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*Full Length Research Paper*

# *In vitro* antioxidant activities of total flavonoids extracts from leaves and stems of *Adenia lobata* (Jacq.) Engl. (Passifloraceae)

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**Oxidant stress is implied in numerous illnesses as a triggering factor or partner to their complications. The present work aimed to determine the antioxidant activity of total flavonoids extracts from *A. lobata* and to identify the molecules that are supposed to be responsible for this activity. The crude extracts, obtained by maceration in 70% (v/v) aqueous methanol, have been extracted successively by hexane, chloroform, ethyl acetate and n-butanol. The crude ($S_1$, $S_2$), ethyl acetate ($S_1^{III}$, $S_2^{III}$) and n-butanol ($S_1^{IV}$, $S_2^{IV}$) extracts underwent a phytochemical screening in order to test the radical scavenging activity against 1, 1' - diphenyl-2-picrylhydrazyl (DPPH). Qualitative analysis by thin layer chromatography permitted the study to put in evidence the flavonoids content of the different extracts. Contents in phenolic compounds were relatively high in the stems (3604 ± 511, 11 µg GAE/g) in relation to the leaves of *A. lobata* (3240 ± 711, 05 µg GAE/g). $EC_{50}$ of 6, 422.5, 430 and 357.5 µg/ml were graphically determined for vitamin C, $S_1^{III}$, $S_2^{III}$, $S_1^{IV}$ and $S_2^{IV}$ respectively; fractions $S_1$, $S_2$ and $S_2^{IV}$ showed an $EC_{50}$ greater than 500 µg/ml.**

**Key words:** *Adenia lobata*, flavonoids, antioxidant activity, DPPH, Ivorian pharmacopeia.

## INTRODUCTION

Few years ago, the world of biological and medical sciences is invaded by a new concept, oxidant stress (OS) ", that means a situation where the cell does not control the excessive presence of oxygenated toxic free radicals (Favier, 2003). OS is involved in many diseases such as cancer, cataract, Alzheimer's disease etc (Halliwell et al., 1992; Finkel and Holbrook, 2000). An antioxidant is a molecule that decreases or prevents the oxidization of other chemical substances.

It plays an important role as a health-protecting factor. Scientists suggest that antioxidants reduce the risk of chronic diseases including cancer and heart disease. The main characteristic of an antioxidant is its ability to scavenge free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or Deoxyribonucleic Acid (DNA) and can initiate degenerative disease. Antioxidant compound like phenolic acids, polyphenols and flavonoids scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Torel et al., 1986; Harborne and Williams, 2000).Species of permanently cool soil, *Adenia lobata*, is spread from Senegal to Cameroon and Angola. In Côte d'Ivoire, one finds it in all secondary formations (Adjanohoun and Aké Assi, 1979). The plant is extensively used in traditional medicine. It is a liana that is often used in the treatment of jaundice (icterus), headaches, otitis, malaria and infantile asthma (Adjanohoun et al., 1986; N'guessan, 1995). The present survey aims to investigate the antioxidant capabilities of *A. lobata*.

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## MATERIALS AND METHODS

### Materials

The plant material which is made up of stems and leaves of *A. lobata*, was collected in March 2008 on the site of the University of Abobo-Adjamé. The plant was authentified by Prof. Aké-Assi Laurent (Centre National Floristique, C.N.F) located at the University of Abidjan-Cocody. A voucher specimen (N°84) was deposited in the herbarium of C.N.F. The plant material (leaves and stems) was cleaned, dried under permanent air-conditioning during one week and was then dried at steam room (50°C) for 24 h after which it was reduced to fine powder.

### Preparation of plant extracts

The total flavonoids extracts were obtained from the crude methanol extracts of leaves ($S_1$) and stems ($S_2$) respectively by the method described by Chen et al. (2006) with some modifications. 200 g of fine powder of the plant material were macerated in 1 L of 70% (v/v) aqueous methanol under permanent agitation for 24 h at ambient temperature (which one). The extract was filtered first through a filter paper and then through cotton wool. The extract was concentrated using a rotary evaporator in water bath set at 65°C. The aqueous residue was kept for 48 h in a refrigerator and then decanted. The aqueous liquor was treated successively by hexane (500 ml), chloroform (500 ml), ethyl acetate (500 ml) and n-butanol (500 ml) to give the total ethyl acetate flavonoids extracts ($S_1^{III}$, $S_2^{III}$) and n-butanol extracts ($S_1^{IV}$, $S_2^{IV}$).

