

# In Vitro Antioxidant Activities and Pancreatic $\alpha$ -Amylase Inhibition of some Cameroonian Medicinal Plants

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## ABSTRACT

The present study was aimed at investigating the antioxidant activities and the inhibition of pancreatic  $\alpha$ -amylase of the aqueous, ethanolic and hydroethanolic extracts of *Laportea ovalifolia*, *Luffa aegyptiaca*, and *Cola nitida*. The phytochemical screening of these extract was also carried out revealing the presence of flavonoids, tannins and saponins. The highest total phenol content was observed in the ethanolic extract of *C. nitida* while the aqueous extract of the same plant and the hydroethanolic extract of *L. aegyptiaca* exhibited the best antioxidant activity by FRAP and DPPH methods, respectively. For all plants tested, the ethanolic extract most inhibited the activity of  $\alpha$ -amylase, although the aqueous extract of *L. aegyptiaca* was a more effective pancreatic  $\alpha$ -amylase inhibitor than that of *L. ovalifolia* and *C. nitida*. The presence of active phytochemical substances with antioxidant properties may provide a substantial basis for the use of these plants in ethnomedicine for the treatment of diabetes.

**Keywords:** DPPH, enzyme, folin, FRAP, herbs, phytochemical substances

**Abbreviations:** DPPH, 1,1-diphenyl-2-picryl-hydrazil; **FRAP**, ferric reducing antioxidant power; **ROS**, reactive oxygen species; **TPTZ**, 2,4,6-tris(2-pyridyl)-s-triazine

## INTRODUCTION

Free radicals can be described as chemical species that have an unpaired electron. The reactivity of free radicals varies from relatively low, as in the case of the oxygen molecule itself, to very high, as in the case of the short-lived and highly reactive hydroxyl radical (OH $\cdot$ ) (Packer 1994). Fatty acids are susceptible to attack by highly reactive oxygen species (ROS) such as OH $\cdot$ ; hence any reaction or process that forms ROS stimulates lipid oxidation. Hydrogen abstraction is easier in unsaturated fatty acids than in their saturated counterparts, thus making them more susceptible to ROS attack. Oxygen and ROS are among the major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro*. Continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Tsen *et al.* 1997). Therefore, antioxidants with free radical scavenging activity may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al.* 1997). In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans 1998; Gil *et al.* 1999). There is no information about the *in vitro* antioxidant activity of water or ethanolic extracts of *Laportea ovalifolia*, *Luffa aegyptiaca* and *Cola nitida*.

These plants are used by traditional practitioners in Cameroon for the management of diabetes. The mechanism of action of these plants is not yet known, but could be related to the reduction of circulating blood glucose levels and oxidative stress, two conditions that need correction in diabetes.

From a toxicological point of view, ethanol and water, as solvents, are safer than other organic solvents, and therefore more suitable for the food and pharmaceutical industries. Thus water, ethanol and hydroethanolic extracts were

used in the present study, the purpose of which was to evaluate the *in vitro* ability of these three Cameroonian medicinal plant extracts to reduce oxidative stress and inhibit the activity of pancreatic  $\alpha$ -amylase, a key enzyme in the control of blood glucose levels.

## MATERIALS AND METHODS

### Plant materials

The material was collected from one plant for each of the 3 species by a botanist from the National Herbarium. The plants were all in the vegetative stage. The plants were collected in the wild and we could not estimate their ages.

*Laportea ovalifolia* (aerial part), *Cola nitida* (leaves) and *Luffa aegyptiaca* (aerial part) were collected. The plants were selected after an ethnobotanical survey in the Yaoundé area in January 2006 of traditional doctors who use them to treat diabetes mellitus. They were identified at the National Herbarium in Yaoundé and assigned voucher numbers (N° 50623/HNC, N° 60621/HNC and N° 51635/HNC respectively for *L. ovalifolia*, *C. nitida* and *L. aegyptiaca*). Each part of the plant used was dried at 50°C for 72 h and extracted using different solvent systems.

### Extract preparation

Three solvent systems were used to extract the active principle in each plant: distilled water (A), ethanol (E) and ethanol/water 1:1 (EH). Three g of each plant powder was macerated in 60 ml of solvent. After 48 h, the mixture was filtered (this was repeated four times until the resulting extract gave no further coloration) and concentrated at 50°C. The resulting materials was then stored at 4°C for further analysis.

### Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, ferric chloride (FeCl<sub>3</sub>), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ),

chloroform, Fehling's solution A and B, potassium ferricyanide 2N, acetic anhydride, catechin, starch and porcine pancreatic  $\alpha$ -amylase were purchased from Sigma Chemical Co. Ltd., USA. All other reagents were of analytical grade.

### Preliminary qualitative phytochemical screening

Standard preliminary qualitative phytochemical screening procedures were used as described by Trease and Evans (1983). Various reagents and tests were used:  $\text{FeCl}_3$  for tannin, frothing test for saponin, magnesium chip and HCl for flavonoids, NaCl and Fehling's solutions A and B for glycoside, diethyl ether, sulfuric acid and anhydride acetic for steroids and triterpens,  $\text{K}_3\text{Fe}(\text{CN})_6$  for phenols and polyphenols. The presence of bioactive compounds was indicated by a colour change. The experiment was repeated three times for each sample.

### Determination of total phenolic compounds using Folin-Ciocalteu phenolic reagent

The total phenolic compounds of different extracts were determined with Folin-Ciocalteu reagent according to the method of Singleton *et al.* (1999) using catechin as a standard phenolic compound. Briefly, 30  $\mu\text{l}$  of each plant extract (1 mg/ml) was added to 1 ml of Folin reagent diluted 10 times. The absorbance at 750 nm was read after 30 min using a Genesys 20 spectrophotometer. The amount of total phenolic compounds in the extracts was determined in mg of catechin equivalent per g of dry powder. All determination was done in triplicate.

### Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) was determined (three repetitions per sample) using the method of Benzie and Strain (1996) which measures the reduction of ferric ion to the ferrous form in the presence of antioxidant components. The FRAP reagent consisted of ten parts acetate buffer (300 mM, pH 3.6), one part of TPTZ (10 mM in 400 mM of HCl) and one part ferric chloride. The colorimetric measurement was performed at 593 nm and the reaction was monitored for up to 12 min on 75  $\mu\text{l}$  of each extract (1 mg/ml) and 2 ml of FRAP reagent.

### DPPH radical scavenging effect

The free radical scavenging activity of plants extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Katalinić *et al.* (2004). Used as a reagent, DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. 0.3 mM solution of DPPH in methanol was prepared and 2 ml of this solution was added to 20  $\mu\text{l}$  of the plant extract (1 mg/ml). After 30 min, the absorbance was measured at 517 nm. Catechin was used as the standard. All experiments were done in triplicate. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage inhibition was calculated as described by Yen and Duh (1994).

### Determination of $\alpha$ -amylase inhibitory activity by iodine-starch assay

The  $\alpha$ -amylase inhibition assay (three repetitions per sample) was carried out by a modified method of Komaki *et al.* (2003). 75  $\mu\text{l}$  of each extract (1 mg/ml) were mixed with 100  $\mu\text{l}$  (0.1%) of soluble starch substrate in phosphate buffer (0.25 M, pH 7.0). After 5 min of incubation at 37°C, 20  $\mu\text{l}$  of 30  $\mu\text{g}/\text{ml}$  solution of  $\alpha$ -amylase in phosphate buffer (pH 7.0) was added to the mixture. After the mixture was further incubated for 10 min, 2 ml of 0.01 N iodine solution was added, followed by measurement of the absorbance at 660 nm. The inhibition activity (%) was calculated as  $[(A-B)/A] \times 100$ , where A is a decrease in the absorbance in the absence of the extract and B is that in its presence.

### Statistical analysis

Measurements of absorbance All the tests were conducted in triplicate and the results presented as means  $\pm$  standard deviations. One-way ANOVA and *post hoc* LSD was used to evaluate the difference between the different extracts of each plant ( $p < 0.05$ ). Spearman and Pearson product moment correlations were used to evaluate the correlation between the various parameters. The software SPSS 10.1 was used for this analysis.

## RESULTS

### Preliminary qualitative phytochemical screening

Tannins, polyphenols and saponins were identified in all plant extracts except for the aqueous extract of *L. aegyptiaca* which was not positively assayed for saponins. Sterols were detected in *L. aegyptiaca* and *L. ovalifolia* while triterpenes were identified in *C. nitida*. Only the hydroethanolic extract of *L. aegyptiaca* and the ethanolic extract of *L. ovalifolia* did not revealed the presence of flavonoids.

