

Full Length Research Paper

Antioxidant activity of eight plants consumed by great apes in Côte d'Ivoire

AHOUA Angora Remi Constant^{1,2}, KONE Mamidou Witabouna^{1,2*}, KONAN Amoin Georgette^{2,3},
TRA Bi Fézan Honora¹ and BONFOH Bassirou²

¹UFR Sciences de la Nature, Université d'Abobo-Adjamé. BP 801 Abidjan 02, Côte d'Ivoire.

²Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS). BP 1303 Abidjan 01, Côte d'Ivoire.

³Laboratoire de Biochimie, UFR Biosciences, Université de Cocody Abidjan. BP 582 Abidjan 22, Côte d'Ivoire.

Accepted 27 April, 2012

Oxidative stress is an aggravating factor involved in a number of pathologies. The source and mobilization of antioxidant compounds are a challenge for the public health sector and new approaches are needed to assess and identify the main sources of antioxidants. Monkeys and great apes are considered to tolerate the Simian immunodeficiency virus (SIV) infection and other diseases. The current study aimed at screening wild chimpanzee's diet to select plants with high antioxidant potential as supplement for improving health status of people under oxidative stress. Bio-cultural approach based on chimpanzee's diet or auto-medication and human traditional medicine was used for selection of eight species, *Ficus elasticoides*, *Ficus lyrata*, *Ficus umbelleta*, *Ficus thonningii*, *Ficus mucuso*, *Xylopiya quintasii*, *Sherbournia calycina* and *Myrianthus libericus*. Further, antioxidant activity of extracts (methanolic and dichloromethane) from these plants was assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. Methanolic extract of leaves from *F. elasticoides* showed the highest radical scavenging activity with 96.69% of DPPH inhibition, followed by extracts of *F. lyrata* (94.53%), *X. quintasii* (94.36%) and *F. mucuso* (94.33%). The IC₅₀ values of extracts were respectively 7.8, 9.3, 8.3 and 8.7 µg/ml and close to those of ascorbic acid (8.00 µg/ml) and gallic acid (8.20 µg/ml). The ferric reducing power of *F. lyrata* (185.01 µM) was the strongest. Active species contain monoterpenoid, secoiridoides and polyphenols. Further investigation on the use of such plants in the traditional medicine will contribute to generate an added value at the interface of human and animal nutrition to provide nutraceuticals for immunocompromised people.

Key words: Côte d'Ivoire, great apes diet, oxidative stress, antioxidant activity.

INTRODUCTION

Oxidative stress has been implicated in more than 200 diseases affecting human (Datta et al., 2000). The main etiologic factors are free radicals when in excess, can

cause damage in the walls, proteins or deoxyribonucleic acid (DNA) of cells (Esterbauer et al., 1992; Cadet et al., 2002). Oxidative stress may affect the immune system elements causing a state of immunodepression, favorable to the emergence of many health problems such as cancer, cardiovascular diseases, Alzheimer's disease, diabetes (Datta et al., 2000) and opportunistic infections (Greenspan, 1993). Oxidative processes are increased in vulnerable people (young, pregnant and immunocompromised) and in particular infants and children due to fragility and immaturity of their immune system (OMS, 2005; Desmoulins, 2006).

*Corresponding author. E-mail: mamidou.kone@csrs.ci. Fax : +225 23 45 12 11.

Abbreviations: SIV, Simian immunodeficiency virus; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power.

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Harman, 1992; Halliwell, 1994). The intake of these compounds contributes to the prevention of oxidative stress and the maintenance of good health (Tammy and Carla, 1994). A wide variety of antioxidants are naturally constituents of plants and as such normal components of human daily diet. Lighter antioxidants are found in the diet, the best known are vitamin E, vitamin C and the carotenoids. Many other non-nutrient food substances, generally phenolic or polyphenolic compounds, display antioxidant properties (Bagchi and Puri, 1998). The consumption of food enriched with antioxidants is of benefit in reducing or preventing oxidative stress and its related complications (Glouchkoff, 2008). Therefore, plant compounds with high antioxidant properties could be considered as a potential nutraceutical or functional ingredient. Such promising plants may come from human traditional medicine, but also from animal auto-medication and food (Luc Montagnier, personal communication).

Human shares the same environment with animals and some transmitted diseases. As such, in the "one health" concept (Zinsstag et al., 2005, 2009, 2011) contributing to generate an added value of species diet, the environmental elements used by one can be explored to face disease in the other. According to Huffman et al. (2000), to solve some health problems in human, we have much to gain by taking a closer look at the pharmacological characteristics of both the proposed auto-medicative and daily dietary items and behavior of wild animals. As a first approach, we focused on chimpanzee, the animal nearest genetically to human with 98% common genome (Leciak, 2009). By this genetic affiliation, expressed in numerous characters, both can be infected by common diseases such as immunodeficiency virus infection, that virus passed from chimpanzees to humans (Keele et al., 2006). In their natural habitat, primates are highly tolerant to Simian immunodeficiency virus (SIV) while human is more vulnerable to human immune virus (HIV) (Kurth and Norley, 1996; Weiss, 2001).