### Dosage of polyphenols

The contents in phenolic compounds of S1 and S2 were valued slightly according to the method of Singleton and Rossi (1965) with modification. To 1 ml of 1/10th dilute extract, 1.5 ml of $Na_2CO_3$ (17%, m/v) and 0.5 ml of (0.5 N) Folin-Ciocalteu reagent were added and was incubated at 37°C for 30 min. The absorbance was measured at 760 nm against blank. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve: $Y= 6.8583X$, $R^2 = 0.9593$; where Y is the absorbance and X is the concentration of gallic acid equivalents. The results are expressed in micrograms equivalent gallic acid by grams of the powder dry mass of the plant material (µg GAE/g).

### Phytochemical screening

The phytochemical screening of $S_1$, $S_2$, $S_1^{III}$, $S_2^{III}$, $S_1^{IV}$, $S_2^{IV}$ was realized by TLC (silica gel 60F254 plates, Merck) according to the methods of Wagner and Bladt (1996) and Chaaib (2004) and Lagnika (2005).

### DPPH scavenging assay for determination of antioxidant activity

The radical scavenging activities of extracts $S_1$, $S_2$, $S_1^{III}$, $S_2^{III}$, $S_1^{IV}$ and $S_2^{IV}$ were determined against 1,1'- diphenyl-2-picrylhydrazyl free radical (DPPH; Carlo-Erba) by UV spectrophotometry at 517 nm by the method previously described (Sanchez-Moreno et al., 1999). Concentrations of 0.5, 0.1, 0.025, 0.01 and 0.001 mg/ml were prepared in absolute ethanol. Vitamin C was used as the antioxidant standard at concentrations of 0.5, 0.1, 0.025, 0.01 and 0.001 mg/ml. 2.5 ml of extract combined with 1 ml of DPPH dissolved in absolute ethanol (0.3 mg/ml) was placed in a test tube. The mixture was shaken vigorously then incubated for 30 min in darkness at room temperature. A blank solution was prepared containing the same amount of ethanol and DPPH. The radical scavenging activity was calculated using the following formula: $I = [(Ab - Ae) / Ab] \times 100$ where I is the percentage of inhibition, Ab is the absorbance of the blank sample and Ae is the absorbance of the extract.

### Statistical analysis

All the tests were done in triplicates. Data were expressed as means ± standard errors. $EC_{50}$ were determined graphically.

## RESULTS

### Dosage of polyphenols

The test performed on crude extracts was used to determine the total polyphenol content of different organs. Contents in phenolic compounds were relatively high in the stems (3604 ± 511, 11 µg GAE/g) in relation to the leaves of *A. lobata* (3240 ± 711, 05 µg GAE/g).

### Phytochemical screening

Phytochemical screening of extracts $S_1$, $S_2$, $S_1^{III}$, $S_2^{III}$, $S_1^{IV}$ and $S_2^{IV}$ revealed the presence of flavonoids by the numerous fluorescent colors of spots observed on the chromatograms under UV at 366 nm (Tables 1 - 3). Fluorescent orange, red, yellow, blue and green observed with Neu's reagent are characteristic of flavonoids. With aluminum chloride; yellow, brown and blue-pale staining were observed, whereas, with the Godin reagent, fluorescent yellow, blue and green were observed.

### Radical scavenging (antioxidant) activity

The results were expressed in percentage of inhibition of DPPH. The capacity of radical scavenging of the crude extracts, ethyl acetate and n-butanol extracts of the leaves and stems of *A. lobata* is shown in Figure 1.

