Journal of Biological Sciences 7 (6): 959-963, 2007
ISSN 1727-3048
© 2007 Asian Network for Scientific Information

Radical Scavenging Activity, Phenolic Content and Cytotoxicity of Bark and Leaves Extracts of *Entada africana* Guill. and Perr. (Mimosaceae)

[1]A. Tibiri, [2]O. Rakotonandrasana, [1]G.O. Nacoulma and [2,3]J.T. Banzouzi
[1]Biochemistry and Applied Chemistry Laboratory (LABIOCA), UFR/SVT,
Ouagadougou University, 03 P.O. Box 7021, Ouagadougou 03, Burkina Faso
[2]Institut de Chimie des Substances Naturelles, 1 avenue de la Terrasse-Bât. 27,
91198 Gif-sur-Yvette Cedex, France
[3]Centre d'Etudes et de Recherche Médecins d'Afrique (CERMA), B.P.45, Brazzaville, Congo

Abstract: Dried ground leaves and barks of *Entada africana* were extracted by maceration in methanol and fractioned with chloroform, ethyle acetate and water. The total phenolic content of each fraction was determined spectrophotometrically according to Folin-Ciocalteu's method and calculated as Tannic Acid Equivalent (TAE). Tannins and flavonoids were also determined. The total phenolic content was quite high, especially in the aqueous fraction (up to 39.7% TAE in the barks and 39.9% in the leaves). The antioxidant activity of lyophilized extracts was determined at room temperature by the means of the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) colorimetric method with a detection scheme at 517 nm and expressed as EC_{50}. The radical scavenging activity was evaluated as the difference in absorbance between a test sample and the control (methanol). Bark extracts had the best EC_{50}, similar to those of rutoside and ascorbic acid for the aqueous and methanol fraction (5.7 and 5.3 μg mL^{-1}, respectively). All the other extracts were moderately active (EC_{50} ranging from 6.9 to 20.0 μg mL^{-1}), except the chloroform extracts (EC_{50} > 69 μg mL^{-1}). Except for the crude and aqueous bark extracts, no extract was cytotoxic on KB or Vero cells.

Key words: *Entada africana*, antioxidant, free radical, $DPPH^{\bullet}$, cytotoxicity, phenolic compounds, flavonoids

INTRODUCTION

Entada africana is a leguminous tree used in traditional medicine throughout all sub-Saharan Africa, for the treatment of numerous diseases, such as fever (Bah, 1998), respiratory tract complaints (Occhiuto *et al*., 1999), diabetes, hypertension and diarrheas (Nacoulma-Ouedraogo, 1996). This tree is indicated in the traditional pharmacopoeias for its wound-healing, haemostatic, anti-rheumatism, anti-inflammatory and hepatoprotective properties (Diallo *et al*., 2001; Burkill, 1995; Oliver-Bever, 1986; Kerharo, 1974). Assays for the hepatoprotective activity were conclusive (Douaré, 1991; Sanogo *et al*., 1998). All plant parts are used, especially bark and leave.

These traditional indications led us to assess the antioxidant activity of *Entada africana*. In fact, very often in the relevant literature, a relationship is observed between hepatoprotective or anti-inflammatory properties and the antioxidant potential of the plant. Many hepatoprotective plants act by relieving liver cells from oxidative stress (Maxwell *et al*., 1999; Tilak *et al*., 2004; Bhattacharjee *et al*., 2006) and free radicals play a role in inflammation (Winrow *et al*., 1993). We therefore decided to assess the free radical scavenging activity of *Entada africana* leaves and bark extracts, using the $DPPH^{\bullet}$ colorimetric method, along with their phenolic content, since antioxidant activity in plants is often due to polyphenols and especially flavonoids and tannins (Yokozawa, 1998).

Indeed, a positive correlation between the antioxidant activity and the total phenolic content can be observed in a number of papers (Kumaran *et al*., 2007; Tawaha *et al*., 2007). Some data are already available on *Entada africana* phenolics, but they had to be completed. Bako *et al*. (2005) determined the phytochemical profile of the leaves and found tannins and flavonoids, without isolating them. Montoro *et al*. (2005) on the other hand isolated flavonoids in the roots, but without looking for the other phenolics. So, in order to look for a correlation between phenolics and radical scavenging activity, we had to get quantitative data and therefore to determine the amount of phenolics in both leaves and barks.

