Antihepatotoxic Potential of Three Bamun Folk Medicinal Plants

Frédéric N. NJAYOU[1], Paul F. MOUNDIPA[1]*, Angèle N. TCHANA[1], Félicité M TCHOUANGUEP[2].

[1]Laboratory of Biochemical Toxicology and Nutrition, University of Yaounde I, [2]Department of Biochemistry, University of Dschang, Cameroon.

ABSTRACT

A survey for medicinal plants with antihepatotoxic activity from the Bamun popular and traditional medicine was done and 56 plants species were recorded. They were then tested for their antihepatotoxic potential activity; their ability to inhibit lipid and microsomal protein oxidation. Ten of them were selected on those basis. We present herein an evaluation of the *in vitro* antihepatotoxic activity for three of the plants. *Entada africana, Erythrina senegalensis* and *Khaya grandifoliola* were shown to possess antihepatotoxic potential properties, as indicated by the ability of their respective stem bark extracts to inhibit microsomal lipid peroxidation initiated with ascorbate (respective IC_{50} values: 50.67± 0.46; 33.11 ± 3.78 and 81.70 ± 3.30) and enzymatically with NADPH (respective IC_{50} values: 18.33 ±0.76; 31.75 ± 3.65 and 102.04 ± 2.52). These extracts also inhibited protein oxidation (respective IC_{50} values: 9.85 ± 0.66; 8.57 ± 0.23 and 42.04 ±0.16) and exhibited antiradicalar activity when tested in the presence of 2,4-dinitrophenyl-1-picrylhydrazyl (2,4-DNPH) (respective EC_{50} values: 17.08 ±0.24; 123.53 ±0.57 and 12.14 ± 0.04). Using rat liver slices system, extracts were effective in decreasing LDH leakage at the dose of 100µg/ml in the 4 *in vitro* hepatitis models with the following respective protective percentage (carbone tetrachloride: 63.33, 65.26 and 65.26 %; D-galactosamine: 59.29, 64.17 and 63.33 %; paracetamol: 96.67, 93.89 and 94 %; tert-butylhydroperoxide: 80, 72.73 and 68.75 %). Phytochemical studies of these extracts revealed the presence of flavonoids and polyphenols. These classes of compounds are known to be antioxidant and therefore support the observed properties.

Key words: medicinal plants; lipid peroxidation; protein oxidation; antiradicalar; hepatitis *in vitro*.

* Corresponding author Paul F. Moundipa, E-mail : pmoundipa@uycdc.uninet.cm, Department of Biochemistry, University of Yaounde I, Cameroon.

INTRODUCTION

Entada africana, Erythrina senegalensis and *Khaya grandifoliola* are three plant species used in the Bamun region for the treatment of hepatitis and other liver related hazards (Moundipa et al., 2002).

Since hepatitis and others degenerative diseases are often associated with the oxidative destruction of lipids and proteins (Dean *et al.*, 1997; Aruoma, 1998; Kehrer , 2000), the plants used by the Bamun in order to cure this disease may contain antioxidant compounds which have been suggested as prophylactic agents (Aruoma, 1997, 2002). Thus, these plants may possess antihepatotoxic properties. However, no systematic pharmacological screening of most of these plants has been reported so far.

This work aimed to evaluate the *in vitro* antihepatotoxic activity of the three above mentionned plants species by testing their efficiency as antioxidant and hepatoprotective.

MATERIALS AND METHODS

Chemicals: All reagents used in this study were purchased from Sigma Chemicals Company (St. Louis, MO, USA) and Prolabo (Paris, France).

Plant extracts: Stem bark powders methanol-methylene chloride (1:1v/v) were used and tested at the doses 10, 100 and 200µg/ml.

Antioxidant activities screening

Lipid peroxidation assays: Liquid peroxidation assays procedure as described by Garle and Fry (1989). Experiments were carried out following the method described by Ulf et al., (1989). Lipid peroxidation was non-enzymatically initiated using ascorbate or enzymatically by NADPH. The samples were assayed for thiobarbituric acid-reactive substances (TBA-RS) as described by Wills (1987).

BSA oxidation assay: BSA was oxidised by a Fenton-type reaction and its carbonyl content determined as 2,4-dinitrophenylhydrazine (DNPH) derivatives by the method described by Martinez et al., (2001).