Chimpanzees' resistance to SIV was found to be in part genetic (Peeter et al., 1989) but also linked to environmental factors such as diet. Chimpanzees are largely frugivorous but also consume leaves, flowers and piths of plant species (Huffman et al., 2000). Study on food behavior of great apes in Côte d'Ivoire revealed that many Moraceae species (*Ficus*) are great part of their diet, dependent on the phenology (Boesch, 1989; Goné Bi, 1999). Some figs are eaten by human in Côte d'Ivoire (Kamanzi, 2002) for nutritional and health needs. Most of these, *Ficus sycomorus* exhibited high antioxidant activity (Abdel-Hameed, 2009). So the nutritious plants eaten by great apes under apparently normal conditions may be sources of antioxidants for reducing progression of oxidative stress. The present study investigates eight plant species, consumed by chimpanzees of Côte d'Ivoire

for antioxidant properties.

MATERIALS AND METHODS

Plant selection

Based on bio-cultural approach using data from human traditional medicine (Gautier-Béguin, 1992; Sofowora, 1996; Tra Bi, 1997; Malan, 2002; Kamanzi, 2002), chimpanzee diet and auto-medication (Boesch, 1989; Goné Bi, 1999), eight plant species only consumed by chimpanzees of Côte d'Ivoire, were selected for evaluation of their antioxidant activity. These plants species were selected on the basis of three criteria which are: the lack of study carried out on these species in relation with antioxidants, the families and genus most represented on the list of species only eaten by chimpanzees and the availability of these species in the surrounding forests of Petit Yapo and Adiopodoumé. These species were available, abundant and accessible at the time of the experiment.

Plant parts were harvested in Southern Côte d'Ivoire, through the forests of Petit Yapo (District of Agboville) and Adiopodoumé (Municipality of Songon) from November to December 2009.

Voucher specimens of the selected plants were collected and processed according to standard practice, identified and then maintained together with photos at the Herbarium of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire. Botanical nomenclature follows the flora of Côte d'Ivoire (Aké Assi, 2001) and the flora of West Tropical Africa (Hutchinson and Dalziel, 1954 to 1972; Lebrun and Stork, 1991, 1992, 1995, 1997).

Preparation of extracts

Plant parts (leaves and fruits) were dried in air conditioning at 18°C for two weeks, and grounded to obtain powders. Powders (15 g) were first extracted with 150 ml of dichloromethane followed by methanol under mechanical stirring (175 rev/min) during 24 h. This operation was performed three times. After filtration, solvents were completely removed in a rotavapor (Büchi R-114, Büchi, Flawil, Suisse) at 40°C and 60 rev/min and extracts were lyophilized.

Evaluation of antioxidant activity

The evaluation of antioxidant activity was performed by thin layer chromatography (TLC) and spectrophotometry. TLC was used for qualitative determination of antioxidant activity and related phytochemicals. Active extracts were then evaluated for quantitative assessment by spectrophotometry dosage.

Detection of antioxidant potential on TLC

Briefly, 10 µl of extract (10 mg/ml) were applied on aluminium backed silicagel 60 F₂₅₄ plates and then developed in two mobile phases depending on the polarity of extracts. These elution systems were chloroform-methanol-water (65:35:5) for methanolic extracts and hexane-ethyl acetate (1:1) for dichloromethane extracts. Two plates were developed for each assay; one for the test and the other as a reference detected with specific reagent for each group of phytochemicals. The retention factor (Rf) values were recorded, using the formula:

$$R_f \text{ value} = \frac{\text{Distance move by the compound}}{\text{Distance move by the solvent front}}$$

For detection of radical scavengers, the chromatograms were sprayed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (2

mg/ml in methanol). The appearance of white spots on a purple background indicates the presence of scavenging compounds (Takao et al., 1994).

Quantitative estimation of antioxidant activity

a) Radical scavenging assay with DPPH

For extracts showing antioxidant activity on TLC, the scavenging activity was quantified using Blois method (Blois, 1958). Briefly, 2.5 ml DPPH radical solution at 0.04 % (4 mg of DPPH in 100 ml of methanol) were added to 100 μ l of each extract. Extracts concentrations vary from 1000 to 15 μ g/ml, obtained from a stock solution (26 mg/ml). The mixtures were incubated in the dark for 30 min and their absorbance was measured at 517 nm, against the control (methanol and DPPH standard solution). The tests were performed in triplicate and the mean of absorbance were calculated. The percentage of scavenged DPPH radical was calculated with the following formula:

$$\% \text{ scavenged DPPH} = [(A_0 - A_1) / A_1] * 100$$

Where, A_0 is the absorbance of the control and A_1 the absorbance of the extract after 30 mn of incubation. Ascorbic acid (Prolabo, VWR, Switzerland) and gallic acid (Fluka chemika, Sigma Aldrich, Switzerland) were used as standards. The IC_{50} (concentration providing 50% inhibition) was determined graphically using curves of standards in the linear range by plotting the extract concentration against the corresponding scavenging effect.

b) Ferric reducing antioxidant power assay

The ability of the extract to reduce ferric ions was measured using the ferric reducing antioxidant power (FRAP) method described by Benzie and Strain (1996, 1999). The stock solutions were 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$; pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM TPTZ in 40 mM HCl) and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The FRAP solution was prepared freshly by mixing acetate buffer, TPTZ solution and $FeCl_3 \cdot 6H_2O$ solution (10:1:1), and then warmed at 37°C before 3 ml of FRAP reagent was added to 100 μ l of each extract (1000 to 15 μ g/ml) prepared from stock solutions (26 mg/ml) and the absorbance was read at the beginning. Then the extracts were incubated in water-bath, at 37°C for 4 min and the absorbance was measured against FRAP standard solution as blank. Experiments were performed in triplicate for each concentration and FRAP values (μ M) of extracts were calculated using the following formula:

$$(FRAP)_{\text{extract}} = (AE_0 - AE_4 / AS_0 - AS_4) * FRAP \text{ value of standard reference as ascorbic acid,}$$

Where, FRAP value of this standard is 2 μ M. AE_0 is the absorbance of extract at $t = 0$ min, AE_4 = absorbance of extract at 4 min, AS_0 and AS_4 are absorbances of standard at 0 and 4 min respectively.