Corresponding Author: Jean Théophile Banzouzi, Institut de Chimie des Substances Naturelles, 1 avenue de la Terrasse-Bât.27, 91198 Gif-sur-Yvette Cedex, France Tel: +33(0)169823011

MATERIALS AND METHODS

Plant material: Fresh *Entada africana* was collected in Gampéla, 15 km east of Ouagadougou (Burkina Faso) in September 2005. A voucher specimen, identified at the department of Biology and Ecology of the University of Ouagadougou, was deposited under the registration number TA05-1 in the herbarium of the University. Leaves and barks were separately dried under ventilation at room temperature then finely ground on an electrical grinder.

Preparation of leaves and bark extracts: Leaves and barks (100 g of each) were extracted by methanol maceration at room temperature. The filtrates of the obtained extracts were concentrated under reduced pressure until all the methanol had evaporated. The concentrates were redissolved in distilled water and lyophilized. One gram of each lyophilized concentrate was dissolved in water and then sequentially extracted with chloroform and ethyl acetate (Ac-OEt). The water residue was then lyophilized. For each fraction and for the crude methanol extract, scavenging activity against DPPH$^{\bullet}$ was determined and the content of total phenolics, tannins and flavonoids was measured.

Free radical scavenging activity: The antioxidant activity of *Entada africana* leaves and barks was determined by the mean of DPPH$^{\bullet}$ colorimetric method as described by Velázquez *et al*. (2003), slightly modified. Three milliliters of a 20 mg mL^{-1} DPPH$^{\bullet}$ methanolic solution were added to 1.5 mL of a methanolic solution of each extract (1-100 μg mL^{-1}). The mixture was shaken vigorously then incubated for 15 min in darkness at room temperature. Absorbance was measured at 517 nm. Methanol was used as control to zero the spectrophotometer (Agilent 8453E). Rutoside, ascorbic acid and quercetin were used as positive controls. The loss of free radical activity of the DPPH- radical stock solution was reduced as much as possible as recommended by Blois (1958). All measurements were performed five times. The difference in absorbance between a test sample and a control (methanol) was taken as the activity and expressed as EC_{50}, that is to say the concentration of antioxidant needed to decrease by 50% the initial concentration. This EC_{50} was calculated from the equation determined by linear regression for the dose/effect results.

Determination of the extracts cytotoxicity: The cytotoxicity of all the extracts of leaves and barks was tested on KB (Human epidermoid carcinoma) and Vero (African green monkey kidney) cells, according to the method described by Mbatchi *et al*. (2006). The reference used was taxotere.

Determination of the plant content in phenolic compounds

Total phenolic content: The amount of total phenolic content in each leaves and barks extract was assessed according to the method of Singleton et Rossi (1965) using Folin-Ciocalteu reagent. For the preparation of calibration curve, 1 mL aliquots of 0.10 to 0.60 mg mL^{-1} ethanolic tannic acid solutions were mixed with 1 mL Folin-Ciocalteu reagent (2N) and 3 mL sodium carbonate (20%), then left 40 min at room temperature. The absorbance was read at 760 nm and the calibration curve was drawn. Zero point twenty-five milliliters of each extract (0.5 g L^{-1}) were completed to 1 mL with distilled water and then mixed with the same reagents as described above. The resulting solution was vortexed and left for 40 min before measuring absorbance. Results were expressed as Tannic Acid Equivalent (TAE). All determinations were performed 5 times.

Non-tannic phenolics: Tannins were precipitated from each extract by polyvinyle polypyrrolidone (PVPP) during their standing at 4°C overnight. The precipitated tannins were removed by centrifugation (3000 rpm, 10 min). The supernatant, containing all phenolic compounds except tannins, was collected. Its absorbance was measured in the same way as for the total phenolics.

Non-flavonoidic phenolics: Flavonoids were precipitated by formaldehyde at pH < 0.8. Five milliliters of a concentrated HCl solution (50/50 v/v) and 5 mL of a formaldehyde solution (8 mg L^{-1}, in distilled water) were added to 10 mL of each extract. The mixture was vortexed, then left 24 h at room temperature. Flavonoids were separated by centrifugation (3000 rmp, 10 min) and the supernatant, containing all phenolic compounds except flavonoids, was collected and filtrated. Its absorbance was measured in the same way as for the total phenolics.

