Research Journal of Chemical Sciences _____ E-ISSN 2231-606X
Vol. **6(10),** 19-23, October **(2016)** *Res. J. Chem. Sci.*

Phytochemical and antioxidant properties of bark and stems extract of *Strychnos camptoneura* Gilg and Busse (Loganiaceae)

Morabandza Cyr Jonas[1*], Amboyi Gloria Stéphanie Aurélia[1], Matini laurent[2], Gouolali Tsiba[3], Ongoka Pascal Robin[2] and Abena Ange Antoine[1]

[1]Department of Biochemistry and Pharmacology, Health Sciences Faculty, University Marien Ngouabi, P .O. Box 69, Brazzaville, CONGO
[2]Departments of Exact Sciences, ENS, University Marien NgouabiI, P.O. Box 69 Brazzaville, CONGO
[3]Vegetable Chemistry and life unity, Technogical and Sciences Faculty, University Marien NgouabiI, P.O. Box 69, Brazzaville, CONGO
cyrmoras@yahoo.fr

Available online at: www.isca.in, www.isca.me
Received 27th June 2016, revised 12th August 2016, accepted 12th October 2016

Abstract

Phytochemical and antioxidants properties of the barks and the stems extracts of Strychnos camptoneura (Loganiaceae) were studied using classic tests. The aqueous, hydroethanolic, ethanolic and chloroformic extract have been prepared. The most important yield was obtained with hydroethanolic extract of the barks. The qualitative analyses by colored reactions in tubes of the aqueous extract have emphasized respectively 13 and 11 chemical families in bark and stems. The thin layer chromatography of the chloroformic extract revealed the presence of sterols and terpenoids; the one of hydroethanolic extract, the presence of flavonoids and phenolic acid in the two organs. The quantitative evaluation showed more contents raised in alkaloids, total phenol (ethanolic extract) and flavonoids (hydroethanolic extract) in the bark that in the stems. Antioxidant properties according 1.1-diphényl-2-picrylhydrazyle (DPPH) method revealed more important effect with ethanolic extract of the bark and stem than with the aqueous and hydroethanolic extracts. These results certainly explain the important use of this plant in traditional medicine.

Keywords: *Strychnos camptoneura*, Phytochemical, Antioxidant, Barks, Stems.

Introduction

Strychnos camptoneura is a liana belonging to the Loganiaceae family. It measures around 120 m long and 25 cm of diameter, trailing with gimlets of 1 to 3 pairs, the shiny branches and green dark, the leaves composed with leafstalk of 7 to 17 mm of long[1,2]. In Congo, *S. camptoneura* is commonly called yindza in Mbéti; iyindza in Mbokô, Ngaré, Mbôchis and Makoua. In Congolese traditional medicine, *S. camptoneura* is widely used against several pathologies and symptoms as malaria, inflammations, pains, diabetes, fever, microbial infections, hernia, parasites infections and sexual weakness[1].

The aqueous macerate of the bark is used in children like laxative[3]. However in spite of his important traditional use, no scientific data on the chemical composition or antioxidant properties of this species is available. Several previous researches have indicated that the pharmacological virtues of plants are intimately related with the presence of chemical families in these species[4-9].

The numerous therapeutic indications assigned to this plant by the farming populations suggest the presence of secondary metabolites that would be responsible for the vaunted virtues. Basing to this hypothesis, we initiated this preliminary study of the phytochemical and antioxidant properties of barks and stems of *S. camptoneura*.

Materials and Methods

Vegetal materials: The barks and stems of *S. camptoneura* constituted material of this study. The plant specimen was collected at M'voula, a village of Itoumbi (Cuvette west of Congo) situated at 765 km from Brazzaville, in June 2013. The specimen was identified in the Institute of Research of Exact and Natural Sciences (I.R.E.N.S.) of Congo-Brazzaville and recorded under the N° 2271. They were previously air dried during 14 days at laboratory temperature (25 ± 1°C) and grounded into powder using a wood mortar.

Preparation of extracts: Four types of extracts have been prepared in accordance with the achieved tests: aqueous, hydroethanolic, ethanolic and chloroformic extracts. 10 g of each powder was subjected to maceration under magnetic agitation in 100 ml of each solvent during 48 hours. The mixtures were filtrated and concentrated at 55°C and the yield determined.