TLC phytochemical screening

The presence of secondary metabolites in the studied plants was qualitatively determined by reference TLC using various reagents. The summaries of methods used are shown in Table 1. Godin's reagent under visible or 366 nm UV light observation was used to identify several types of compounds (Wagner et al., 1984). Purple, blue and red spots in visible spectrum indicate presence of monoterpenoids (including essential oils, saponins, iridoids). Yellow spots correspond to polyphenols such as flavonoids, xanthenes and

naphthoquinones. Brown spots characterize secoiridoids. At 366 nm UV light, yellow and orange fluorescences show presence of flavonoids (Kouri, 2004). With aluminum chloride ($AlCl_3$) solution (5% in ethanol), yellow spots or fluorescence eye visible or with 366 nm UV light indicate the presence of flavonoids (Merck, 1980). In the presence of ammoniac solution (25%), yellow, green (Georgievskii et al., 1990) and blue fluorescences (Dawson et al., 1991) characterize coumarins. Eye visible blue spots with Folin-ciocalteu reagent indicate the presence of phenolic compounds (Mallikharjuna et al., 2007).

Statistical analysis

The software STATISTICA 7.1 was used for data analysis (Statistica, 1999). Results are presented as mean followed by standard deviation (SD) of triplicate experiments. The influence of different concentrations of the extracts on the percentage scavenging of DPPH was performed by one-way analysis of variance (ANOVA). Significant differences between extracts were determined at $P < 0.05$. The least significant difference (LSD) test was used to determine the difference in the percentage of scavenged DPPH among extracts. The correlation between FRAP and DPPH assays is given by the regression analysis, with $R^2 \geq 0.90$ as the highest correlation value (Prabhjit et al., 2008).

RESULTS AND DISCUSSION

Qualitative assessment of antioxidant activities of extracts

Of the 22 extracts tested, 14 (64%) including three dichloromethanes and 11 methanolics, showed radical scavenging activity (Table 2). No antioxidant activity in DPPH screening was found for dichloromethane extracts of *Myrianthus libericus* leaves and *Ficus umbellata* fruits. The results show that all the tested plants contain antioxidant compounds. Methanolic extracts from these plants are more active than dichloromethane extracts. This is explainable as phenolic compounds which are most extracted by methanol, are more often responsible for antioxidant activity of many plants (Hennebelle et al., 2004). To the best of our knowledge, this is the first report on the antioxidant activity of these African plants and the daily dietary of primates, except for *Ficus lyrata*.

Quantitative estimation of antioxidant activities of extracts

Antioxidant activities assessed by spectrophotometry are presented in the Tables 3 and 4. There was a very high significant difference ($\alpha = 5\%$, $P < 0.001$, $F = 53.41$) between the percentages of DPPH inhibition of extracts, ascorbic acid and gallic acid (Table 3). The multiple comparison with the LSD test showed that the highest scavenging activity was achieved by leaves methanolic extracts of *Ficus elasticoides*, *Ficus lyrata*, *Xylopia quintasii* and *Ficus mucuso*. Methanolic extracts of *Ficus thonningii* (leaves), *Sherbournia calycina* (leaves), *F.*

Table 1. Summaries of different methods used to determine the nature of active antioxidant compounds.

Reagent	Condition	Color	Result
Godin	Visible	Purple, blue, red	Monoterpenoids
		Yellow	Flavonoids
Aluminium chloride (AlCl ₃)	366 nm UV light	Brown	Secoiridoids
		Yellow, orange	Flavonoids
Aluminium chloride (AlCl ₃)	366 nm UV light	Yellow	Flavonoids
		Yellow	Flavonoids
Ammonia solution (NH ₃) 25%	366 nm UV light	Yellow, green, blue	Coumarins
Folin-Ciocalteu	Visible	Blue	Polyphenols

Table 2. Antioxidant activity and possible related compounds of studied plants.

Methanolic extract	Before derivatization				After derivatization							Possible compound
	Rf	Visible	254 nm	366 nm	DPPH		AlCl ₃	NH ₃	Godin		Folin	
					Activity	Rf	366 nm	366 nm	Visible	366 nm	Visible	
<i>Ficus elasticoides</i> (leaves)	0.00		Visible		+++	0.00			Red			Monoterpenoids
					+++	0.05	Purple				nd	
<i>Ficus elasticoides</i> (fruits)	0.00	Yellow	Visible	Yellow	+++	0.00			Brown		Grey	Secoiridoids
					+	0.09					nd	
					+	0.23	Yellow		Blue		Blue	Polyphenols (flavonoids)
<i>Ficus lyrata</i> (leaves)	0.00		Visible		+++	0.00			Red			Monoterpenoids
					+++	0.05					nd	
					+++	0.17					nd	
					+++	0.39					nd	
					++	0.51					nd	
<i>Ficus mucoso</i> (leaves)					+++	0.00	Yellow					Flavonoids
					+++	0.05					nd	

Table 2. Contd.

