gastroenteric diseases [5]. In the Central African Republic, the seeds of this plant are traditionally used as a spice. Moreover, pharmacological studies have been carried out by several researchers and have shown that the extracts of seeds of *A. lepidophyllus* possess very interesting properties. [6] demonstrated antifungal activity and identified Afrostyraxthioside A, Afrostyraxthioside B and Afrostyraxthioside C. The work of [7] showed that seed extracts possess an antioxidant property. Also, the studies carried out by [8] showed the insecticidal activity of the extracts of the seeds of *A. lepidophyllus*. [9] showed that the essential oil of *A. lepidophyllus* seeds reduces free radicals (DPPH and ABTS) and also evaluated the cytotoxicity of this oil against a panel of cancer cell line. This work aims at making a phytochemical study of the extracts of *A. lepidophyllus* seeds and then evaluating their biological activity.

MATERIALS AND METHODS

Reagents and products

Products and reagents used for the various analyzes are provided by Sigma-Aldrich-Fluka (Saint Quentin France).

Collection of plant material

A. lepidophyllus seeds was collected in October 2016 in southern Central African Republic, precisely M'Baiki (Boukoko). The specimen (AFM102012) was identified by botanist (Dr Olga Yongo and Dr Denis Beina) in the Department of Life Sciences and the Center for Studies in Pharmacopoeia and Traditional Medicine (CERPHAMETRA) from the University of Bangui.

Preparation of extract

The plant sample was dried in the shade at room temperature and was then put into powder. 200 g of this powder was macerated successively with 2 liters for 4 hours with different solvents of increasing polarity: cyclohexane, dichloromethane, ethyl acetate and methanol. The extracts were concentrated by a rotary evaporator under vacuum at 35 ° C.

Determination of total phenolics

The total polyphenols of extracts were determined by the method of Folin-Ciocalteu George described by [10]. 20 µL of solution was mixed with 100 µl of solution of sodium carbonate (75 g / L in 20 ml H2O) were dissolved in 100 ml of solution of Folin 0.2 N. The mixture was stirred for 30 minutes and then allowed to incubate for 5 min. Added. After 15 min incubation, the absorbance was measured at 765 nm. The white was measured under the same conditions as the Folin is replaced by water. Gallic acid (GA) was used as standard. The results are expressed in mg of gallic acid equivalent by gram of dry mass (DM).

Determination of flavonoids

The rate of flavonoids was obtained using the method described by [10]. In 96-well microplates, 100 µL of different plant extracts were mixed with 100 µL of aluminum trichloride (AC13) in methanol (2%) and the absorbance was read at 415 nm after 15 minutes incubation at room temperature. White was measured under the same conditions by mixing 100 µL extract and 100 µL of methanol. Quercetin is used as a standard and is expressed as a quercetin equivalent by gram of dry mass.

Determination of tannins

Condensed tannins extracts were determined by the method of vanillin [10]. Solution of 50 µL of extract was mixed with 150 µL of vanillin (1% H2SO4 in 7 M), the mixture was incubated at 25 ° C. After 15 min, the absorbance of the solution was read at 500 nm. The measurement was made of white vanillin being replaced by water. Catechin was used as standard. The results were expressed in mg of catechin equivalent by gram of dry mass.

Determination of total anthocyanins

The total anthocyanins extracts were determined by the method described by different pH [10], using two solutions: hydrochloric acid, potassium chloride (pH 1.0, 0.2 M) and acetic acid, sodium acetate). The 96 well plates were used. 20 µL of the solution was mixed with 180 µL of each solution of Ph and the corresponding absorbance was calculated using the following formula.

$$A = [(A510-A700)\ pH1.0- (A510-A700).\ PH4.5].$$

We used the molar extinction coefficient of 29600. Final results were expressed in mg of Cyanidin-3-Glucoside equivalent of dry mass.

