Antioxidant Properties and α-amylase Inhibition of *Terminalia superba*, *Albizia* sp., *Cola nitida*, *Cola odorata* and *Harungana madagascarensis* Used in the Management of Diabetes in Cameroon

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Aqueous, ethanolic, hydroethanolic and methanolic extracts of *Albizia* sp., *Cola nitida*, *Terminalia superba* (*T. superba*), *Cola odorata* and *Harungana madagascarensis* were screened for phytochemical constituents. The evaluations of the antioxidant potential and α-amylase inhibitory activity of these extracts were also carried out. Tests for tannins and phenols were positive in all extracts, with the highest total phenol content observed in the ethanolic extract of *T. superba*. This extract, as well as the aqueous and hydroethanolic extracts of the same plant had the highest antioxidant properties as determined by the ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods. For all the plants tested, at least one extract inhibited the activity of α-amylase. The most effective was the hydroethanolic extract of *T. superba*. The presence of active phytochemical substances with antioxidant properties may account for the above mentioned properties, and provide substantial basis for the use of these plants in ethno-medicine for the treatment of diabetes.

Key words——phytochemicals, antioxidant activity, α-amylase, 1,1-diphenyl-2-picrylhydrazyl, ferric reducing antioxidant power

INTRODUCTION

In the past few years, reactive oxygen species (ROS) have been linked to several pathological situations. The oxidation induced by ROS-like highly reactive free radicals, especially oxygen-derived radicals which are formed by exogenous chemicals or endogenous metabolic processes in the human body can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease.[1–3] Almost all organisms are well protected against free radical damage by enzymes such as gluthation peroxidase, superoxide dismutase and catalase or compounds such as vitamins (E and C), glutathione and phenolic compound.[4, 5] When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur resulting in diseases and accelerating aging. An example of oxidative stress in disease is observed in diabetes mellitus, which is aggravated by an increase in oxidative stress.[6)]

As a new strategy for alleviating the oxidative damage in diabetes, interest has grown in the usage of natural antioxidants. It has been postulated that supplementation with antioxidants such as vitamin E, C and other non-nutrient antioxidant such as plant derived natural antioxidants have been shown to reduce the oxidative stress in experimental diabetes.[7, 8] It has been also postulated that supplementation of antioxidant vitamin C showed to lower glycosylated haemoglobin is diabetic patient.[9]

There is high demand for natural antioxidants in the food, cosmetic and therapeutic industries, due to their low cost, high stability, high compatibility with dietary intake and no harmful effects to the human body, like some synthetic antioxidants butylated hydroxy anisole (BHA) and butylated hydroxy

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toluene (BHT) are carcinogenic in nature. On the basis of above facts, natural antioxidants are potentially promising alternatives for synthetic antioxidants.[10, 11]

Plants like *Terminalia superba* (*T. superba*), *Albizia* sp. (*A*. sp.), *Cola nitida* (*C. nitida*), *Cola odorata* (*C. odorata*) and *Harungana madagascarensis* (*H. madagascarensis*) are used by tradipractitioners in Cameroon for the management of diabetes. The mechanism of action of these plants are however not known, but could be related to the reduction of circulating blood glucose levels and oxidative stress, two conditions that need correction in diabetes.

In this paper, we report the *in vitro* ability of these Cameroonian medicinal plant extracts to reduce oxidative stress and inhibit the activity of pancreatic α-amylase, a key enzyme in the control of blood glucose levels.

MATERIALS AND METHODS

Plants Materials —— *T. superba* (roots), *C. nitida* (stem), *A*. sp. (stem), *H. madagascarensis* (roots) and the aerial part (leaves and stem) of *C. odorata* were collected from the Yaoundé environs in January 2006. They were identified at the National Herbarium in Yaoundé and assigned voucher numbers. The plants were dried and extracted using different solvent systems.

Preparation of Different Extracts —— Four solvent systems were used to extract the active principle in each plant: distilled water, ethanol, ethanol/water 1 : 1 and methanol. Three g of each plant powder was macerated in 60 ml of solvent. After 48 hr, the mixture was filtered 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 ($FeCl_3$), 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 α-amylase were purchased from Sigma Chemical Co. Ltd. (Saint Louis, Missouri, U.S.A.). All others reagents were for analytical grade.

Preliminary Qualitative Phytochemical Screening —— Standard preliminary qualitative phytochemical screening procedures were used as described by Trease and Evans.[12] Various reagents and tests were used: $FeCl_3$ 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, $K_3Fe(CN)_6$ for phenols and polyphenols. The presence of a bioactive compound was indicated by a colour change.