<i>Ficus thonningii</i> (leaves)	0.16	Yellow	+++	0.03			nd
			+++	0.16	Pale yellow		Flavonoids
			+++	0.00		Blue	Monoterpenoids
<i>Ficus umbellata</i> (leaves)	0.49	Visible	+++	0.16	Yellow		Flavonoids
	0.64	Blue	+++	0.49			nd
			+++	0.64		Blue	Polyphenols
<i>Ficus umbellata</i> (fruits)			+++	0.03			
			+++	0.16	Blue		nd
			+++	0.49			nd
			+++	0.72		Purple	nd

nd = No determined.

Table 3. DPPH radical scavenging power of plant species and reference compounds.

Reference compounds and plant species	Parts of plant	Extract	% inhibition \pm SD	IC ₅₀ (μ g/ml)
Ascorbic acid			97.65 \pm 0.49 ^a	8.00
Gallic acid			96.53 \pm 1.21 ^{ab}	8.20
<i>Ficus elasticoides</i>	Leaves	MeOH	96.69 \pm 2.05 ^{ab}	7.75
<i>Ficus lyrata</i>	Leaves	MeOH	94.53 \pm 4.72 ^{ab}	9.25
<i>Xylopiya quintasii</i>	Leaves	MeOH	94.36 \pm 2.20 ^{ab}	8.25
<i>Ficus mucuso</i>	Leaves	MeOH	94.33 \pm 3.55 ^{ab}	8.70
<i>Ficus thonningii</i>	Leaves	MeOH	89.86 \pm 12.67 ^b	13.00
<i>Sherbournia calycina</i>	Leaves	MeOH	88.94 \pm 9.62 ^b	11.50
<i>Myrianthus libericus</i>	Leaves	MeOH	87.16 \pm 14.44 ^b	14.40
<i>Ficus umbellata</i>	Fruits	MeOH	74.74 \pm 25.71 ^c	33.25
<i>Ficus umbellata</i>	Leaves	MeOH	73.03 \pm 25.91 ^c	41.50
<i>Ficus elasticoides</i>	Fruits	MeOH	71.44 \pm 30.93 ^c	46.10
<i>Sherbournia calycina</i>	Fruits	MeOH	68.42 \pm 28.01 ^c	53.50
<i>Ficus umbellata</i>	Leaves	DCM	28.18 \pm 18.07 ^d	904
<i>Ficus lyrata</i>	Leaves	DCM	26.09 \pm 23.99 ^d	416
<i>Xylopiya quintasii</i>	Leaves	DCM	18.10 \pm 11.83 ^d	> 1000
F			53.41	
P			< 0.001	

MeOH, Methanol; DCM, dichloromethane; IC₅₀, concentration providing 50% inhibition; F, fisher statistical; P, probability; SD, standard deviation; DPPH, 2, 2-diphenyl-1-picrylhydrazyl.

Table 4. Iron chelating potential of active extracts.

Plant species	Parts of plant	Extract	FRAP values \pm SD (μM)
<i>Ficus lyrata</i>	Leaves	MeOH	185.01 \pm 99.70 ^a
<i>Ficus umbellata</i>	Leaves	MeOH	149.94 \pm 57.26 ^a
<i>Sherbournia calycina</i>	Leaves	MeOH	127.74 \pm 52.91 ^a
<i>Ficus mucuso</i>	Leaves	MeOH	114.65 \pm 41.93 ^a
<i>Myrianthus libericus</i>	Leaves	MeOH	112.63 \pm 39.93 ^a
<i>Ficus thonningii</i>	Leaves	MeOH	111.56 \pm 30.02 ^a
<i>Xylopiya quintasii</i>	Leaves	MeOH	85.07 \pm 42.74 ^a
<i>Ficus elasticoides</i>	Leaves	MeOH	47.66 \pm 25.74 ^a
<i>Sherbournia calycina</i>	Fruits	MeOH	29.75 \pm 6.27 ^a
<i>Ficus umbellata</i>	Fruits	MeOH	9.04 \pm 1.74 ^a
F			1.32
P			0.23

MeOH, Methanol; FRAP, ferric reduction antioxidant power; F, fisher statistical; P, probability; SD, standard deviation.

elasticoides (fruits), *S. calycina* (fruits), *M. libericus* (leaves) and *F. umbellata* (fruits and leaves) exhibited moderate radical scavenging activities, while low radical scavenging ability was found in leaves dichloromethane extracts of *F. umbellata*, *F. lyrata* and *X. quintasii*. The determination of percentage scavenging of DPPH radical showed that the most promising extracts were leaves methanolic extracts from *F. elasticoides*, *F. lyrata*, *F. mucuso* and *X. quintasii* which showed the highest radical scavenging activities with IC₅₀ values of 7.75, 9.25, 8.25 and 8.70 $\mu\text{g/ml}$, respectively (Table 3). These values are close to those of ascorbic acid (8.00 $\mu\text{g/ml}$) and gallic acid (8.20 $\mu\text{g/ml}$), two strong antioxidant compounds. Consequently, the concerned plant species are rich in radical scavengers. *F. elasticoides* leaves has the highest antioxidant activity with IC₅₀ (7.75 $\mu\text{g/ml}$) lower to those of reference compounds.