Antiradicalar effect assay: The 2,4-DPPH in methanol was used and dicolorated according to the method described by Brand-Williams et al., (1995).

Hepatoprotective properties study

The hepatoprotective action of the extracts was studied by assessing the LDH leakage from rat liver slices according to the method described by Benford and Hubbard (1987). Liver slices were intoxicated either with carbon tetrachloride (CCl4 40mM), D-galactosamine (D-galN 100mM), paracetamol (APAP 30mM) or ter-butyl hydroperoxide (tBH 15mM) as indicated by Wormser and Zakine (1990).

Phytochemical study

Groups of phytochemical compounds (flavonoids, polyphenols, leucoanthocyanins, alkaloids, tannins, triterpens and sterols, anthranoids) were tested.

Calculations and statistical analysis

Different IC_{50} values were estimated using the EPA probit analyses. LDH leakage percentages were analysed by ANOVA test and the probabilities $P< 0.05$, $P< 0.001$considered as significant.

RESULTS

Antioxidant activities screening

For lipid peroxidation and protein oxidation, all the extracts strongly inhibited these biochemical phenomena as indicated by their low IC_{50} in Figures 1 and 2. Values were less than 200µg/ml irrespective the mode of lipid peroxidation initiation. The same observation was done with regard to their antiradicalar activities (Figure 3).

Hepatoprotective activity study

Generally, the inhibition of the enzyme leakage was dose dependant and all the three plant extracts significantly increased ($P< 0.05$) the LDH leakage in

Table I: Phytochemical composition of plant extracts

Classes of compounds / Species	Flavonoids	Triterpens	Sterols	Alcaloids	Polyphenols	Tannins	Anthranoids	Leucoanthocyans
Erythrina senegalensis	+	-	-	-	+	-	+	-
Khaya grandifoliola	+	-	-	-	+	+	-	+
Entada africana	+	-	-	-	+	+	-	+

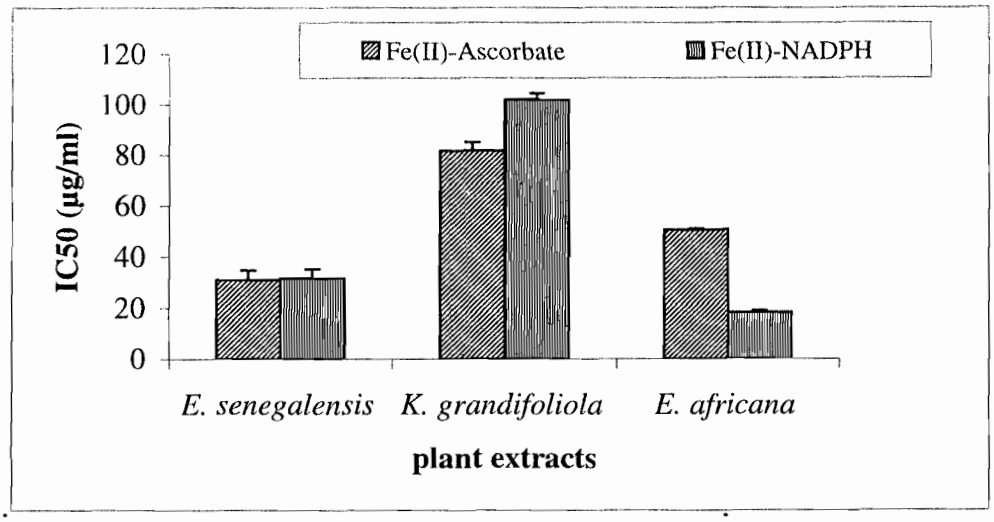

Figure 1: Half inhibiting concentration of plant extracts depending on the mode of lipid peroxidation initiation.