Determination of Polyphenols Contents Using Folin-ciocalteu Phenolic Reagent —— The total phenolic compounds of different extracts were determined with Folin-ciocalteu reagent according to the method of Singleton[13] using catechin as a standard phenolic compound. Briefly, 30 µl 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 polyphenols contents in the extracts was determined in milligrams of catechin equivalent per gram of dry powder.

Ferric Reducing Antioxidant Power (FRAP) —— The FRAP was determined using the method of Benzie[14] which measures the reduction of ferric ion to the ferrous form in the presence of antioxidant components. The FRAP reagent consist of ten part acetate buffer (300 mM, pH 3.6), one part of TPTZ (10 mM in 400 mM of HCl) and one part of ferric chloride. The colorimetric measurement was performed at 593 nm and the reaction was monitored for up to 12 min on 75 µl 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 DPPH using the method of Katalinié.[15] Used as a reagent, DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. A solution of 0.3 M DPPH in methanol was prepared and 2 ml of this solution was added to 20 µl of the plant extract (1 mg/ml). After 30 min, the absorbance was measured at 517 nm. Catechin was used as the standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage inhibition was calculated as earlier described by Yen.[16]

Determination of α-Amylase Inhibitory Activity by Iodine-starch Assay —— The α-amylase inhibition assay was carried out by a modified method of Komaki.[17] Seventy five µl of each extract (1 mg/ml) were mixed with 100 µl (0.1%) of soluble starch substrate in phosphate buffer (0.25 M, pH 7.0). After 5 min of incubation at 37°C, 20 µl of 30 µg/ml solution of α-amylase in phosphate buffer (pH 7.0) was added to the mixture. After the mix-

ture 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 were made in triplicate and the results presented as mean ± standard deviation. Analysis of variance (ANOVA) one way, post hoc Low Statistical Difference (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 soft wear Statistical Package for Social Science (SPSS) was used for this analysis.

RESULTS

Preliminary Qualitative Phytochemical Screening —— Polyphenols and tannins were identified in all plant extracts. Only aqueous and hydroethanolic extracts of *A.* sp., the ethanolic extract of *C. odorata*, the aqueous extract of *C. nitida* and the hydroethanolic extract of *C. odorata* did not showed respectively the presence of flavonoids, saponins and glycosides. Triterpens were identified in *T. superba*, *H. madagascarensis*, *C. nitida* but not in *C. odorata*, while only the hydroethanolic extract of *A.* sp. showed the presence of this metabolite.

Total Phenolic Compounds of Plant Extracts —— The results of total phenolics content of different plant extracts are presented in Fig. 1. There were a significant difference ($p < 0.05$) in the polyphenolics contents of *T. superba* and *H. madagascariensis* for the different plant extracts. The lowest content was found in the aqueous extract of *C. nitida* (185.67 ± 2.4 mg catechin equivalent/g of dry material) while highest content was found in ethanol extract of *T. superba* (637.31 ± 4.19 mg catechin equivalent/g of dry material). *T. superba* and *H. madagascarensis* have their highest polyphenols contents when extracted with ethanol (637.31 ± 4.19 and 390.12 ± 1.836 mg catechin equivalent/g of dry material respectively) while the aqueous and methanol solvent extract more phenolics compound from *A.* sp., *C. odorata* and *C. nitida* respectively.

Ferric Reducing Antioxidant Power of Plant Extracts —— Figure 2 shows the FRAP of different

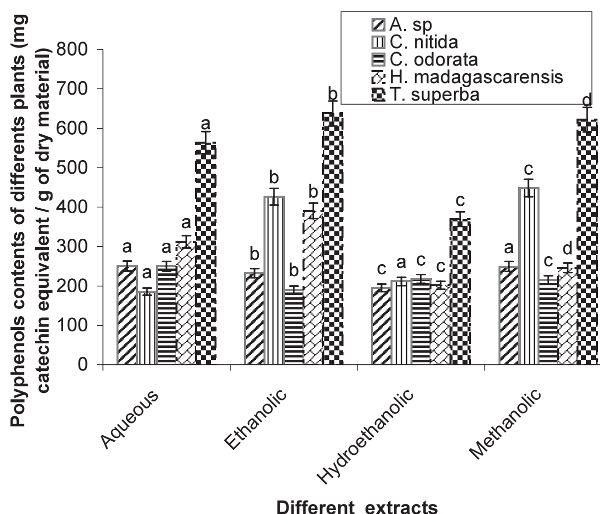

Fig. 1. Polyphenols Contents of Plant Extracts (mg catechin equivalent/g of dry material)

Results are presented as means ± standard deviation, $n = 3$; the extracts of the same plant are compared; the data affected with different letters are significantly different ($p < 0.05$).

