# Antiradicalar, Antioxidant and *In Vitro* Hepatoprotective Effects of Fractions isolated from *Xylopia phloiodora*

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## ABSTRACT

*Xylopia phloiodora* is an annonaceae which antihepatotoxic activity has been proven. In order to isolate its active principle, successive fractionation of the $CH_2Cl_2$/MeOH extract from the plant bark has been done. The fractions obtained were tested for their *in vitro* hepatoprotective effects using the liver slices system. Furthermore, these fractions were also tested for their antioxidant and antiradicalar activities. Antioxidant and antiradicalar activities were studied respectively using rat hepatic microsomes and a solution of 2,2- diphenyl-1-picrylhydrazyl (DPPH). All fractions were investigated at doses of 10, 50, 100 and 200µg/ml. The antihepatotoxic effect was studied using carbon tetrachloride-induced hepatitis in rat liver slices, by assessing LDH leakage. Silymarin was used as reference compound. Two of the ten fractions inhibited lipid peroxidation initiated by ascorbic acid with the respective percentage of 81.89 and 65.14 % at the dose 50µg/ml, and 82.38 and 78.5 % at the dose of 100µg/ml. With respect to the antihepatotoxic activity, the two fractions were found to be the most effective in respectively decreasing LDH leakage by 16.68 and 19.98% at 50µg/ml and 14.34 and 16.70 % at 100µg/ml. The percentage of discoloration for the two fractions was respectively 12.34 and 25.26% at the dose of 50µg/ml and 21.89 and 41.68% at 100µg/ml.

**Keys words:** Antioxydant, hepatoprotective, liver slices, *Xylopia phloiodora.*

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## INTRODUCTION

Free radicals are inevitable by products of biological redox reactions. They are formed constantly in human body either for useful metabolic purposes or as accidents of chemistry from external influence. There are many pathologies in which free radicals play an important role (Aruoma, 1998). Reaction of the reactive oxygen species (ROS) with bio–molecules generally lead to impairment or loss of biological functions (Martinez *and coll.*, 2001). The resulting cell injury causes a number of pathophysiological conditions leading to degenerative diseases. Since the liver is the site of metabolism in general, the cell injury lead to hepatitis.

Traditional medecine use plants to cure many diseases, *Xylopia phloiodora*, an annonaceae, is a plant which antihepatotoxic activity has been proven.

The present work was an attempt to isolate the active principle of this plant extract

## MATERIALS AND METHODS

**Chemicals:** All reagents used in study were purchased from SIGMA Chemicals Co.

**Animals**: Male wistar albinos rats from the Biochemistry Department Animal House, University of Yaounde I weighing 180 g – 200 g were used.

**Plant extract:** Stem bark of *X. P.* were collected in the Centre Province (Cameroon), dried and pulverised. The plant was cold extracted with a mixture of methylene chloride/methanol evaporated to dryness and use as crude extract. The crude extract was successfully separated in ether to obtain a precipitate (PE) and ether extract (EE) which was evaporated. The PE obtained were separated on colunn chromatography and fractions obtained (EB, PE, A, B, C, D, $F_1$, $F_2$, $F_3$, $F_4$ and $F_5$).

**Assay of lipid peroxydation:** Lipid peroxidation was investigated using rat liver microsomes. The microsomes were isolated by calcium aggregation procedure as described by Garle and Fry (1988). Lipid peroxidation was non-enzymatically initiated using ascorbate as described by Ulf *et al.*, (1989). After incubation for an appropriate time, the reaction stopped and lipid peroxidation was assayed for thiobartituric acid-reactive substance (TBA-RS) as described by Wills (1987). Four concentrations of each fraction in the system were used. The percentage of inhibition calculated.

**Assay of anti-hepatotoxic activity:** The antthepatotoxic activity was studied using carbon tetrachloride induced hepatitis in rat liver slices, by assessing lactate dehydrogenase (LDH) using the modified method described by Wormser (1992). Two doses were used in the incubation medium. The percentage of leakage of LDH calculated.

**Assay of antiradicalar activity**: Antiradicalar potentialities were tested against a solution of 2-2-diphenyl-1-picrylhydrazyl (DPPH). The change from the radical to the non- radical from leads to the disappearance of the purple coloration of DPPH. Four doses were also use.The percentage of discoloration calculated.