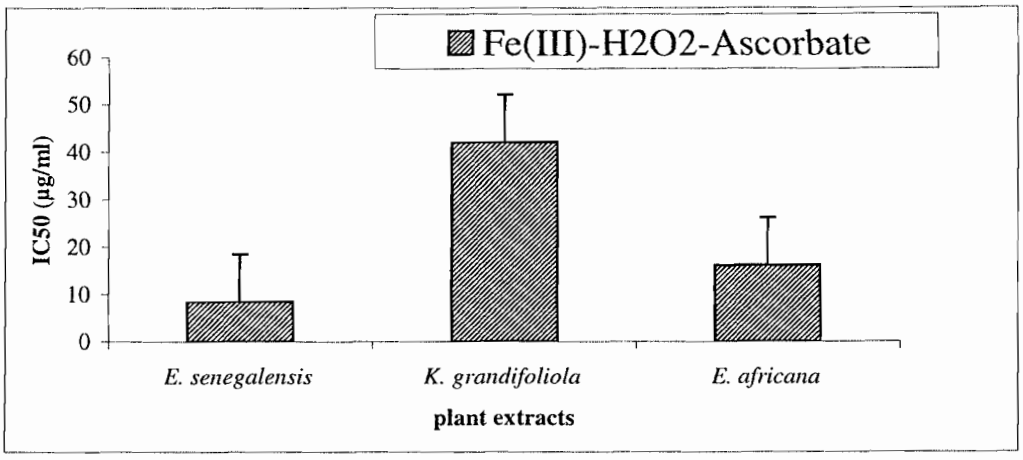

Figure 2: : Half inhibiting concentration of plant extracts in protein oxidation

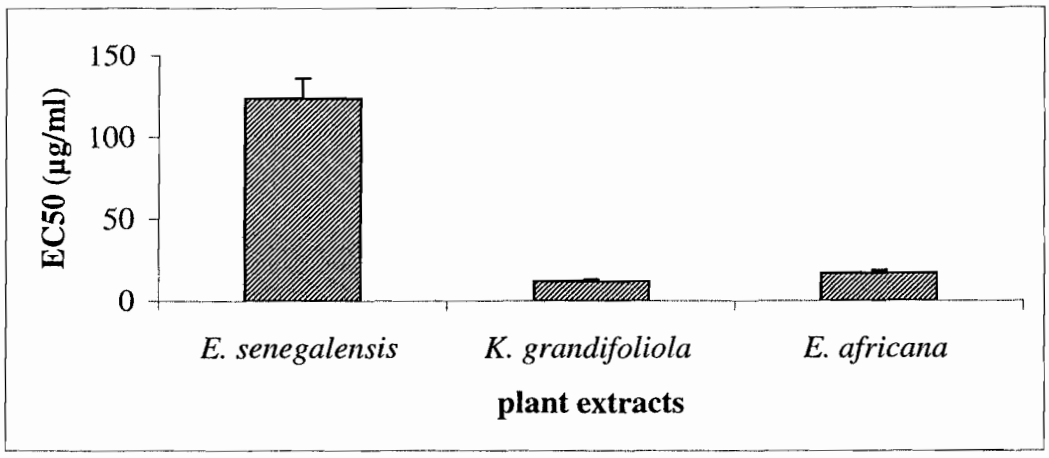

Figure 3: : Half effective concentration of plant extracts in DPPH discoloration

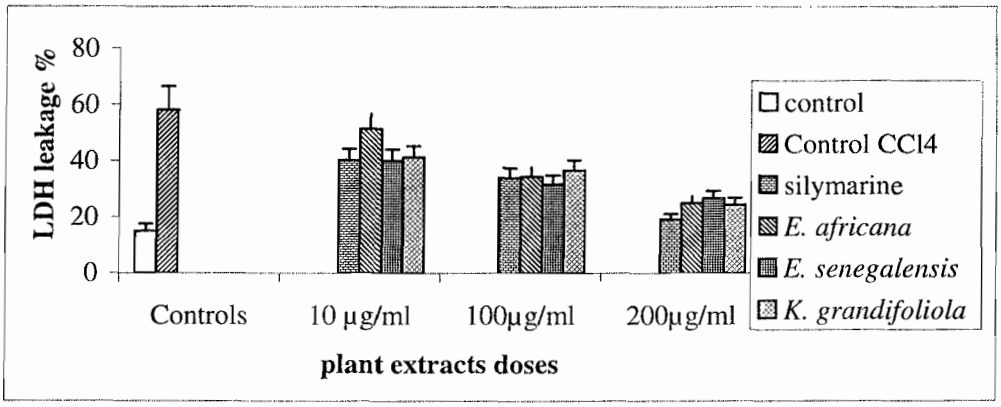

Figure 4: LDH leakage from rat liver slices incubated in the presence of CCl4 and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to*p< 0.05; **p< 0.001