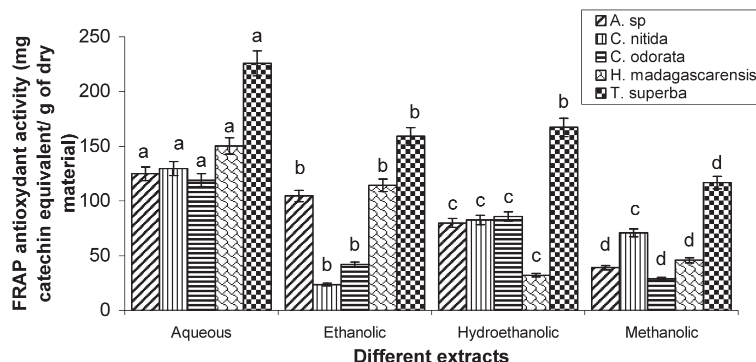

Fig. 2. FRAP Antioxidant Capacity of Plant Extracts (mg catechin equivalent/g of dry material)

Results are presented as means ± standard deviation, $n = 3$ the extracts of the same plant are compared; the data affected with different letters are significantly different ($p < 0.05$).

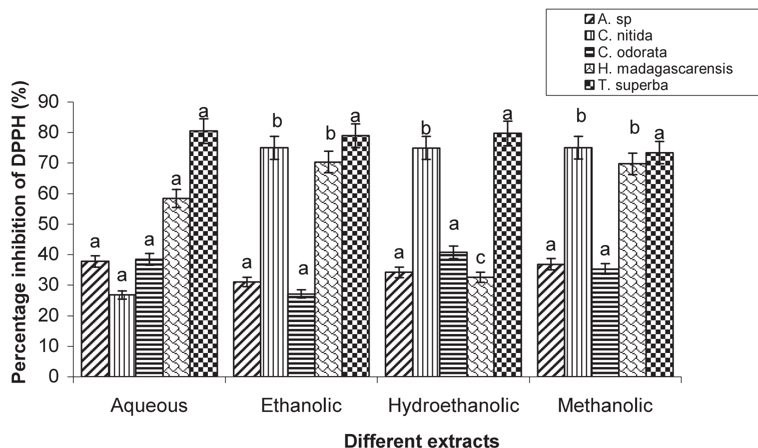

Fig. 3. Percentage Inhibition of DPPH Radical Scavenging Activity (%)

Results are presented as means ± standard deviation, $n = 3$; the extracts of the same plant are compared; the data affected with different letters are significantly different ($p < 0.05$).

plant extracts. *T. superba* showed the highest FRAP in all solvent extracts. Statistical analysis revealed that all extracts of *A.* sp., *C. odorata* and *H. madagascarensis* had significantly different FRAP activities at $p < 0.05$. High antioxidant activity was obtained from the aqueous extract of *T. superba* (225.79 ± 8.22 mg catechin equivalent/g of dry material) while low antioxidant activity were found from ethanolic extract of *C. nitida* (23.66 ± 0.96 mg catechin equivalent/g of dry material).

DPPH Radical Scavenging Effect of Plant Extracts —— Radical scavenging activities (RSA) of different plant extracts are shown in Fig. 3. There was a significant difference ($p < 0.05$) between different plant extracts. In the extracts tested for each plant, the hydroethanolic extract from *T. superba* reacted faster than the other extracts and was the most effective DPPH radical scavenger (80.53 ± 0.39%). This was followed by the methanolic extract of *C. nitida* (75.04 ± 2.47%) and the ethanolic extract of *H. madagascarensis* (70.35 ± 3.76%) while the aqueous extract of *A.* sp. (37.72 ± 3.35%) showed the lowest RAS activity. It was also noticed that only *A.* sp., *C. odorata*, the hydroethanolic extract of *H. madagascarensis* and the aqueous extract of *C. nitida* showed less than 50% inhibition of DPPH.

Figures 4 and 5 summarise the relationship between Folin, FRAP and DPPH. There is a significant correlation ($p < 0.01$) between the polyphenols contents and FRAP (Fig. 4); between phenolic content and DPPH radical-scavenging activity ($p < 0.01$; Fig. 5).