Results of FRAP assay showed that, among the 22 tested extracts, only 10 exhibited ferric reducing activity (Table 4). Statistical analysis showed no significant difference ($\alpha = 5\%$, $P > 0.05$, $F = 1.32$) between the tested extracts. Only methanolic extracts presented significant FRAP antioxidant capacity. Leaves of *F. umbellata* and *S. calycina* showed high ferric reducing antioxidant power, while their fruits led to low FRAP values. Also, leaves of *F. lyrata*, *F. mucuso*, *F. thonningii* and *M. libericus* showed high ferric reducing antioxidant power. *X. quintasii* and *F. elasticoides* achieved low ferric reducing antioxidant potential. However the strongest ferric reductants were *F. lyrata*, *F. umbellata* and *S. calycina* leaves extracts with FRAP values of 185.01; 149.94 and 127.74 μM , respectively.

Intermediate mutual positive correlation was observed, as coefficient determination (R^2) of 0.599 (Figure 1) was obtained between DPPH and FRAP assays for most of the active plants; a R^2 value of 0.90 is required to have high significant correlation (Prabhjit et al., 2008). Leaves extracts of *F. elasticoides* and *X. quintasii* exhibited

strong scavenging activity in DPPH assay but had low antioxidant potential according to FRAP value. For *F. umbellata*, leaves showed low radical scavenging activity in DPPH test, but high activity with FRAP assay. However, leaves of *F. lyrata*, *F. mucuso*, *S. calycina*, *F. thonningii* and *M. libericus* showed a strong antioxidant activity for DPPH radical scavenging as well as FRAP value. Fruits of *F. umbellata* and *S. calycina* showed low antioxidant activity with both FRAP and DPPH methods. However, *F. lyrata* and *F. mucuso* leaves extracts showed high antioxidant potential in both assays. This observation suggests that antioxidant compounds are probably different from one plant to another.

Many species of the genus *Ficus* are involved in the daily diet of Chimpanzees in Côte d'Ivoire (Goné Bi, 1999). Interestingly, most of the tested *Ficus* showed high antioxidant potential, particularly *F. lyrata* and *F. mucuso*. Previous studies reported antioxidant activity of several other *Ficus* species. The aqueous extract of *Ficus religiosa* modulated enzymes of antioxidant defense system, hence reducing oxidative stress induced by type 2 diabetes in rats (Kirana et al., 2009). Green fruits of *Ficus glomerata* contain phenolic compounds such as gallic, chlorogenic and ellagic acids, responsible for antioxidant properties and protection against DNA damage (Verma et al., 2010). The high radical scavenging activity of *F. lyrata* also was shown (Abdel-Hameed, 2009). However, an additional data is provided here concerning its high iron chelating power, which increases the interest of this species against oxidative stress. *Ficus* sp. is promising in the control of oxidative stress which is an aggravating factor of certain diseases such as HIV/acquired immune deficiency syndrome (AIDS) (Greenspan, 1993; Montagnier et al., 1998; Datta et al., 2000). Therefore, it is possible that antioxidant rich content plants (especially *Ficus* sp.) in daily dietary of chimpanzees being one of the main components of their resistance to HIV infection.

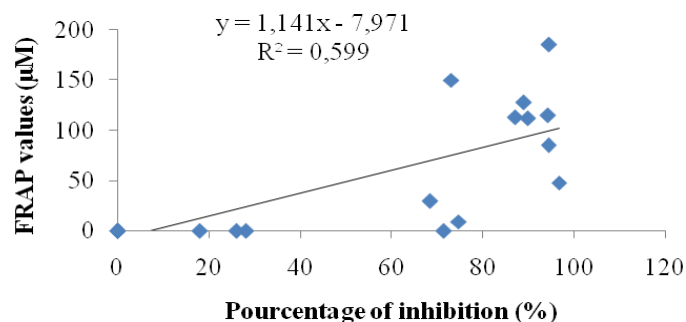


Figure 1. Correlation between DPPH and FRAP activities. DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power.

Phytochemical composition of plant species

TLC phytochemical screening revealed that the eight tested plant species contain monoterpenoids and polyphenols such as flavonoids, anthocyanins and coumarins (Table 2). Only *F. elasticoides* contains secoiridoids in addition to monoterpenoids and flavonoids. Monoterpenoids were present in *F. lyrata*, *F. thonningii*, *M. libericus*, *S. calycina* and *F. umbellata*. Coumarins were found in *M. libericus* and *F. umbellata*. Flavonoids were revealed in *F. mucoso*, *S. calycina*, *M. libericus* and *F. umbellata*. Most of these secondary compounds such as flavonoids (Montagnier et al., 1998; Plumb et al., 1998; Yokozawa et al., 1998; Chan-Bacab and Peña-Rodríguez, 2001; Singh et al., 2005), monoterpenoids (Elmastas et al., 2006; Souza et al., 2007) and secoiridoids (Carrasco-Pancorbo et al., 2005) are known to exhibit high antioxidant activity. Secoiridoids found only in *F. elasticoides* may certainly explain its strongest radical scavenging activity, with 96.69% scavenging of DPPH radical and IC_{50} (7.75 µg/ml) lower than the reference values.