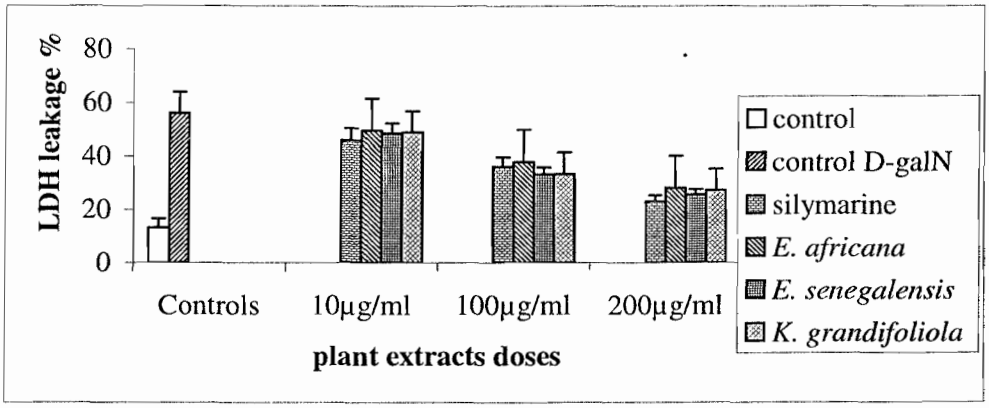

Figure 5: LDH leakage from rat liver slices incubated in the presence of D-galN and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to*p< 0.05

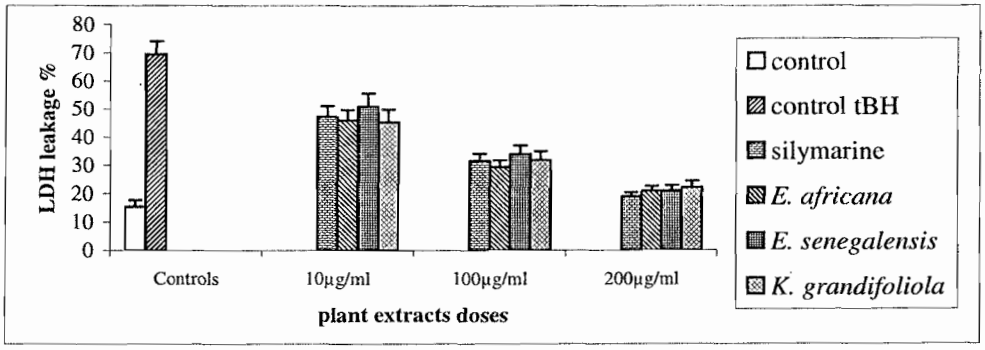

Figure 6: LDH leakage from rat liver slices incubated in the presence of tBH and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to*p< 0.05; **p< 0.001

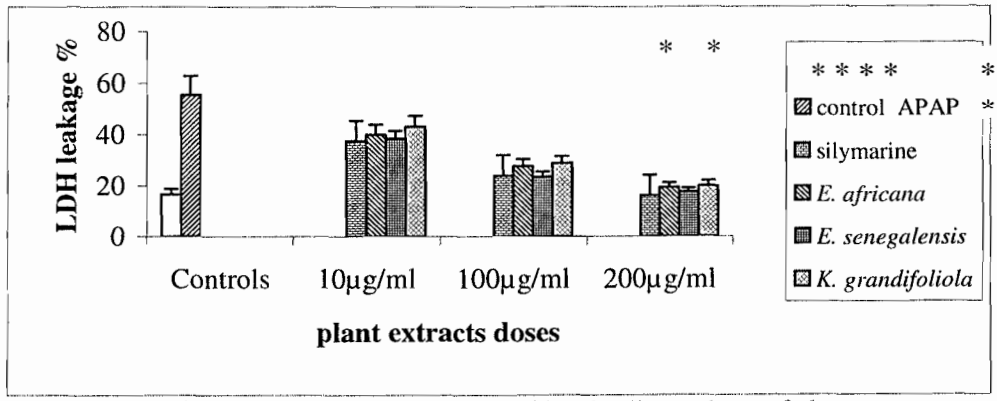

Figure 4: LDH leakage from rat liver slices incubated in the presence of CCl4 and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to*p< 0.05; **p< 0.001

all the four models of hepatitis. Leakage percentages values were not significantly different from that of silymarine. Data are presented in figures 4 to 7.