## RESULTS AND DISCUSSION

The results in table 1 show the inhibition percentage of microsomal lipid peroxidation of all fractions. We noted that the inhibition percentage of lipid

**Table 1 :** Inhibition percentage of microsomal lipid peroxidation of differents fractions of X.P.

| Fractions → / Doses ↓ | EB | PE | A | B | C | D | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 µ/ml | 05.85 ± 0.00 | 15.17 ± 15.02 | 13.2 ± 04.17 | 04.64 ± 01.62 | 32.2 ± 0.69 | 20.98 ± 05.48 | 37.33 ± 01.72 | 24.64 ± 03.79 | 17.81 ± 04.14 | 24.65 ± 0.01 | 21.95 ± 03.46 |
| 50 µg/ml | 35.85 ± 07.14 | 56.83 ± 03.10 | 61.72 ± 00.34 | 25.86 ± 04.14 | 42.94 ± 05.92 | 40.50 ± 02.07 | 81.89 ± 0057 | 6514 ± 0655 | 4513 ± 05.87 | 40.98 ± 12.42 | 35.12 ± 09.66 |
| 100 µg/ml | 46.59 ± 05.86 | 66.59 ± 03.10 | 74.65 ± 02.,06 | 37.33 ± 06.55 | 45.38 ± 04.14 | 46.11 ± 03.,10 | 82.38 ± 00.12 | 78.56 ± 05.52 | 56.11 ± 01.39 | 55.57 ± 00.35 | 62.93 ± 06.21 |
| 200 µg/ml | 69.27 ± 03.44 | 79.27 ± 02.42 | 83.92 ± 01.39 | 60.5 ± 01.39 | 69.53 ± 01.32 | 70.75 ± 06.21 | 82.95 ± 02.76 | 84.41 ± 00.69 | 70.26 ± 02.06 | 64.15 ± 03.10 | 57,81 ± 00,35 |

Data are given as means ± SD of two experiments

**Table 2 :** Inhibition percentage of microsomal lipid peroxidation of fractions EB, PE, A, $F_1$ and $F_2$.

| Fractions→ *Doses*↓ | EB | PE | A | $F_1$ | $F_2$ |
|---|---|---|---|---|---|
| 10 µg/ml | 05.85 ± 0.00 | 15.17±15.02 | 13.2 ± 4.17 | 37.33 ± 1.72 | 24.64 ± 3.79 |
| 50 µg/ml | 35.85 ± 7.14 | 56.83 ± 3.10 | 61.72 ± 0.34 | 81.89 ± 0.57 | 65.14 ± 6.55 |
| 100 µg/ml | 46.59 ± 5.86 | 66.59 ± 3.10 | 74.65 ± 2.06 | 82.38 ± 0.,12 | 78.56 ± 5.52 |
| 200 µg/ml | 69.27 ± 3.44 | 79.27 ± 2.42 | 83.92 ± 1.39 | 82.95 ± 2.71 | 84.41 ± 0.69 |

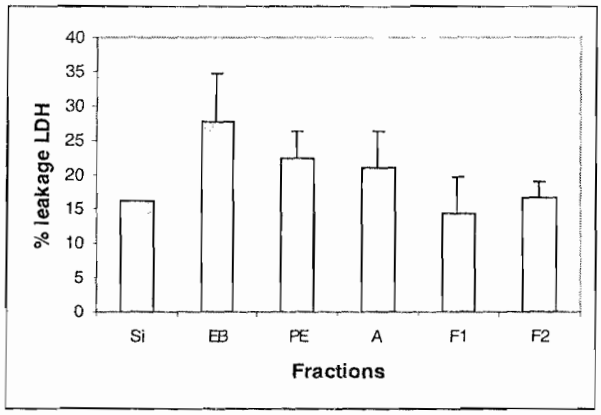
Data are given as means ± SD of two experiments

**Table 3:** LDH leakage percentage of fractions EB, PE, A, $F_1$ and $F_2$.

| Fractions | % leakage of LDH |
|---|---|
| Si | 16.22 ± 0.00 |
| EB | 27.69 ± 7.08 |
| PE | 22.45 ± 3.80 |
| A | 20.98 ± 5.22 |
| F1 | 14.34 ± 5.38 |
| F2 | 16.70 ± 2.31 |