Antioxidant activity by DPPH

Antioxidant activity was studied using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the method described by [10]. 7 mg of 1,1-diphenyl-2-picrylhydrazyl was dissolved in 20 ml of methanol. This solution was diluted 10 times and keeps refrigerated up to 2 days. Prepare 3 mg / ml mother extract solution in DMSO. Dilute the stock solution so as to have three different concentrations of dilute solution. In each microplate well, put 20 µL of each extract and 180 µL of DPPH. For white, put 20 µL of DMSO and 180 µL methanol. Place the plate in the reader and shake for 30 seconds, then incubate 25 min protected from light and 524 nm was read out. The yields inhibition of the extract was calculated by the following equation:

$$I\% = \frac{\text{White Absorbance} - \text{Absorbance of the sample}}{\text{White Absorbance}} \times 100$$

The antioxidant activity of the extract is expressed as IC_{50} which defines the concentration of the extract that reduces by 50% the free radical (DPPH). Ascorbic acid was used as standard.

Anti-inflammatory activity

The anti-inflammatory activity of the extracts was measured on 5-lipoxygenase, according to the method described by [10] then changed slightly. 20 µL of sodium phosphate buffer at pH 7.4, 60 µL of linoleic acid (3.5 mM) and 30 µL of enzyme. White is determined under the conditions except that the enzyme is replaced by the buffer. The extracts are dissolved in 5% DMSO. The set was incubated for 10 min at 25 ° C then the reading was made at 234 nm. The nordihydroguaiaretic acid (NDGA) is used as standard.

Xanthine oxidase (XOD) inhibition assay

Anti-hyperuricemia test to inhibit xanthine oxidase activity and hence reducing the formation of uric acid. The xanthine oxidase inhibition can be explained by the uric acid's absorbance. Method of [11] was used with some modifications. The assay mixture was composed of 50 µL of extract solution, 60 µL of 70 mM phosphate buffer (pH = 7.5) and 30 µL of enzyme solution (0.1 units/mL in the same buffer). After the first incubation during 15min at 25°C, the reaction were then initiated by addition of 60 µL of substrate solution (150 µM xanthine in the same buffer) all in a microplate of 96 wells and the final volume of each well was 200 µL. Absorbance of the mixture was measured at 295nm after the second incubation for 5min against a blank consisting of the same sample without enzyme solution. Allopurinol was used as a positive control. Each experiment was performed three times. Percentage inhibition was calculated in the following way:

$$(\%)\ \text{Inhibition} = 100 - (\text{Asample}/\text{Acontol}) \times 100.$$

Where A sample is the real absorbance of the extract containing reaction and A control the real absorbance of the reaction control.

Data analysis

Data were processed by the software Excel 2007 where and slopes, variances, standard deviations, and IC_{50} were determined. The threshold of 5% margin of error was used as a criterion for significance in all cases.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 below shows the results of the chemical family assay. The ethyl acetate extract shows a high total polyphenol content (51.8 ± 1.6 mg eq AG / g MS), the extract cyclohexane, dichloromethane and methanol have a polyphenol content of (13.1 ± 0.4 30.5 ± 0.5, 33.2 ± 4.0 mg eq AG / g MS), respectively. The best level of tannins and anthocyanins are obtained respectively with dichloromethane and ethyl acetate (10.68 ± 0.1 mg eq Cat / g MS and 1.65 ± 0.07 mg eq Cya / g MS). The work of [7] allowed to quantify the total polyphenols in the methanolic extract of the seeds (9, 12 ± 0, 37 mg eq Cat / g MS). Our results are superior to those of the latter; this difference could be explained by the fact that we did not use the same protocol. It could be due to climatic, soil and geographic variations.

Studies conducted by [9] showed a total polyphenol content of 0.0068 mg eq AG / ml in the essential oil of the *A. lepidophyllus* seeds. The presence of total polyphenols in our study is consistent with previous work ([7] and [9]). The majority of phenolic compounds make this plant a non-negligible source of phyto-drug.

Table 1: Chemical composition of *A. lepidophyllus* seed extracts

Screening chemical / Sample	Total phenolics (AG)^a	Tanins (CE)^a	Flavonoïds (QE)^a	Anthocyanins (C3GE)^a
Cyclohexane	13,1±0,4	2,35±0,3	nd	0,79±0,04
Dichloromethane	30,5±0,5	10,68±0,1	nd	0,65±0,02
Ethyl Acetate	51,8±1,6	7,78±0,2	nd	1,65±0,07
Methanol	33,2±4,0	nd	nd	0,18±0,03

no detectednd; mg/g dw^a

Antioxidant activity by DPPH

The histogram below represents the result of the antioxidant activity as a function of the extracts (Figure 1).