### Total phenolic compounds of plant extracts

The results of total phenolics content of different plant extracts are presented in **Table 1**. There was no significant difference between the polyphenol concentration of the three different extracts of *L. aegyptiaca*. There was, however, a significant difference ( $P < 0.05$ ) between the polyphenol concentration of the ethanolic extract and both the aqueous and hydroethanolic extracts of *C. nitida* and *L. ovalifolia*. The lowest content was found in the ethanolic extract of *L. ovalifolia* while highest content was found in ethanol extract of *C. nitida*, *L. aegyptiaca* and *L. ovalifolia* have their highest polyphenol content when extracted with water while the ethanolic solvent extract more phenolics compound from *C. nitida*.

### Ferric reducing antioxidant power of plant extracts

**Table 2** shows the ferric reducing antioxidant power of different plant extracts. *C. nitida* showed the highest reducing ferric antioxidant power amount all extracts. Statistical ana-

**Table 1** Polyphenol concentration of plant extracts (mg catechin equivalent/g dry weight).

Plants	Aqueous extract	Ethanolic extract	Hydroethanolic extract
<i>L. aegyptiaca</i>	195.66 $\pm$ 7.60 a	181.02 $\pm$ 1.50 a	191.32 $\pm$ 6.30 a
<i>L. ovalifolia</i>	209.80 $\pm$ 6.32 a	180.28 $\pm$ 3.62 b	195.66 $\pm$ 2.69 a
<i>C. nitida</i>	188.03 $\pm$ 4.88 a	453.71 $\pm$ 8.33 b	194.11 $\pm$ 10.08 a

Results = means  $\pm$  standard deviation, n = 3, within the same row, the values affected with different letters are significantly different ( $P < 0.05$ ) according to One-way ANOVA and *post hoc* LSD.

**Table 2** FRAP antioxidant capacity of plant extracts (mg catechin equivalent/ g dry weight).

Plants	Aqueous extract	Ethanolic extract	Hydroethanolic extract
<i>L. aegyptiaca</i>	193.29 $\pm$ 1.00 a	10.27 $\pm$ 1.31 b	37.71 $\pm$ 12.84 c
<i>L. ovalifolia</i>	44.55 $\pm$ 2.83 a	8.07 $\pm$ 1.90 b	37.07 $\pm$ 1.69 a
<i>C. nitida</i>	199.56 $\pm$ 0.38 a	120.44 $\pm$ 4.37 b	179.99 $\pm$ 0.15 c

Results are presented as means  $\pm$  standard deviation, n=3, within the same row, the values affected with different letters are significantly different ( $P < 0.05$ ) according to One-way ANOVA and *post hoc* LSD.

**Table 3** Percentage inhibition of DPPH radical scavenging activity (%).

Plants	Aqueous extract	Ethanollic extract	Hydroethanollic extract
<i>L. aegyptiaca</i>	72.30 ± 0.98 a	77.03 ± 0.42 b	60.86 ± 8.94 a
<i>L. ovalifolia</i>	26.45 ± 0.52 a	27.08 ± 0.41 a	37.44 ± 0.52 a
<i>C. nitida</i>	26.31 ± 0.17 a	27.80 ± 0.12 a	27.87 ± 0.47 a

Results are presented as means ± standard deviation, n=3, within the same row, the values affected with different letters are significantly different ( $P<0.05$ ) according to One-way ANOVA and *post hoc* LSD.

**Table 4** Percentage inhibition of plant extracts on  $\alpha$ -amylase activity.

Plants	Aqueous extract	Ethanollic extract	Hydroethanollic extract
<i>L. aegyptiaca</i>	26.22 ± 1.31 a	83.98 ± 10.3 b	27.41 ± 2.25 a
<i>L. ovalifolia</i>	67.78 ± 0.30 a	83.32 ± 0.09 b	28.74 ± 0.75 c
<i>C. nitida</i>	76.02 ± 0.94 a	82.90 ± 0.10 a	82.01 ± 0.44 a

Results are presented as means ± standard deviation, n=3, within the same row, the values affected with different letters are significantly different ( $P<0.05$ ) according to One-way ANOVA and *post hoc* LSD.

lysis revealed that all extracts of *L. aegyptiaca* and *C. nitida* had significantly different FRAP activities at  $P<0.05$ . High antioxidant activity was obtained from the aqueous extract of *C. nitida* while low antioxidant activity was found from ethanolic extract of *L. ovalifolia*.