Statistical analysis: Experimental values are means +/- SEM of the number of experiments indicated in the legends. Data were evaluated for statistical significance with one-way ANOVA followed by Dunnett's multiple-range tests when appropriate (GraphPad Prism version 4.03 pour Windows, GraphPad Software, San Diego California USA). A p value of 0.05 or less was considered as statistically significant.

RESULTS

Scavenging activity against DPPH• radicals: The aqueous and methanol extracts of barks were found to have the highest antioxidant activity (EC_{50} values of 5.7 and 5.3 μg mL^{-1}, respectively), of the same order as those of rutoside and ascorbic acid. The Ac-OEt extract of bark is not far behind, with an EC_{50} value of 6.9 μg mL^{-1}. However, their scavenging activity was twice inferior to the activity of quercetin. Both chloroform extracts (for barks and leaves) were inactive (EC_{50}: 69 and 226 μg mL^{-1} respectively). All the other fractions (leaves extract) were moderately active, with EC_{50} values ranging from 9.5 μg mL^{-1} (methanol extract) to 20 μg mL^{-1} (aqueous extract; Table 1).

Cytotoxicity: Nearly all the barks and leaves extracts of Entada africana have a very low cytotoxic activity against Vero and KB cells at the concentration of 10 μg mL^{-1}. Only two of the bark extracts (the crude extract and the aqueous extract) showed a measurable cytotoxicity against KB cells (respectively 53 and 57% of cell growth inhibition) (Table 2).

Phenolic content of *Entada africana* leaves and barks: Tannins and flavonoids concentrations were calculated from the values obtained for the non-tannic and non-flavonoidic phenolic compounds, respectively. The methanolic crude extract has a quite high content in total phenolics, especially in the aqueous fraction (up to 39.7% TAE in the barks and 39.9% in the leaves). Tanins and flavonoids are more abundant in the barks than in the leaves. Same proportions were observed for the aqueous fraction, as could be expected (Table 3).

Table 1: Extraction yield and EC_{50} values against DPPH• of *Entada africana* leaves and bark extracts

	Fractions							
	Methanol (crude extract)		Chloroform		Ac-OEt		Water	
	Yield (% w/w)	EC_{50} (μg mL^{-1})	Yield (% w/w)	EC_{50} (μg mL^{-1})	Yield (% w/w)	EC_{50} (μg mL^{-1})	Yield (% w/w)	EC_{50} (μg mL^{-1})
Barks	20.68	5.3[a,b] (4.9; 5.6)	1.2	69.0 (66.0; 72.0)	14.5	6.9 (6.7; 7.2)	84.6	5.7[a,b] (4.9; 6.5)
Leaves	8.93	9.5 (8.9; 10.0)	2.1	226.0 (207.0; 246.0)	26.1	15.0 (13.0; 18.0)	67.8	20.0(18.0;22.0)

Rutoside, 5.2 (5.0; 5.5); Ascorbic acid, 5.3 (5.1; 5.5); Quercetine, 2.5 (2.4; 2.6) Data in parenthesis represent the confidence interval at 95% (n = 5) a,b p>0,05 compared to rutoside and ascorbic acid

Table 2: Cytotoxicity of *Entada africana* leaves and bark extracts (Percentage of cell growth inhibition)

	Fractions							
	Methanol (crude extract)		Chloroform		Ac-OEt		Water	
	KB	Vero	KB	Vero	KB	Vero	KB	Vero
Barks	53	10	11	15	0	3	57	11
Leaves	1	7	5	18	0	5	17	12

Taxotere (2.5 10^{-10} M): 65 (KB)/0 (Vero)

Table 3: Phenolic content of *Entada africana* leaves and barks, expressed in TAE (Tannic Acid Equivalent)