In tropical areas such as Côte d'Ivoire, many bacterial, viral or parasitic infections can threaten the health of people, in particular those infected with HIV (Staal et al., 1993) due to the warm temperatures. Non-nutritional antioxidant plant secondary compounds found in chimpanzee diet also have antibacterial, antiviral and antiparasitic properties. For example, flavonoids have exhibited antiviral, antimicrobial, anticancer, anti-allergic, anti-inflammatory, anti-thrombotic, anti-tumor, hepatoprotective and leishmanicidal effects (Plumb et al., 1998; Yokozawa et al., 1998; Middleton, 2000; Chan-Bacab and Peña-Rodríguez, 2001; Narayana et al., 2001; Singh et al., 2005; Seyoum et al., 2006). Flavonoids showed a wide anti-HIV spectrum (Kirana et al., 2009; Verma et al., 2010). Secoiridoids also have leishmanicidal activity (Chan-Bacab and Peña-Rodríguez, 2001). The presence of these phytochemicals (monoterpenoids, secoiridoids, flavonoids, etc) with high antioxidant power and probable properties against opportunistic diseases strengthens

more the hypothesis of the role of tested plants in resistance of chimpanzees to HIV/AIDS infection. These secondary compounds from the diet of primates may slow down the rate of opportunistic diseases, hence enhance the immune system.

Conclusion

The work carried out on eight plants from the diet of chimpanzees has noted a wealth of plants studied in compounds with strong antioxidant potential. Of the eight selected species, six belong to the Moraceae family and the antioxidant tests performed on these plant species showed that all have more or less important antioxidant properties. These results suggest that the species of this family could be potential targets for the search for new antioxidant molecules. The compounds identified in these different plants may strengthen the immune system of chimpanzees. For active plants, studies are ongoing for evaluating antimicrobial and immunomodulatory activities, cytotoxicity and isolating active compounds.

ACKNOWLEDGEMENTS

We sincerely thank Centre Suisse de Recherches Scientifiques en Côte d'Ivoire and Programme d'Appui Stratégique à la Recherche Scientifique for financial support. Thanks are also due to Profs. Montagnier Luc and Colizi Vittorio for inspiring this study. We are also grateful to Mr. Téré Henri for botanical assistance, and Dr. Kouassi Kouadio Ignace (University of Abobo-Adjamé) for statistical analysis. This article was prepared with the support of the consortium Afrique One "Ecosystem and Population Health: Expanding Frontiers in Health". Afrique One is funded by the Wellcome Trust (WT087535MA).

REFERENCES

- Abdel-Hameed ESS (2009). Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. *Food Chem.*, 114: 1271-1277.
- Aké Assi L (2001). Flore de la Côte d'Ivoire: catalogue systématique, biogéographique et écologie. *Boissiera*, 57: 1-396.
- Bagchi K, Puri S (1998). Free radicals and antioxidants in health and disease. *East Mediterr. Health J.*, 4: 350-360.
- Benzie F, Strain J (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power; The FRAP assay. *Anal. Biochem.*, 239: 70-76.
- Benzie F, Strain J (1999). Ferric Reducing/Antioxidant Power Assay: Direct Measure of Total antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. *Meth. Enzym.*, 299: 15-23.
- Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181: 1199-1200.
- Boesch C (1989). Hunting behaviour of wild chimpanzees in Tai national Park. *Am. J. Phys. Anthropol.*, 78: 547-573.
- Cadet J, Belem S, Berger M, Bourdat AG, Donki T, Duarte V, Frelon S, Gasparutto D, Muller E, Ravanat JL, Sauvaigo S (2002). Recent