Phytochemical study

Phytochemical studies of active plant extracts revealed the presence of flavonoids polyphenols among others classes of compounds as shown in table I.

DISCUSSION

The low values of different IC_{50} allow us to suggest that these extracts were all active against lipid peroxidation process (non-enzymatically or enzymatically induced) and BSA oxidation.

. The protection of hydroxyl-mediated oxidation of BSA takes place essentially by reducing the H_2O_2 concentration, a fundamental component in Fenton-type reaction, by chelating iron or by scavenging the hydroxyl formed on the immediate side during oxidation on the target protein (Kingu and Wei, 1997). Thus, it may suggest that plant extracts tested in this study were able to scavenge hydroxyl or chelate iron.

Plant extracts were otherwise tested for their antiradicalar activity in order to appreciate their efficiency to scavenge free radicals. According to the EC_{50} values obtained, *Entada africana* and *Khaya grandifoliola* extracts may be classified as extracts that quickly discolorate the DPPH solution and that of *Erythrina senegalensis* as the one which slowly discolorates it. Since the DPPH discoloration takes place by H° atomes transfer to the DPPH° radical, structural differencies of chemical compounds bearing the transferable hydrogen atome and present in each extract may reasonably explain the varied behaviour of the extracts as suggested by Brand-Williams et al., (1995) when analysing the antiradicalar efficacy of 20 phenolic compounds.

The liver slice system in four different models was used to assess the hepatoprotective effect of the extracts. Considering the results model by model, all the 3 plant extracts, at the dose of 100µg/ml, exhibited significant($p< 0.05$) protective effect, compared to intoxicated control, against injuries induced in rat liver slices by any of the toxins used in this study. Since there are differencies between the mechanisms of CCl4, D-galN, APAP and tBH toxicity (Recknagel and Glende, 1973; Mandl et al., 1989; Masaky et al., 1989; Fleurentin and Joyeux, 1990), these extract may contain a mixture of hepatoprotective compounds. Some of these compounds may protect against certains toxins and others against others toxins as it was shown for other hepatoprotective plants (Hikino and kiso, 1988) and *Melothria maderaspanata*(Thabrew et al., 1995).

Flavonoids and polyphenols were identified in these 3 plant extracts. In another studies(Wandji et al., 1995), flavonoids had been detected in *Erythrina senegalensis* extract. Thus, the observed inhibitory effect against lipid peroxidation, free radical-mediated degradation of BSA, LDH leakage from intoxicated rat liver slices by *Entada africana, Erythrina senegalensis* and *Khaya grandifoliola* extracts and their antiradicalar effect may be attributed to the presence of flavonoids and polyphenols as many of these phytoconstituents are known to be antioxidants and antiradicalar (Faurè *et al.*, 1990, Markus et al., 1996).

CONCLUSION

Entada africana, Erythrina senegalensis and *Khaya grandifoliola* extracts exhibited strong antioxidant and hepatoprotective effects in four different *in vitro* hapatitis models. These extracts also contain flavonoids and polyphenols. The potential antihepatotoxic of these species is therefore likely. The confirmation of this antihepatotoxic potential activity by *in vivo* studies is indicated.

ACKNOWLEDGMENTS

We are grateful to Professor Martinez Gregorio Sanchez (Center for Evaluation and Biological Research, institute of Pharmacy, Havana University, Cuba) for the useful literature assistance and advices.

REFERENCES

Aruoma, O. I. (1997) : Extracts as antioxidant prophylactic agents. INFORM, 8(12) :1236-1242

Aruoma, O. I. (1998) : Free radicals, oxidative stress, and antioxidants in human health and disease. JAOCS, 75(2) : 199-212

Aruoma, O. I. (2002) : Free radicals and antioxidants in health and diseases : a case for plant extracts as antioxydant prophylactic agents. Third international conference of the Society for Free Radical Research-Africa, Cape Town-South Africa, P.12 October 2002(Abstracts)

Benford, D. J. and Hubbard, S. A. (1997) : Preparation and culture of mammalian cells. In : SNELL, K. and MULLOCK, B. Ed. Biochemical Toxicology. A Practical Approch series. IRL press Oxford Washington pp. 73-75

Bradford, M. M. (1976) : A rapid sensitive method for quantitation of microgram quantities of protein utilising the principle of protein dye binding. Anal. Biochem., 72, 248-254

Brand-Williams, W., Cuvelier, M. E. and **Berset, C.**(1995) : Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 28: 25-30