		Fractions							
		Methanol (crude extract)		Chloroform		Ethyle acetate		Water	
		(μg mL^{-1})	(%)	(μg mL^{-1})	(%)	(μg mL^{-1})	(%)	(μg mL^{-1})	(%)
Total phenolics	Barks	4.91 (4.71; 5.11)	38.74	5.18 (4.01; 6.34)	5.03	6.55 (5.38;7.73)	14.14	10.44 (8.65; 12.23)	39.70
	Leaves	4.09 (3.76; 4.42)	32.24	9.60 (8.88; 10.32)	7.62	9.64 (9.17;10.11)	33.24	10.49 (9.62;11.35)	39.89
Non-tanic phenolics	Barks	4.33 (4.09; 4.56)	17.01	1.43 (1.05; 1.82)	2.78	2.60 (2.27; 2.92)	9.78	2.13 (1.77; 2.48)	16.18
	Leaves	6.47 (6.37; 6.56)	25.56	1.95 (1.70; 2.19)	3.09	8.83 (8.29; 9.36)	30.44	3.05 (2.78; 3.32)	23.20
Tannins*	Barks	-	21.73	-	2.25	-	4.36	-	23.52
	Leaves	-	6.68	-	4.53	-	2.08	-	16.69
Non-flavonoidic phenolics	Barks	0.87 (0.73; 1.00)	9.90	1.64 (0.66; 2.62)	3.18	3.95 (2.95; 4.95)	7.75	1.27 (0.96; 1.59)	14.53
	Leaves	1.37 (1.16; 1.58)	15.74	1.39 (0.60; 2.18)	3.32	6.25 (6.23; 6.27)	25.88	2.18 (1.97; 2.40)	24.92
Flavonoids**	Barks	-	28.84	-	1.85	-	6.39	-	25.17
	Leaves	-	16.53	-	4.30	-	7.36	-	14.97

Data in parenthesis represent the confidence interval at 95% (n = 5), *Calculated from the values of non-tannic phenolics, **Calculated from the values of non-flavonoidic phenolics

DISCUSSION

As we had expected after having reviewed the traditional uses of the plant, *Entada africana* has a high antioxidant power, of the same level as ascorbic acid and rutoside, well-known free-radical scavengers. Our results confirm the previous study from Cook *et al*. (1998), who obtained with the trolox assay a relatively high antioxidant activity for *Entada africana* leaves (more active than potato and spinach). In our assay, the barks were more active than the leaves. The EAT values obtained for the total phenolic content, the tannins and the flavonoids are consistent with the results for antioxidant activity, since they are higher in the barks than in the leaves. We therefore think that the compounds responsible for the radical scavenging activity of the plant are likeliest to be found among the flavonoids or the tannins of the plant. This is supported by the results of Montoro *et al*. (2005), who isolated in the roots of *Entada africana* three myricetin-derived flavonols. These molecules had an interesting antioxidant activity, though lower than the standard, quercetin. Since this team used TEAC (Trolox Equivalent Antioxidant Capacity) and not $DPPH^{\bullet}$ as we did, results are not easy to compare. However, they seem coherent with the EC_{50} we obtained for leaves and bark extracts, which are lower than EC_{50} of quercetin but near EC_{50} of rutoside and ascorbic acid.

The cardio-protective and anti-tumor properties attributed to some flavonoids (Cook *et al*., 1998) make them useful compounds for medicine. Indeed, free radicals or oxidative injury underlay quite a number of human neurological and immune disorders and diseases such as diabetes, through mechanisms beginning to be well known. They also play a role in carcinogenesis and their level increase with exercise, fever, infection, etc. (Atawodi, 2004). Therefore, finding new radical scavenging compounds with a low cytotoxicity would be very interesting. The low cytotoxicity and the good antioxidant activity of *Entada africana* leaves and barks extracts give us good reasons to continue with our studies of this plant. Our next step will be to isolate this or these compounds through a bio-guided fractionation, in order to determine whether they are already known compounds or new ones. We will also check if there is a synergistic between the different phenolic compounds contained in the barks and leaves of *Entada africana*.

ACKNOWLEDGMENTS

We thank the researchers from CERMA, especially Ms Aline Prost, who helped in the writing and the translation of this study.

REFERENCES

Atawodi, SE., 2004. Antioxidant potential of African medicinal plants. Afr. J. Biotechnol., 4: 128-133.

Bah, A., 1998. Sensibilité d'*Anopheles gambiae* aux insecticides organiques de synthèse et à divers extraits de plantes médicinales du Mali (Sensibility of Anopheles gambiae to organic pesticides and to several Malian medicinal plant extracts). Thèse Pharmacie. Bamako (Mali).

Bako, S.P., M.J. Bakfur, I. John and E.I. Bala, 2005. Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. Int. J. Bot., 1: 147-150.