Identification of chemical family: The chemical families have been identified by colored reactions in tubes method with aqueous extract[9] and thin layer chromatography (TLC) with chloroformic extract for sterols and terpenoides on hydroethanolic extracts for flavonoids and phenolic acids[10].

Sterols and terpenoides identification: It has been achieved with the chloroformic extract of the two organs in migration

solvent constituted by Petrol ether/acetate (7:3). After extract deposit, the chromatographic layers are placed at 110°C in the steam room during 10 min and the revelation of the spotlights gets used sulphuric anisaldehyde. The sterols and terpenoids were revealed by the presence of the brown, blue, green and purple colors.

Flavonoids and phenolic acids identification: The hydroethanolic extract of the two organs of *S. camptoneura* have been deposited on chromatographic layers with the capillary tube. The migration solvent was Ethyl acetate/ formic acidic/water (8:1:1); the revelation gets used to the U.V 254 nm and 365 nm after pulverization with the reagent of Neu. After migration and revelation, the spotlights are fluorescent for the phenolic acid; fluorescent, blue, green or orange for the flavonoids.

Quantitative evaluation of some chemical families: Total alkaloids: In order to determine the alkaloids content, 5g of powder of every organ have been macerated in 20 ml of acetic acid 10% and 180 ml of ethanol during 4 hours. After filtration, the mixtures were concentrated to the quarter (1/4) of his initial volume. The ammonium hydroxide (NH4OH) has been added drop by drop to the extract until complete precipitation. The precipitate was collected and has been washed with the diluted ammonium hydroxide, and filtered. The final product is the alkaloid that has been dried and weighed for the calculation of the content[11].

Total phenols (TP): The reagent of Folin-Ciocalteu was used for the evaluation of total phenols of aqueous, hydroethanolic and ethanolic extracts. Folin-Ciocalteu is a mixture of phosphotungstene acid ($H_3PW_{12}O_{40}$) and phosphomolybdène ($H_3PMo_{12}O_{40}$) of yellow color. The method is based on the oxidation of the phenolic compounds by this reagent. This oxidation draws the formation of a new complex molybdenum-tungsten of blue color that absorbs to 725 nm. The evaluation of TP is done by comparison of the optic density (D.O) observed to the one obtained from a stallion of known acid Gallic concentration. The total phenol compounds are measured as follow: 0.1ml of the extract hydroethanolic is introduced in an Eppendorff tube of 2 ml, the extract is diluted with 0.9 ml of distilled water. 0.9ml of the reagent of Folin-Ciocalteu (1N) is immediately added after addition of 0.2 ml of Na_2CO_3 (20%) solution. The obtained mixture is hatched to the ambient temperature during 40 minutes safe from light. The absorbance is measured with the spectrophotometer at 725 nm against a solution of ethanol used like white (control). A right of standardization achieved previously with the Gallic acid in the same conditions that the samples to analyze, permitted to calculate the total phenols contain. The results are expressed in mg equivalent Gallic acid by gram of dry matter (mg E GA/g Ms) [12].

Total Flavonoids (TF): The colorless solutions of sodium nitrite ($NaNO_2$, 5%) and of aluminum chloride ($AlCl_3$, 10 %) have been used for the evaluation of total flavonoids in aqueous, hydroethanolic and ethanolic extracts. The method is based on the oxidation of the flavonoids by these reagents; oxidation that draws the formation of a brownish complex that absorbed at 510 nm. The comparison of the optic density (D.O) observed to the one obtained from a stallion of known concentration Rutin permits to value the total content in flavonoids by colorimetric effect. In a ball of 10 ml are introduced 250 µl of extract and 1 ml of distilled water successively. To the initial time (0 minute) are added 75 µl of a $NaNO_2$ (5%) solution. After 5 min 75µl of $AlCl_3$ (10%) are added; 6 minutes later, 500µl of NaOH (1N) and 2.5 ml of distilled water are added successively to the mixture. A curve of standardization is elaborated with solutions standards of Rutin prepared at different concentrations[12].