Data are given as means ± SD of two experiments

peroxidation increases with the concentrations of extract in all fractions. Table 2 shows that the inhibition percentage of lipid peroxidation increases for one step of fractionation to the next step at the same dose. Table 3 shows the percentage of lactate dehydrogenage (LDH) leakage of some fractions. We also noted that the LDH leakage decrease when concentrations increases. And from one step to the next step of fractionation at the same dose (Figure 1). The




*Si: Silymarin, a reference compound*

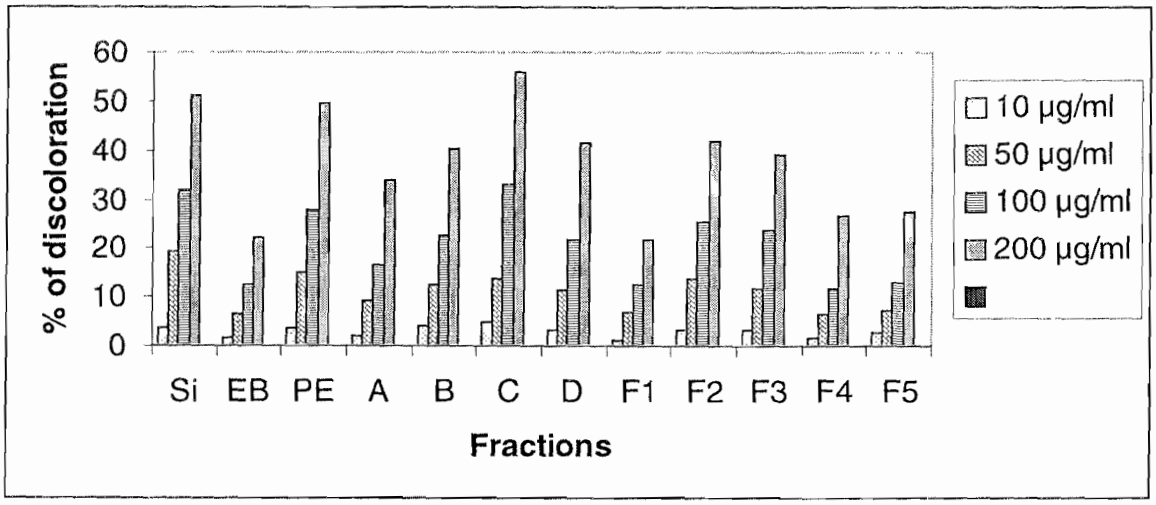
**Figure 1:** LDH leakage percentage of different fractions of *X.P.* at 100 µg/ml

same observations are noted when the antiradicalar activity was evaluated (Figure 2).

As indicated in table 1, inhibition percentage obtained ascorbate and $Fe^{3+}$ were used to initiate lipid peroxidation (Ulf *et a.,* 1989). showed that the fractions $F_1$ and $F_2$ contained a compound that reduced TBA-RS production by 82.4% and 78.6 % when tested at 100 µg/ml respectively.

Carbone tetrachloride ($CCl_4$)-induced hepatitis and other toxins as paracetamol are usually used as experimental model in the search for new antihepetotoxic compounds (Fleurentin and Joyeux, 1990). Once introduced in the organism, $CCl_4$ is converted in the liver into a radical which reacts with $O_2$ to form trichloromethyl peroxyl radical. This compound attacts membrane polyunsaturated fatty acids and cause lipid peroxidation (Recknagel, 1983), which lead to impairment of membrane function. The consequences is the leakage of some enzymes including LDH.

LDH leakage percentage obtained at 100 µg/ml with $F_1$ and $F_2$ were respectively 14.34 and 16.70%. This showed that these fractions inhibited the damage of cell membrane. At that concentration, $F_1$ and $F_2$ protected cell membrane similary as




*Si: Silymarin, a reference compound*

**Figure 2:** Discoloration percentage of different fractions of *X.P.*

silymarin, a reference compound with which percentage of LDH leakage is 16.22 at 100 µg/ml.

According to the present results, the fractions $F_1$ and/or $F_2$ contain active principles against and may be considered as active fractions.

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