$EC_{50}$ of 6, 422.5, 430 and 357.5 µg/ml were determined for vitamin C, $S_1^{III}$, $S_2^{III}$, $S_1^{IV}$ and $S_2^{IV}$ respectively; fractions $S_1$, $S_2$ and $S_2^{IV}$ showed an $EC_{50}$ greater than 500 µg/ml. We noticed a relatively high antioxidant potential with ethyl acetate extracts and butanolic extracts, their effective concentrations were lower.

## DISCUSSION

The method (Singleton and Rossi, 1965) used for the determination of total polyphenols is based on the oxidation of the hydroxyl groups of phenols in basic media

**Table 1.** TLC analysis of $S_1$ and $S_2$; gradient (*n*-BuOH/ AcOH /$H_2O$ 4: 1: 5; v/v/v).

| Crude extracts | 366 nm | | $AlCl_3$/366 nm | | Neu/366 nm | | Godin/366 nm | |
|---|---|---|---|---|---|---|---|---|
| | Rf | Color | Rf | Color | Rf | Color | Rf | Color |
| $S_1$ (Leaves) | 0.93 | Red[1] | | | 0.93 | Orange[3] | 0.93 | Pale yellow [4] |
| | | | 0.86 | Brown[2] | | | 0.84 | Yellow[4] |
| | | | 0.79 | Blue- pale[2] | | | | |
| | | | 0.72 | Blue- pale[2] | | | | |
| | | | 0.66 | Yellow[2] | 0.66 | Yellow[3] | | |
| | | | 0.55 | Yellow[2] | 0.62 | Yellow[3] | | |
| | 0.35 | Yellow[1] | 0.39 | Blue [2] | 0.42 | Orange -yellow[3] | | |
| | | | 0.20 | Yellow[2] | | | | |
| $S_2$ (Stems) | | | 0.90 | Blue [2] | | | 0.95 | Blue [4] |
| | | | 0.76 | Blue- pale[2] | 0.76 | Yellow[3] | | |
| | | | | | 0.72 | Orange -yellow[3] | 0.72 | Yellow[4] |
| | | | 0.61 | Yellow-green[2] | 0.61 | orange-Yellow[3] | | |
| | | | 0.46 | Yellow[2] | 0.46 | Orange[3] | 0.46 | Yellow[4] |
| | | | 0.32 | Yellow-green[2] | 0.35 | Orange[3] | | |
| | | | 0.24 | Blue [2] | | | | |
| | | | 0.17 | Blue-white[2] | | | | |

[1]Flavonoids detected at 366 nm; [2]Flavonoids detected with $AlCl_3$; [3]Flavonoids detected with Neu reagent; [4] Flavonoids detected with Godin reagent.

**Table 2.** Detection of chemical compounds of $S_1^{III}$ and $S_2^{III}$; gradient ($CHCl_3$/ AcOEt/ AcOH 6: 5: 0.5; v/v/v).

| Crude extracts | UV/366 nm | | $AlCl_3$/366 nm | | Godin/366 nm | | Neu/366 nm | |
|---|---|---|---|---|---|---|---|---|
| | Rf | Color | Rf | Color | Rf | Color | Rf | Color |
| $S_1^{III}$ (Leaves) | | | 0.93 | Blue[2] | | | 0.93 | Blue[4] |
| | 0.78 | Purple[1] | | | | | 0.87 | Red[4] |
| | 0.71 | Red[1] | | | | | 0.71 | Yellow-pale[4] |
| | 0.60 | Green[1] | 0.60 | Yellow[2] | | | 0.65 | Blue[4] |
| | | | | | | | 0.58 | Red[4] |
| | | | 0.52 | Blue- pale[2] | | | 0.52 | Yellow[4] |
| | 0.41 | Mallow[1] | 0.46 | Yellow[2] | | | 0.41 | Blue[4] |
| | | | | | 0.38 | Yellow[3] | 0.38 | Orange[4] |
| | | | | | | | 0.34 | Yellow[4] |
| | | | | | | | 0.30 | Blue[4] |
| | | | | | | | 0.27 | Yellow[4] |
| | | | 0.22 | Brown[2] | | | 0.21 | Mallow[4] |
| | | | 0.15 | Blue-pale[2] | | | | |
| | | | 0.09 | Yellow[2] | 0.09 | Yellow[3] | 0.09 | Yellow[4] |
| | | | | | | | 0.05 | Yellow- pale[4] |
| | 0.03 | Yellow[2] | 0.03 | Yellow[2] | | | | |
| $S_2^{III}$ (Stems) | 0.92 | Purple[1] | | | | | 0.92 | Blue[4] |
| | | | 0.53 | Blue[2] | | | | |
| | 0.45 | Blue[1] | 0.46 | Blue[2] | | | | |
| | 0.36 | Blue[1] | 0.36 | Blue[2] | | | 0.36 | Blue- fluor[4] |
| | | | | | | | 0.31 | Yellow-green[4] |
| | | | | | | | 0.27 | Bleu-fluor[4] |