### DPPH radical scavenging effect of plant extracts

Radical scavenging activities (RSA) of different plant extracts are shown in **Table 3**. There was not a significant difference between different extracts of *C. nitida* and those of *L. ovalifolia* meanwhile the hydroethanolic and ethanolic extracts of *L. aegyptiaca* showed a significant difference ( $P<0.05$ ) between their RSA. In the extracts tested for each plant, the ethanolic extract from *L. aegyptiaca* reacted faster than the other extracts and was the most effective DPPH radical scavenger. This was followed by the hydroethanolic extract of *L. ovalifolia* (37.44 ± 0.52%) while the aqueous extract of *C. nitida* showed the lowest RSA activity. It was also noticed that only *L. aegyptiaca* extracts showed more than 50% inhibition of DPPH.

### Effect of plant extracts on $\alpha$ -amylase activity

The results of different plant extracts on inhibition of porcine pancreatic  $\alpha$ -amylase are showed in **Table 4**. All extracts of *C. nitida* showed more than 50%  $\alpha$ -amylase inhibition. The aqueous extract of *L. aegyptiaca* showed the lowest percentage of  $\alpha$ -amylase inhibition while the ethanolic extract of the same plant showed the highest percentage of inhibition.

## DISCUSSION

Phenolic compounds are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts (Okwu 2005). Phenolics, triterpenes, flavonoids, tannins and saponins detected in the extracts are compounds that have been reported to possess medicinal properties and health-promoting effects (Kameswara 1997).

All the plant extracts tested contained phenolic compounds, with *C. nitida* having the highest polyphenol content when extracted with ethanol. These plants, thus, have antioxidant activity which could be attributed to these polyphenolic compounds, secondary metabolites and principal compounds of plants (Rice-Evans *et al.* 1996). They include tannins; flavonoids and phenolic acids and act as hydrogen donors to free radicals by stopping lipid peroxidation at the stage of initiation (Glücin 2004).

FRAP measures the ferric reducing ability of the antioxidant molecule at low pH. Both FRAP and Folin values have been cited to reflect the antioxidant capacity of samples (Juliani and Simon 2002). The aqueous extracts of the plants showed high FRAP activity. These results confirm others obtained by Then (2003) who stipulated that alcoholic extracts do not have an influence on the antioxidant activity of the aqueous extract measure by FRAP method.

In the present investigation, all the plants tested with different solvents demonstrated significant DPPH scavenging activity indicating their abilities to act as radical scavengers. From the results obtained, we noticed that the ethanolic extract of *L. aegyptiaca* with a low phenolic content had a very high DPPH scavenging activity. This goes to support the hypothesis of Brand-Williams *et al.* (1995) that the DPPH kinetics is proportional to the amount of OH groups present on the phenolic compound. Thus, this plant can be "poor" in phenolic compounds but the phenolic compounds present have many OH groups leading to its high DPPH scavenging activity.

The antidiabetic properties of plants can be evaluated by many methods among which we have the determination of the  $\alpha$ -amylase inhibitory activity by 2-chloro-4-nitro phenyl  $\alpha$ -maltotrioxide (CNP-G<sub>3</sub>) assay, the determination of the  $\alpha$ -glucosidase inhibitory activity and the determination of the  $\alpha$ -amylase inhibitory activity by the iodine starch assay (Miliauskas *et al.* 2004; Maher 2006).  $\alpha$ -amylase (EC 3.2.1.1) is an enzyme found in the salivary, intestinal mucosa and pancreatic secretions, functioning in the breakdown of the  $\alpha$ -1-4-glycosidic bonds in starch. Thus, this enzyme increases the bioavailability of glucose in blood. For a substance to be antidiabetic it should be able to reduce the amount of glucose in blood or increase the efficacy of insulin. It has been demonstrated that the inhibition of  $\alpha$ -amylase reduces the bioavailability of glucose (Braodhurst *et al.* 2000; Matsui 2001). This result suggests that phenolic compounds involved in the DPPH scavenging activity may also be involved in the  $\alpha$ -amylase inhibition or may directly or indirectly intervene in the enzyme activity mechanism (McDougall *et al.* 2005).

## CONCLUSIONS

In conclusion, this study indicates that these plants exhibit some antioxidant activity irrespective of the method used for the analysis. They also inhibit the activity of  $\alpha$ -amylase.

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