On the other hand no correlation was observed between FRAP antioxidant activity and DPPH.

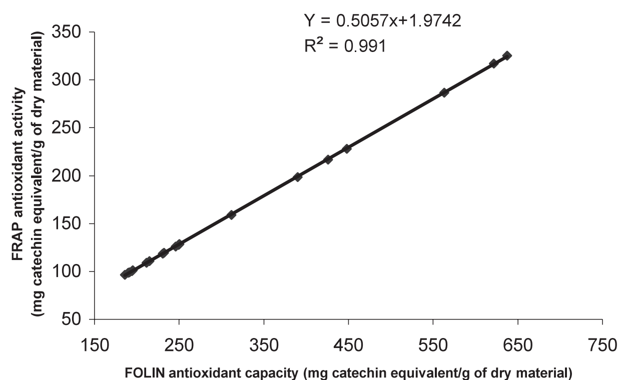

Fig. 4. Correlation Analysis between Folin Antioxidant Capacity and FRAP Antioxidant Activity of the Studied Samples

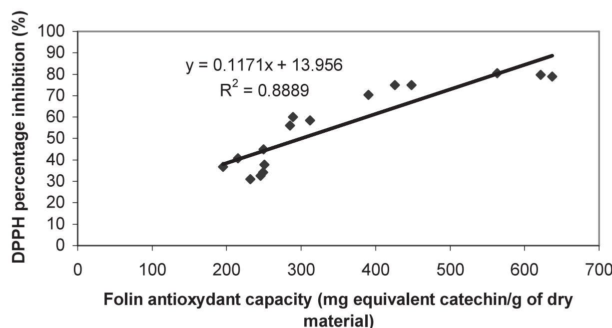

Fig. 5. Correlation Analysis between Folin Antioxidant Capacity and the Inhibition of DPPH Radical of the Studied Samples

Effect of Plant Extracts on Pancreatic α-amylase Activity —— The results of different plant extracts on inhibition of porcine pancreatic α-amylase are showed in Fig. 6. The lowest α-amylase inhibition

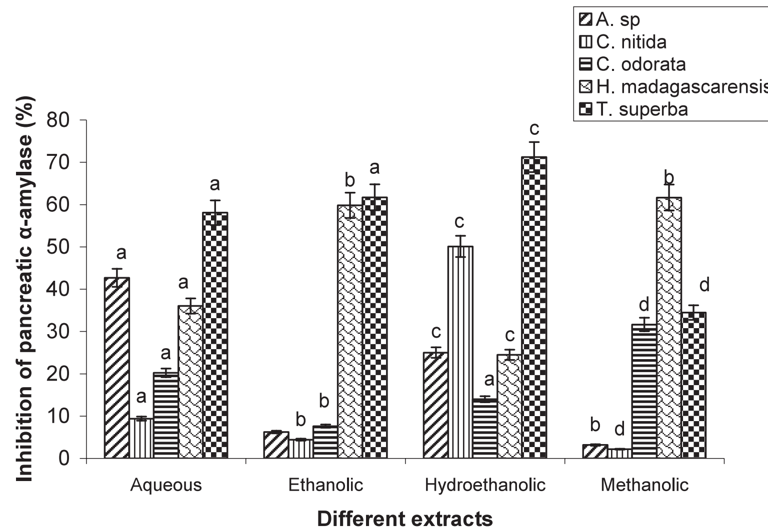

Fig. 6. Percentage Inhibition of Plant Extracts on Pancreatic α-amylase

Results are presented as means ± standard deviation, $n = 3$; the extracts of the same plant are compared; the data affected with different letters are significantly different ($p < 0.05$).

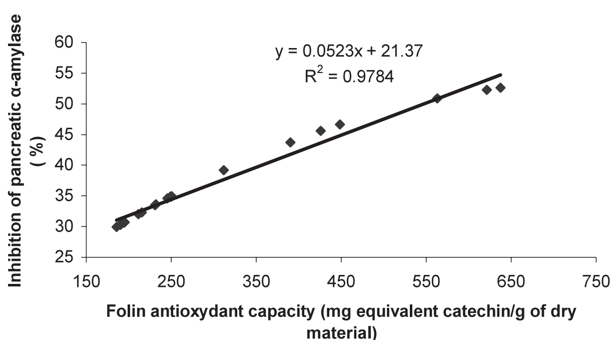

Fig. 7. Correlation Analysis between Folin Antioxidant Capacity and the Inhibition of Pancreatic α-amylase