Antioxidant evaluation: The antioxidants properties of aqueous, hydroethanolic and ethanolic extracts of the barks and the stems of *S. camptoneura* was evaluated quantitatively by mixing 2 ml of the solution of 1,1-diphényl-2-picrylhydrazyle (DPPH) to 10 mg /250 ml in the ethanol and, 100 µl of extract at the concentrations of 10; 5; 2,5; 1,25 and 0,625 mg/ml. The activity was measured at 517 nm safe from light after 30 minutes of incubation in obscurity with the help of an UV-Visible spectrophotometer in comparison with the quercetin[13]. The percentage of inhibition has been calculated by the following relation:

$$\% I = \frac{O.D_{white} - O.D_{El}}{D.O_{white}} x100$$

$D.O_{white}$=*control Optic Density* $O.D._{El}$= *extract/inhibitor Optic Density*

The concentration which inhibits 50 % of DPPH (C.I50) was determined proportionally.

Results and Discussion

The aim of this study was to research phytochemical and antioxidant properties of the bark and stems extracts of *Strychnos camptoneura* (Loganiaceae). Table-1 presents the yield of different extractions of the two organs. It shows that the hydroethanolic extract presents the most important yield comparatively to aqueous, ethanolic and chloroformic extracts respectively with the barks and the stems. The qualitative analysis by colored reactions in tube method permitted to obtain 13 chemical families in barks and 11 families in the stems. Table-2 indicates that the alkaloids, anthraquinons, flavonoids, mucilage, saponins and tannins are more abundant in the barks whereas carotenoids are in the stems. Anthocyans, coumarins, cardiotonic heterosids, sterols and terpenoids are in the same proportions in the two organs. We note however, the absence of the reducing compounds and quinons in the stems. This result oriented the revelation of the sterols, terpenoids and phenolic acid by thin layer chromatography (TLM). The chromatogram of chloroformic extract (Figure-1) obtained in the eluant system of Ether of petrol / ethyl acetate (7/3) and revealed by

anisaldehyde after heating to 110°C, show the presence of the identical tasks of purple colors and chestnuts in the barks and the stems. According to the literature, these different tasks could be assigned to the presence of phytosterols and terpenoides[10,14]. This result is confirmed by the values of the frontal ratio presented in Table-3, showing that the two organs contain the same compounds. Whereas the chromatogram (Figure-2 and 3) of hydroethanolic extract remains dominated by fluorescents tasks that orient toward the phenolic acid and flavonoids[10,14] as indicated in the Table-4 of frontal ratio. These results are in agreement with those of the colored reactions in tubes and, inform that *S. camptoneura* organs are rich of secondary metabolites. That is the reason why we undertook to evaluate quantitatively some chemical families. Table 5 shows that the alkaloids are more important in the barks: 8.03 ± 0.15% than in the stems: 4.50 ± 0.36%. The same tendency is observed with the total phenol and flavonoids, after establishment of the standard curves (R^2 = 0,987; R^2 = 0,993) with Gallic acid and Rutin respectively as control. Table 6 shows that the contents of phenol are 22.10 ± 0.14 mg EqAG/g.M.S in the barks against 16.13 ± 0.31 mg EqAG/g.M.S in the stems with the ethanolic extracts. This fact certainly explains itself by the polarity of the solvents; indeed, the ethanol is a more polar solvent than the two other solvents.

On the other hand, Table-7 reveals that the contents in flavonoids are 10.59 ± 0.31 mgEqRt/g.M.S in the barks against 7.58 ± 0.26mg EqRt/g.M.S in the stems with hydroethanolic extract followed by the ethanolic and aqueous extracts. These results are in agreement with those of the chemical screening which shows more alkaloids, phenol in the barks than in the stems. Several authors revealed that the chemical families identified in our analysis present important pharmacological properties notably the antioxidant potentialities[14-16]. Besides, the literature recognizes that phenols are molecules endowed the antioxidant capacity[14]. Their setting in evidence in this study lets think that *S. camptoneura* would present antioxidant potentialities. Also, other studies proved that aqueous, ethanolic and hydroethanolic extracts were the seat of phenolic compounds sensors of the free radicals[17-19]. These observations explain the quantitative determination of antioxidant effect.