Davies, K. J. and **Goldberg, A. L.**(1987) : Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in Erythrocytes. The Journal of Biological Chemistry, 262, (17), pp. 8220-8226

Dean, R. T., Fu, S., Stocker, R. and **Davies, M. J.** (1997) : Biochemistry and pathology of radical-mediated protein oxidation. Biochem. J., 324 : 1-18

Faurre , M., Torres, R. and **Vidella, L. A.** (1990) : Antioxidant activities of lignan and flavonoids. Phytochemistry, 29: 3773-75

Fleurentin, J. et **Joyeux, M.** (1990) : Les tests in vivo et in vitro dans l'évaluation des propriétés antihépatotoxiques de substances d'origine naturelle. . In : Fleurentin, J., Cabaillon, P., Mazarj, G., Santos, J. D. et Younos, C. (Eds.) Ethnopharmacologie : Sources, méthodes, objectifs. Actes du 1er colloque Européen d'Ethnopharmacologie- Metz (France) – ORSTOM, PP.248-269

Garle, M. J. and **Fry, J. R.** (1989) : Detection of reactive metabolites in vitro. Toxicology, 54: 101-110

Hikino, H. and **Kiso,Y.** (1988) : Natural products for liver disease. In : WAGNER, H., HIKINO, H. and FARNSWORTH, R. Ed. Economic and Medicinal Plant Research , vol. 2, Academic press London, pp. 39-72

Kehrer, J. P. (2000) : The Haber-weiss reaction and mechanisms of toxicity. Toxicology, 149 : 43-50

Kingu, C. S. and **Wei, R.** (1997) : Alcohol dehydrogenase catalysed reduction of protein carbonyl derivatives. J. Biochem. Biophys. Methods, 34, 61-68

Mandl, J., Meszaros, K., Antoni, F., Spolarics, Z. and **Garzo, T.** (1982) : Reversible inhibition of RNA synthesis by D-galactosamine in isolated mouse hepatocyte. Mol. Cell. Biochem., 46: 25-30

Markus, B. (1996) : Electrochemical behaviour and antioxidant activity of some natural polyphenols. Helv-Chim. Acta, 79 : 1147-58

Martinez, G. M., Giuliani, A., Leon-Fernandez, O. S., Davison, G. P. and **Nunez-Selles, A. J.** (2001) : Effect of *Mangifera indica* L. extract (QF808) on protein and hepatic micosome peroxidation. Phytother. Res., 15(7) : 581-5

Masaki, N., Kyle, M. E. and **Farber, J. L.** (1989): Tert-buthylhydroperoxide kills culture hepatocytes by peroxidizing membrane lipids. Arch. Biochem. Biophys., 269, 390-399

Moundipa F. P., Njayou F. N., Yanditoum S., Sonke B. and **Mbiapo T. F.** (2002). Medicinal plants used in the Bamoun region of the Western province of Cameroon against jaundice and other liver disorders. Cam. J. Biochem. Sc. 12 (2): 39-46

Recknagel, R O. and **Glende, E. A. Jr.** (1973): Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. CRC Crit. Rev. Toxicol., 2, 263-297

Thabrew, M. I., Gove, C. D., Robbin, D. H., Mc Farlane, I. G. and **Williams, R.** (1995) : Protection against galactosamine and Tert-buthylhydroperoxide induced hepatocyte damage by *Melothria maderaspanata* extract. Phytother. Res., Vol. 9, 513-517

Ulf, A. N., Lars-I, O., Gunnar, C. and **FALLENIUS, A. C. B.** (1989) : Inhibition of lipid peroxidation by spin labels. The Journal of Biological Chemistry, 264(19) : 11131-11135

Wandji, J., Tanee, F. Z., Tillequin, F., Seguin, E. Koch, M. (1994) : Two isoflavones from Erythrina senegalensis. Phytochemistry, 35(1) : 245-248

Wills, E. O. (1987) : Evaluation of lipid peroxidation and biological membranes. In : Snell, K. and Mullock, B. Ed. Biochemical Toxicology. A practical approch series. IRL Press Oxford Washington pp. 127-151

Wormser, U. and **Zakine B.. S.** (1990) : The slice liver system : An in vitro acute toxicity test for assessment of hepatotoxins and their antidotes. Toxicology In Vitro, 4, 449-451