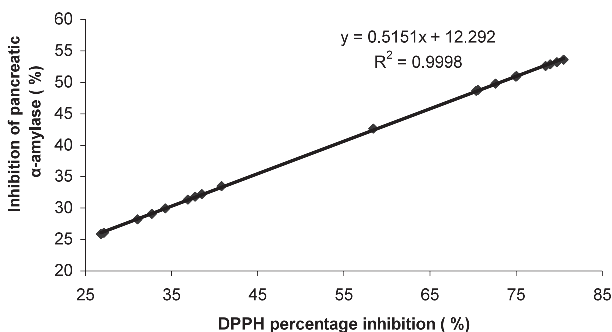

Fig. 8. Correlation Analysis between the Inhibition of DPPH Radical and the Inhibition of Pancreatic α-amylase

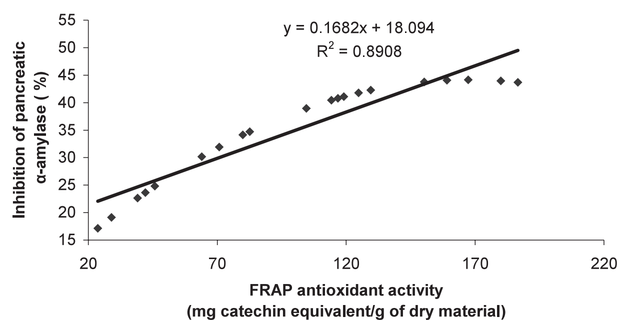

Fig. 9. Correlation Analysis between FRAP Antioxidant Activity and the Inhibition of Pancreatic α-amylase

observed in the methanolic extract of *C. nitida*, while the highest inhibition was with the hydroethanolic extract of *T. superba*.

The results obtained from the inhibition of porcine α-amylase reveal that there was a significant correlation between polyphenols contents and pancreatic α-amylase ($p < 0.05$; Fig. 7); between DPPH radical-scavenging activity and-α-amylase ($p < 0.05$; Fig. 8) and between FRAP antioxidant and pancreatic α-amylase ($p < 0.001$; Fig. 9).

DISCUSSION

Phenolic compounds are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts.[18] Phenolics, triterpens, flavonoids, tannins and saponins detected in the extracts are compounds that have been reported to possess medicinal properties and health-promoting effects.[19]

All the plant extracts tested contained polyphenols compounds, with *T. superba* having the highest polyphenol content when extracted with ethanol.

These plants have thus an antioxidant activity which would be granted to these polyphenolic compounds, secondary metabolites and principal compounds of plants.[20] They include tannins; flavonoids and phenolic acids and act as hydrogen donors to free radicals by stopping lipid peroxidation at the stage of initiation.[21]

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.[22] All the plant extracts showed the high FRAP activity with aqueous extract. These results confirm others obtained by Then *et al.*[23] who stipulated that alcoholic extracts do not have an influence on the antioxidant activity of the aqueous extract measured by FRAP method.

In the present investigation, all the plants tested at different solvent demonstrated significant DPPH scavenging activity indicating their abilities to act as radical scavengers. From the results obtained, we notice that the hydroethanolic extract of *C. nitida* a low polyphenols contents had a very high DPPH scavenging activity. This goes to support the hypothesis of Brand-Williams *et al.*[24] 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.

Significant correlations between polyphenols contents and FRAP, polyphenols contents and DPPH radical-scanvenging activity suggesting that FRAP and the radical scanvaging activity of the extracts are due mainly to its content of polyphenols compounds.

The antidiabetic properties of plants can be evaluated by many methods amongst which we have the determination of the α-amylase inhibitory activity by 2-chloro-4-nitro phenyl α-maltotrioside (CNP-G_3) assay, the determination of the α-glycosidase inhibitory activity and the determination of the α-amylase inhibitory activity by the iodine starch assay.[25,26] α-amylase (EC 3.2.1.1) is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions, functioning in the breakdown of the α-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 α-amylase reduces the bioavailability of glucose.[27,28] The correlations noticed between polyphenols contents (especially tannins, flavonoids and phenolic acids) and the inhibition of pancreatic α-amylase stipulate that this can be used as a marker to antidiabetic extracts. This suggests that polyphenols compounds involved in the DPPH scavenging activity may also be involved in the pancreatic α-amylase inhibition or may directly or indirectly intervene in the enzyme activity mechanism.[29]

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 α-amylase, to different extracts.

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