According to the Table-8, the different extracts are interesting antioxidant potentialities according to the organ and the used solvent. According to obtained results, the ethanolic extract of the barks presents more important antioxidant effect than those of stems. Indeed, the values of IC 50 are 3.14 ± 0.01 and 5.57 ± 0.01 mg/ml respectively with the barks and the stems, compared to the quercetin 0.12 ± 0.05 mg/ml (control). More is the inhibitory concentration capable to trap 50% of free radicals (IC 50) is raised, weaker is the antioxidant effect; least is this concentration more important is the antioxidant effect. These suggest that the barks of *S. camptoneura* have a better antioxidant effect than the stems. Our results are in agreement with the qualitative and quantitative analysis and would also explain the important use of this plant in treatment of some pathology as malaria. Indeed, a study showed that the antioxidant plants would be appropriated to treat malaria since at the time of the multiplication of the parasite[20], oxidative stress observed with excessive production of the free radicals[21,22]. And the production of hémozoïne weakens the potential antioxidant generating other serious pathologies[23].

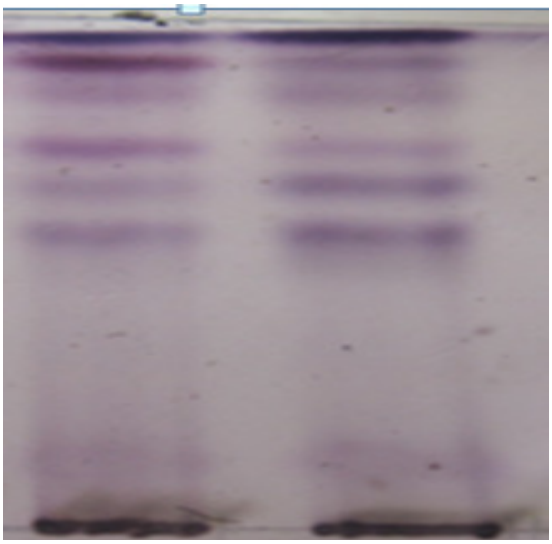
Table-1
Yield (%) of extraction of the barks andstems of *S. camptoneura* with different solvents

Extracts	Barks	Stems
Aqueux	4.70 ± 0.18	2.27 ± 0.07
Ethanolic	5.70 ± 0.04	5.44 ± 0.13
Hydroethanolic	10.34 ± 0.14	9.67 ± 0.16
Chloroformic	2.22 ± 0.07	1.09 ± 0.24

Table-2
Chemical screening of aqueous extract of the barks and stems of *S. camptoneura*

ChemicalFamily	Barks	Stems
Alkaloids	+ + +	+ +
Anthocyans	+	+
Anthraquinons	+ + +	+ +
Carotenoids	+ +	+ + +
Coumarines	+	+
Red. Comp.	+ + +	-
Flavonoids	+ +	+
Card. Hete.	+ + +	+ + +
Mucilags	+ +	+
Quinons	+ +	-
Saponosids	+ +	+
Sterols/Terpenoides	+ +	+ +
Tannins	+ +	+

- : Absent; **+** : present ; **+ +** : average; **+ + +**:abundant, Card Hete. : Cardiotonic heterosids, Red. Comp.: Reducing compounds.

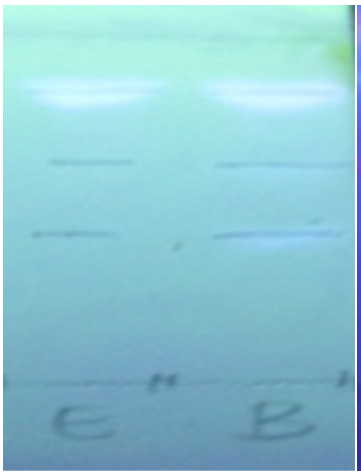


B S

Figure-1
TLC of chloroformic extract of the barks and stems of *S. camptoneura,* Petrol ether / ethyl acetate (7: 3) revealed by anisaldehyde at visible light

Table-3
Frontal ratioof chloroformic extracts of the barks and stems of *S. camptoneura*

Organs	F.R							
Barks	0.15	-	-	0.57	0.67	0.75	0.87	0.92
Stems	0.15	-	-	0.57	0.67	0.75	0.87	0.92



B S

Figure-2
T.L.C of hydroethanolic extracts of the barks and stems of *S. camptoneura*. Ethyl acetate / formic Acid / water (8:1:1), UV 254 et 365 nm, with Neu

B S

Figure-3

Table-4
Frontal ratio of hydroethanolic extracts of the barks and stems of *S. camptoneura*

Organs	**F R**					
Barks	0.38	0.56	-	-	0.79	0.93
Stems	0.38	0.56	-	-	0.79	0.93

Table-5
Weight (g) and contains (%) in alkaloids of the barks and stems of *S. camptoneura*