Bhattacharjee, R. and C.S. Parames, 2006. Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties. Food Chem. Toxicol., (In Press).

Blois, M.S., 1958. Antioxidant determination by the use of a stable free radical. Nature, 181: 1199-1201.

Burkill, M.H., 1995. The Useful Plants of West Tropical Africa, Families J-L, Vol. 3. Royal Botanic Garden Kew, pp: 229-230.

Cook, J.A., D.J. VanderJagt, A. Dasgupta, G. Mounkaila, R.S. Glew, W. Blackwell and R.H. Glew, 1998. Use of the trolox assay to estimate the antioxidant content of seventeen edible wild plant of Niger. Life Sci., 63: 105-110.

Diallo, D., B.S. Paulsen, T.H.A. Liljebäck and T.E. Michaelsen, 2001. Polysaccharides from the roots of *Entada africana* Guill. et Perr., *Mimosaceae*, with complement fixing activity. J. Ethnopharmacol., 74: 159-171.

Douaré, I., 1991. Contribution à l'étude clinique de *Entada africana* dans le traitement des hépatites virales (Contribution to the clinical study of Entada africana for the treatment of viral hepatitis). Thèse Médecine. Bamako (Mali).

Kerharo, J. and J.G. Adam, 1974. La Pharmacopée Sénégalaise Traditionnelle, Plantes Médicinales et Toxiques (Traditional Senegalese Pharmacopoeia, Medicinal and Toxic Plants). Paris, Editions Vigots et frères, pp: 575-576.

Kumaran, A. and R.J. Karunakaran, 2007. *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT- Food Sci. Technol., 40: 344-352.

Maxwell, A.G., Y. Masato and A. Yoko, 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. Gen. Pharmacol., 32: 661-667.

Mbatchi, S.F., B. Mbatchi, J.T. Banzouzi, T. Bansimba, G.F. Nsonde Ntandou, J.M. Ouamba, A. Berry and F. Benoit-Vical, 2006. *In vitro* antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. J. Ethnopharmacol., 104: 168-174.

Montoro, P., A. Braca, C. Pizza and N. De Tommasi, 2005. Structure-oxidant activity relationship of flavonoids isolated from different plant species. Food Chem., 92: 349-355.

Nacoulma-Ouedraogo, O., 1996. Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: Cas du plateau central (Traditional medicinal plants and medical practice in Burkina Faso: Case of plateau central). Thèse de doctorat d'Etat ès-sciences, Faculté des Sciences et Techniques, Université de Ouagadougou.

Occhiuto, F., R. Sanogo, P. Germano, A. Keita, V. D'Angelo and R. De Pasqale, 1999. Effects of some Malian medicinal plants on the respiratory tract of guinea pigs. J. Pharm. Pharmacol., 51: 1299-1303.

Oliver-Bever, B.E.P., 1986. Medicinal Plants in Tropical West Africa. Cambridge University Press, Cambridge, pp: 178.

Sanogo, R., M.P. Germano, V. D'Angelo, M. Guglielmo, and R. De Pasquale, 1998. Antihepatotoxic Properties of *Entada africana* (Mimosaceae). Phytother. Res., 12: 157-159.

Singleton, C.P. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. J. Enol. Viticult., 16: 144-158.

Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohammad and T. El-Elimat, 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry, (In Press).

Tilak, J.C., S. Adhikari and T.P.A. Devasagayam, 2004. Antioxidant properties of *Plumbago zeylanica*, an Indian medicinal plant and its active ingredient, plumbagin. Redox Report, 9: 219-227.

Velázquez, E., H.A. Tournier, P. Mordujovich de Buschiazzo, G. Saavedra and G.R. Schinella, 2003. Antioxidant activity of Paraguayan plant extracts. Fitoterapia, 74: 91-97.

Winrow, V.R., P.G. Winyard, C.J. Morris and D.R. Blake, 1993. Free radicals in inflammation: Second messengers and mediators of tissue destruction. Br. Med. Bull., 49: 506-522.

Yokozawa, T., C.P. Chen, E. Dong, T. Tanaka, G.I. Nonaka and I. Nishioka, 1998. Study on the inhibitory effect of tannins and flavonoids against the 1,1 diphenyl-2-picrylhydrazyl radical. Biochem. Pharmacol., 56: 213-222.