- aspects of oxidative DNA damage: guanine lesions, measurement and substrate specificity of DNA repair glycosylases. *Biol. Chem.*, 383: 933-943.
- Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Del Carlo M, Gallina-Toschi T, Lercker G, Compagnone D, Fernández-Gutiérrez A (2005). Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. *J. Agric. Food Chem.*, 53: 8918-8925.
- Chan-Bacab MJ, Peña-Rodríguez LM (2001). Plant natural products with leishmanicidal activity. *Nat. Prod. Res.*, 18: 674-688.
- Datta K, Sinha S, Chattopadhyay P (2000). "Reactive oxygen species in health and disease. *Natl. Med. J. India*, 13: 304-310.
- Dawson R, Elliott D, Elliott W, Jones K (1991). Dictionnaire de biochimiste. Edition Mir, Moscou. In: N'gaman K. *et al.*: Sur la composition en métabolites secondaires et l'activité anti-oxydante d'extraits bruts de *Gmelina arborea* Roxb (Verbenaceae) de Côte d'Ivoire, Afrique de l'ouest: analyse par chromatographie en couche mince. *E. J. Sci. Res.*, 36: 161-171.
- Desmoulins C (2006). Faim de sécurité alimentaire. In: *Revue critique de l'actualité scientifique internationale sur le VIH et les virus des hépatites n° 129-Automne 2006*, Pistes-Paris (France).
- Elmastas M, Dermistas I, Isidak O, Aboul-Enein H (2006). Antioxidant activity of S carvone isolated from spearmint (*Mentha spicata* L. Fam Lamiaceae). *J. Liq. Chromato. Rel. Technol.*, 29: 1465-1475.
- Esterbauer H, Gebicki J, Puhl H, Jurgens G (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Rad. Biol. Med.*, 13: 341-350.
- Gautier-Béguin D (1992). Plantes de cueillettes alimentaires dans le Sud du V-Baoulé en Côte d'Ivoire; description, écologie, consommation et production. *Boisseira*, 46: 1-341.
- Georgievskii VP, Komissarenko NF, Dmitrouk SE (1990). *Substances bioactives des plantes médicinales*, édition « Naouka » Novosibirsk, 336p. (Traduit du russe). In: N'gaman K. *et al.* (2009). Sur la composition en métabolites secondaires et l'activité anti-oxydante d'extraits bruts de *Gmelina arborea* Roxb (Verbenaceae) de Côte d'Ivoire, Afrique de l'ouest : analyse par chromatographie en couche mince. *EJSR*, 36 : 161-171.
- Glouckhoff A (2008). Un projet de l'UE confirme les bienfaits d'une alimentation riche en antioxydants. <http://www.idietetique.pro>.
- Goné Bi ZB (1999). Phénologie et distribution des plantes dont divers organes (principalement les fruits) sont consommés par les chimpanzés dans le Parc National de Taï. DEA Ecologie tropicale option végétale, Laboratoire de Botanique, UFR Biosciences, Université de Cocody-Abidjan (Côte d'Ivoire), p. 95.
- Greenspan HC (1993). The role of reactive oxygen species, antioxidants and phytopharmaceuticals in human immunodeficiency virus activity. *Med. Hypoth.*, 40: 85-92.
- Halliwell B (1994). Free radicals, antioxidants, and human disease curiosity, cause, or consequence? *Lancet*. 344: 721-724.
- Harman D (1992). Role of free radicals in aging and disease. *An. NYA Sci.*, 673: 126-141.
- Hennebelle T, Sahpaz S, Bailleul F (2004). Polyphénols végétaux, sources, utilisations et potentiel dans la lutte contre le stress oxydatif. *Phytothérapie* 1: 3-6.
- Hutchinson J, Dalziel JM (1954-1972). *Flora of West Tropical Africa*, vol. I, II et III, Crown agents for overseas governments and administrations millbank, 2nd ed. London, p. 651.
- Huffman MA, Waller PJ, Ohigashi H, Yole D, Mwenda J, Nkunya MHH, Mbago F, Elias R, Gasquet M, Boegh H, Kalunde MS (2000). Great Ape Self-Medication and Human Traditional Medicine—A Bio-Cultural Approach to Evaluating Herbal Anthelmintics for Humans and Production Animals in Africa. Conference paper prepared for Medicinal Plants, Traditional Medicines and Local Communities in Africa: Challenges and Opportunities of the new Millennium. Nairobi, Kenya, 16-19 May, 2000.
- Kamanzi AK (2002). Plantes médicinales de Côte d'Ivoire : Investigations phytochimiques guidées par des essais biologiques. Thèse Biologie Végétale option Phytochimie, U.F.R. Biosciences. Université de Cocody-Abidjan (Côte d'Ivoire), p. 176.
- Keele BF, Heuverswyn FV, Li Y, Bailes E, Takehisa J, Santiago ML, Bibollet-Ruche F, Chen Y, Wain LV, Liegeois F, Loul S, Ngole EM, Bienvenue Y, Delaporte E, Brookfield JFY, Sharp PM, Shaw GM, Peeters M, Hahn BH (2006). Chimpanzee Reservoirs of Pandemic and Nonpandemic HIV-1. *Science*, 313: 523-526.
- Kirana H, Agrawal SS, Srinivasan BP (2009). Aqueous extract of *Ficus religiosa* Linn. Reduces oxidative stress in experimentally induced type 2 diabetic rats. *Indian J. Exp. Biol.*, 47: 822-826.
- Kouri CF (2004). Investigation phytochimique d'une brosse à dents africaine *Zanthoxylum zanthoxyloides* (Lam.) Zepernick et Timler syn. *Fagara zanthoxyloides* L. (Rutaceae). Thèse de doctorat, Institut de Pharmacognosie et Phytochimie, Faculté des Sciences, Université de Lausanne, p. 211.
- Kurth R, Norley S (1996). Why don't the natural hosts of SIV develop simian AIDS? *J. NIH Res.*, 8: 33-37.
- Lebrun JP, Stork AL (1991). Enumération des plantes à fleurs d'Afrique Tropicale: Généralités et Annonaceae à Pandaceae, vol I. Conservatoire et Jardin botaniques, Genève, p. 249.
- Lebrun JP, Stork AL (1992). Enumération des plantes à fleurs d'Afrique Tropicale: Chrysobalanaceae à Apiaceae, vol II. Conservatoire et Jardin botaniques, Genève, p. 257.
- Lebrun JP, Stork AL (1995). Enumération des plantes à fleurs d'Afrique Tropicale: Monocotyledones: Limnocaritaceae à Poaceae, vol III. Conservatoire et Jardin botaniques, Genève, p. 341.
- Lebrun JP, Stork AL (1997). Enumération des plantes à fleurs d'Afrique Tropicale: Gamopétales: Clethraceae à Lamiaceae, vol IV. Conservatoire et Jardin botaniques, Genève, p. 712.
- Leciari E (2009). Les chimpanzés, eux aussi, victimes du Sida? Institut Jane Goodall. http://www.univers-nature.com/inf/inf_actuelite1.cgi?id=3598, (accessed 12/02/2009).
- Malan DF (2002). Utilisation de la diversité floristique des forêts villageoises de la périphérie du parc national de Taï, réserve de biosphère et patrimoine mondial: cas de Gouleako II. DEA de Gestion et Valorisation des Ressources Naturelles option Biodiversité et Gestion Durable des Ecosystèmes, UFR des Sciences de la Nature, Université d'Abobo-Adjamé, Abidjan (Côte d'Ivoire), p. 99.
- Mallikharjuna PB, Rajanna LN, Seetharam YN, Sharanabasappa GK (2007). Phytochemical studies of *Stychnos potatorum* L.f. a medical plant. *EJ Chem.*, 4: 510-518.
- Merck E (1980). Révélateurs pour la chromatographie en couche mince et sur papier. Darmstadt, 153 p. In: N'gaman K. *et al.* (2009). Sur la composition en métabolites secondaires et l'activité anti-oxydante d'extraits bruts de *Gmelina arborea* Roxb (Verbenaceae) de Côte d'Ivoire, Afrique de l'ouest : analyse par chromatographie en couche mince. *EJSR*, 36: 161-171.
- Middleton E, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol. Rev.*, 52: 673-751.
- Montagnier L, Olivier R, Parquier C (1998). Oxidative stress in cancer, AIDS and neurodegenerative disease. Marcel Dekker, New York, p. 546.
- Narayana KR, Reddy MS, Chaluvadi MR, Krishina DR (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J. Pharmacol.* 33: 2-16.
- OMS (2005). Executive summary of a scientific review-consultation on nutrition and HIV/AIDS in Africa: Evidence, lessons and recommendations for action. Durban (Afrique du Sud), 10-13/04/2005.
- Peeter M, Honoré C, Huet T, Bendjabaga L, Ossari S, Bussi P, Cooper R, Delaporte E (1989). Isolation and partial characterization of an HIV related virus occurring naturally in chimpanzees in Gabon. *Aids*, 3: 625-630.
- Plumb GW, De Pascual-Teresa S, Santos-Buelga C, Cheynier V, Williamson G (1998). Antioxidant properties of catechins and proanthocyanidins: Effect of polymerisation, galloylation and glycosylation. *Free Rad. Res.*, 29: 351-358.
- Prabhjit K, Bikram S, Subodh K, Satwinderjeet K (2008). *In vitro* evaluation of free radical scavenging activity of *Rubia cordifolia* L. *J. Chinese Clin. Med.*, 3: 278-284.
- Seyoum A, Asres K, El-Fiky FK (2006). Structure-radical scavenging activity relationships of flavonoids. *J. Phytochem.*, 67: 2058-2070.
- Singh IP, Bharate SB, Bhutani KK (2005). Anti-HIV natural products. *Curr. Sci.*, 89: 269-290.
- Sofowora A (1996). *Plantes médicinales et médecine traditionnelle d'Afrique*. Académie Suisse des Sciences Naturelles, éditions