[1]Flavonoids detected at 366 nm; [2] Flavonoids detected with $AlCl_3$. [3] Flavonoids detected with Godin reagent; [4] Flavonoids detected with Neu reagent.

**Table 3.** Detection of chemical compounds of $S_1^{IV}$ and $S_2^{IV}$; gradient (n-BuOH/ AcOH/ $H_2O$ 4: 1: 5; v/v/v).

| Crude extracts | UV/366 nm | | $AlCl_3$/366 nm | | Godin/366 nm | | Neu/366 nm | |
|---|---|---|---|---|---|---|---|---|
| | Rf | Color | Rf | Color | Rf | Color | Rf | Color |
| $S_1^{IV}$(Leaves) | 0.83 | Yellow[1] | | | 0.83 | Yellow[3] | | |
| | 0.73 | Red[1] | | | 0.73 | Yellow[3] | | |
| | 0.67 | Brown[1] | 0.67 | Yellow[2] | 0.64 | Yellow[3] | 0.64 | Yellow[4] |
| | 0.61 | Yellow[1] | 0.58 | Yellow[2] | | | 0.60 | Yellow[4] |
| | | | | | | | 0.51 | Orange[4] |
| | 0.40 | Yellow[1] | | | 0.46 | Yellow[3] | 0.40 | Yellow[4] |
| $S_2^{IV}$(Stems) | 0.74 | Yellow- pale[1] | 0.74 | Blue[2] | 0.74 | Yellow[3] | | |
| | | | 0.64 | Yellow[2] | 0.67 | Yellow[3] | 0.67 | Orange[4] |
| | | | | | 0.61 | Yellow[3] | 0.61 | Yellow[4] |
| | 0.55 | Yellow[1] | | | | | 0.55 | Red[4] |
| | | | | | 0.53 | Yellow[3] | 0.50 | Yellow[4] |
| | | | 0.40 | Yellow[2] | | | 0.35 | Orange[4] |

[1]Flavonoids detected at 366 nm; [2] Flavonoids detected with $AlCl_3$. [3] Flavonoids detected with Godin reagent; [4] Flavonoids detected with Neu reagent.