The antioxidant activity measured resulted in a better IC_{50} with the methanol extract with IC_{50} = 248.4 ± 0.1 mg / L. The cyclohexane, dichloromethane, ethyl acetate extract slightly reduced the DPPH free radicals. The IC_{50} of the methanol extract found was higher than that of vitamin C (6.4 ± 0.1 mg / L), used as a reference in this study. The work of [7] showed that the methanolic extract of the seeds of *A lepidophyllus* has an antioxidant activity with an IC_{50} of 98.6 mg / L. Our results are much higher than those obtained by the latter, this difference could be due to the adopted protocol, but also to climatic and soil variations. [9] found an IC_{50} = 21.6 mg / L with the essential oil of the seeds of *A lepidophyllus*. The results obtained with the various extracts show that the extracts of this plant are not antioxidants. Analysis of the correlation between chemical families and antioxidant activity showed a low correlation between total polyphenols and DPPH, a low correlation between tannins and DPPH. Same for anthocyanins and DPPH with respectively (R^2 = 0.30 and 0.19). These weak correlations justify the inability of these plant extracts to trap DPPPH free radicals.

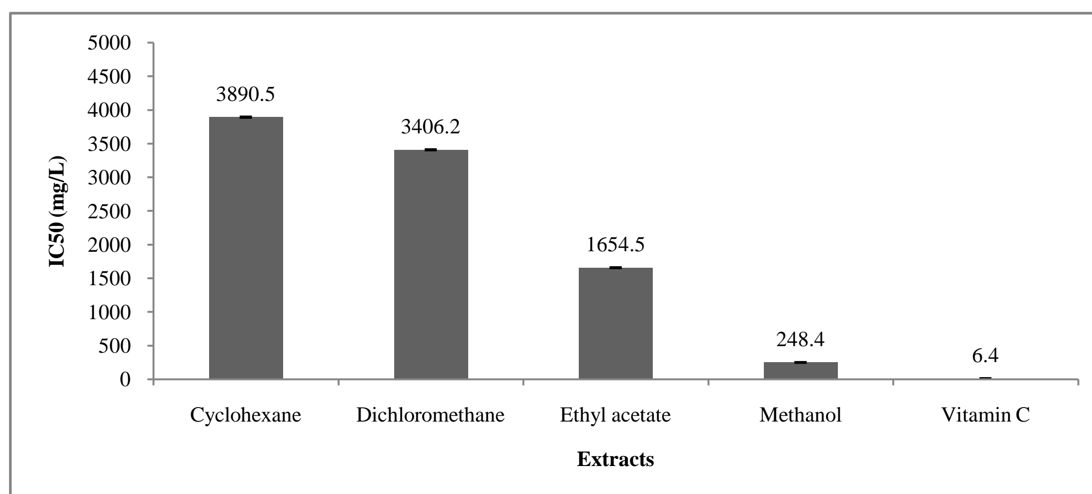

Figure 1: Antioxidant activity of *A. lepidophyllus* extracts by DPPH

Anti-inflammatory activity

Figure 2 shows the results of the anti-inflammatory activity of the extracts of *A. lepidophyllus* and NDGA used as standard in this study.

The anti-inflammatory activity evaluated shows that no extract showed a percentage greater than or equal to 50% to 40 mg / L. The ethyl acetate, methanolic, dichloromethane and cyclohexane extract inhibited 5-lipoxygenase activity with 36%, 33%, 29.5% and 30%, respectively, although no extract gave 50% inhibition , These results remain appreciable at this concentration (40 mg / L). The literature review shows that studies have not been conducted on the inhibition of this enzyme.