- Karthala, Paris (France), p. 375.
- Souza TJJ, Apel MA, Bordignon S, Matzenbacher NI, Zuanazzi JAS, Henriques AT (2007). Composição química e atividade antioxidante do óleo volátil de *Eupatorium polystachyum* DC. Rev Bras Farmacogn, 17, 368-372. In Maria Rodríguez *et al.* (2008). Antidiabetic and antiradical activities of plants from Venezuelan Amazon. Braz. J. Pharmacog., 18: 331-338.
- Staal FJT, Roederer M, Raju PA, Anderson M, Ela SW, Herzenberg LA (1993). Antioxidants inhibit stimulation of HIV transcription. AIDS Res. Hum. Retrovir., 9: 299-306.
- Statistica (1999). *Statistica pour Windows, release 5.5*. Statoft Inc, France.
- Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Bios. Biotech. Biochem., 58: 1780-1783.
- Tammy MB, Carla GT (1994). Enhancement of tissue glutathione for antioxidant and immune functions in malnutrition. Biochem. Pharmacol., 47: 2113-2123.
- Tra Bi FH (1997). Utilisation des plantes par l'homme dans les forêts classées du Haut-Sassandra et de Scio en Côte d'Ivoire. Thèse Botanique option ethnobotanique ; U.F.R. Biosciences, Université de Cocody-Abidjan (Côte d'Ivoire), p. 215.
- Verma AR, Vijayakumar M, Rao CV, Mathela CS (2010). *In vitro* and *in vivo* antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata*, Food Chem. Toxicol., 48: 704-709.
- Wagner H, Blatt S, Zgainski EM (1984). Plant drug analysis. In: *A thin layer Chromatography Atlas*. Springer-Verlag, Berlin, p. 368.
- Weiss R (2001). Perspective Gulliver's Travels in HIV land. Nature, 410: 963-967.
- Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I (1998). Study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radical. Biochem. Pharmacol., 56: 213-222.
- Zinsstag J, Schelling E, Wyss K, Mahamat MB (2005). Potential of cooperation between human and animal health to strengthen health systems. Lancet, 366: 2142-2145.
- Zinsstag J, Schelling E, Bonfoh B, Fooks AR, Kasymbekov J, Waltner-Toews D, Tanner M (2009). Towards a "one health" research and application tool box. Vet. Ital., 45: 121-133
- Zinsstag J, Schelling E, Waltner-Toews D, Tanner M (2011). From "one medicine" to "one health" and systemic approaches to health and well-being. Prev. Vet. Med., 101: 148-156.