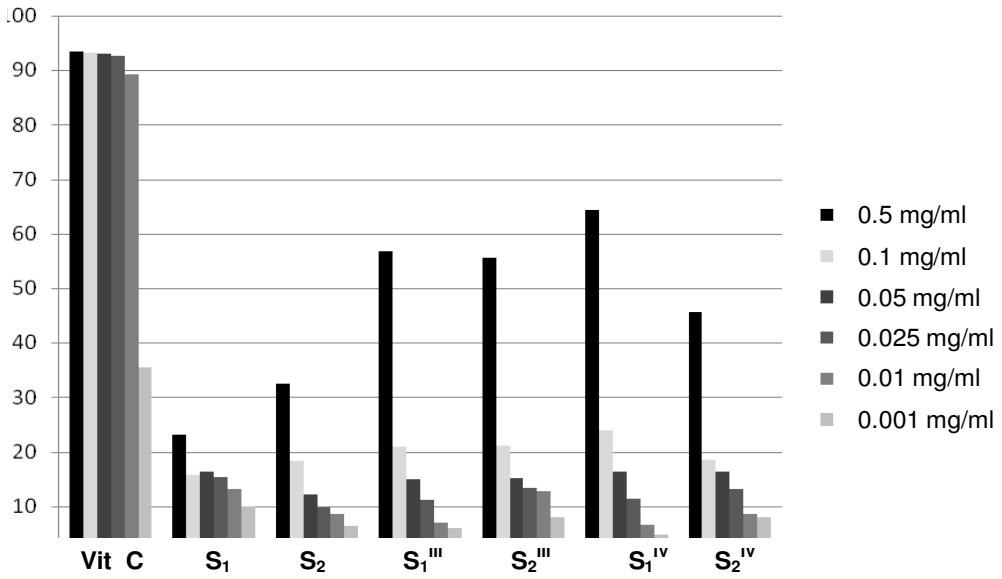


**Figure 1.** Percentage inhibition of DPPH by $S_1$, $S_2$, $S_1^{III}$, $S_2^{III}$, $S_1^{IV}$ and $S_2^{IV}$.

by the Folin-Ciocalteu reagent (mixture of phospho-tungstic and phosphomolybdic acids of yellow colour). The reduction of this reagent produces a mixture of tungstic and molybdic oxides that present a characteristic blue colouration with a maximum absorption wavelength at 760 nm. The dosage of the polyphenols showed a content that is relatively raised in phenolic compounds in the stems of *A. lobata* than in the leaves. This unequal distribution of the polyphenols in different parts of a plant has been observed by Falleh et al. (2006). The difference in contents of polyphenols in different parts of the same plant could be attributed to the influence of environmental conditions or purely by genetic.

The qualitative analysis by TLC permitted to put in evidence of numerous characteristic fluorescences of the flavonoids which are groups of naturally occurring compounds are widely distributed, as secondary metabolites in the plant kingdom. Indeed, flavonoids form with several specific reagents ($AlCl_3$, Neu, Godin) complexes are well colored in daylight or under UV at 366 nm. These reagents have been used to characterize the flavonoids among the spots observed under UV irradiation. Furthermore, by referring to the work of Markham (1982) taken by Mohamed (2006), the allocations were also

made with different fluorescence. Flavonoids are primary antioxidants or free radical scavengers (Polterait, 1997). Nakayama et al. (1993) reported that flavonoids possess antioxidant and antiradical activities.

DPPH method is commonly used to assess radical scavenging of any antioxidant substance because it is a quick, reliable and reproducible method to search *in vitro* general antioxidant of pure compounds as well as plant extracts (Koleva et al., 2002). The DPPH test provides information on the potential reactivity of the test extracts with a stable free radical. It gives a strong absorption band at 517 nm in visible spectrophotometer. As the electron becomes paired off in the presence of a free radical scavenger, the absorption and the DPPH solution is decolorized as the color changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the antioxidant activity of the crude extracts. The ethyl acetate ($S_1^{III}$, $S_2^{III}$) and n-butanol ($S_2^{IV}$) extracts of *A. lobata* ($S_1$) have the highest antioxidant activity; their $EC_{50}$ are the lowest among all study samples. Ethyl acetate and n-butanol extracts are concentrated flavonoids; their high antioxidant activity compared to crude fraction, suggests that the potential antiradical *A. lobata* is due to flavonoids. This study highlights the antioxidant potential of flavonoids reported by several authors (Harbone and Williams, 2000; Mamyrbékova-Békro et al., 2008). The survey suggests that *A. lobata* possess antioxidant potential activities which can counteract the oxidative damage induced by the illnesses for which the populations use it to take care of themselves.

## Conclusion

*A. lobata* is a potential source of flavonoïdes with antioxidant activity. The flavonoid extracts have a relatively high antioxidant activity against DPPH radical compared to crude extracts. This study confirms the antioxidant activity of flavonoid extracts reported by various studies. A deepened chemical survey will permit the isolation of new natural molecules. The results of this study can be a rational scientific explanation to the large use of *A. lobata* in non conventional medicine by the populations.

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