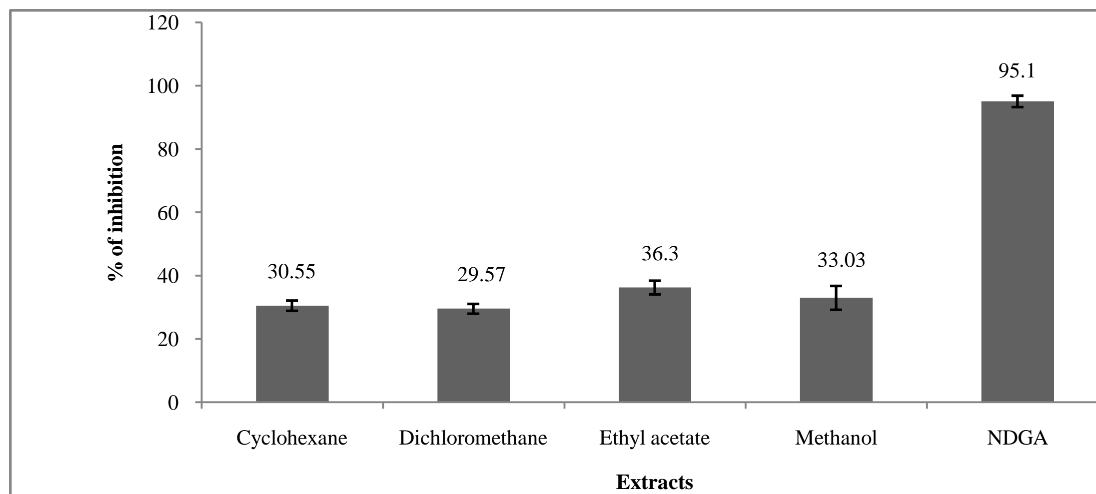

Figure 2: Anti-inflammatory activity at 40 mg / L of *A. lepidophyllus* extracts

Anti-xanthine oxidase activity

The results of the anti-xanthine oxidase activity of the extracts and of the allopirinol employed as reference molecule are represented by the histogram below (Figure 3).

The activity of inhibition of xanthine oxidase estimated at 50 mg / L of the extracts (dichloromethane, acetate ethyl and methanol) was 22.68%, 24.13% and 17.97%, respectively. These results show that no extract inhibited the action of xanthine oxidase. The literature does not mention any studies carried out on the anti-xanthine oxidase activity of the extracts of this plant.

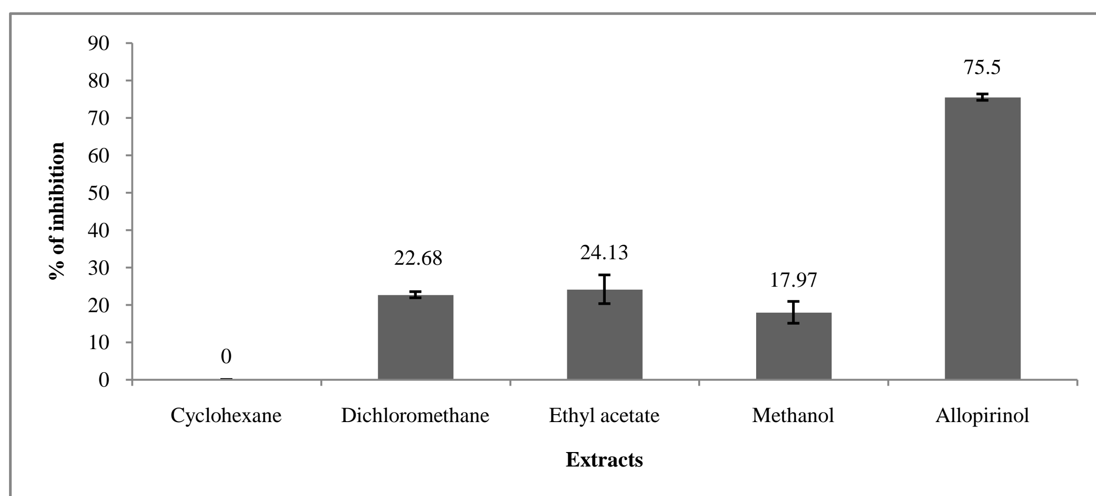

Figure 3: Inhibition of xanthine oxidase by *A. lepidophyllus* (50 mg / L)

CONCLUSION

In conclusion, these results demonstrate that the methanol extract of the *A. lepidophyllus* seeds exhibits antioxidant activity. Phenolic compounds such as total polyphenols, tannins and anthocyanins were quantified. Indeed, few studies have been carried out to highlight the antioxidant activity of the seed extracts of this plant, and its composition in phenolic compounds. Thus, we note a weak correlation between the antioxidant activities and the phenolic compound composition. These data suggest that this plant could be a valuable source of secondary metabolites with beneficial properties, and a promising source of health products.

Conflict of interest

Authors declare that there is no conflict of interest to reveal.

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