	Organs	**Barks**	**Stems**
Alkaloids	Weight (g)	0.200 ± 0.004	0.112 ± 0.009
	Percentage (%)	8.030 ± 0.150	4.500± 0.360

Table-6
Total phenol of the barks and stems extracts of *S. camptoneura*

Organs	**Total phenol of extracts (mg EAG/g.M.S)**		
	Aqueous	**Ethanolic**	**Hydroethanolic**
Barks	10.60 ± 0.19	22.10 ± 0.14	16.30 ± 0.52
Stems	09.54 ± 0.22	16.13 ± 0.31	12.23 ± 0.32

Table-7
Total flavonoids of the barks and stems extracts of *S. camptoneura*

Organs	**Total flavonoids of extracts (mg ERt/g.MS).**		
	Aqueous	**ethanolic**	**Hydroethanolic**
Barks	04.84 ± 0.30	06.71± 0.41	10.59 ± 0.31
Stems	04.35 ± 0.21	05.45 ± 0.22	07.58 ± 0.26

Table-8
Antioxidants effect (C.I 50 mg/ml) of barks and stems extracts of *S. camptoneura*

	C.I 50 (mg/ml)	
Extracts	**Ecorces**	**Tiges**
Aqueous	05.42 ± 0.01	06.73 ± 0.13
Ethanolic	03.14± 0.01	05.05 ± 0.11
Hydroethanolic	04.43 ± 0.03	06.57 ± 0.07
Quercetin	0.12 ± 0.05	

Conclusion

The present study showed that the barks and the stems of *S. camptoneura* are rich in secondary metabolites. The quantitative analysis revealed the high contents in alkaloids, moderate in total phenol with ethanolic extract and average in total flavonoids with hydroethanolic extract. The evaluation of antioxidant properties with the three types of extracts showed that the barks and stems of *S. camptoneura* are interesting antioxidant activity. These results could explain the abundant use of this plant in traditional medicine and, open perspectives of research for the pharmacological activities.

Références

1. Bouquet A. (1969). Féticheurs et Médecine Traditionnelle du Congo-Brazzaville, Mémoire ORSTOM. Brazzaville, 36, 48-249.

2. Leeuwenberg A.J.M. (1969). The *Loganiaceae* of Africa 8. *Strychnos 3*. Revision of the African species with notes on the extra-African. Mededelingen Landbouwhoge school Wageningen, Netherlands, 69-1, 316.

3. Bouquet A. (1972). Plantes médicinales du Congo-Brazzaville: Uvariopsis, Pauridiantha, Diospyros. Travaux et Documents de l'ORSTOM, N° 13. Office de la Recherche Scientifique et Technique Outre-Mer. Paris, France, 113.

4. Bamidele V.O., Olubunmi O.S., Dare K., Ogunbiy B.A. Elizabeth AdeolaAruboula and Ayodele Olufemi Soladoye. (2008). Analgesic, anti-inflammatory and antipyretic activities from flavonoids fractions of *Chromolaena odorata*. *Journal of Medicinal Plants Research*, 2(9), 219-225.

5. Ogbe A.O. and George G.A.L. (2012). Nutritional and Anti-nutrient Composition of Melon Husks: Potential as Feed Ingredient in Poultry Diet. *Res.J.Chem.Sci.,* 2(2), 35-39.

6. Morabandza C.J, Ongoka R.P., Matini L., Epa C., Nkounkou L.C. and Abena A.A. (2013). Chemical Composition of the Mesocarp of *Garcinia kola Heckel* (*Clusiaceae*) Fruit. *Res.J.Recent Sci*., 2(1), 1-8.

7. Morabandza C.J., Okemy A.N., Ongoka R.P., Okiemy-Akieli M.G. and Attibayeba Abena A.A. (2014). Effets antimicrobiens, anti-inflammatoires et antioxydants du mésocarpe de Garcinia kola Heckel (Clusiaceae). *Phytothérapie*., 12, 164-169.

8. Elion Itou R.D.G., Sanogo R., EtouOssibi A.W., NsondeNtandou G.F., Ondele R. and

Peneme B.M. et al. (2014). Anti-inflammatory and analgesic effects of aqueous extract of stem bark of *Ceiba petandra* Gaertn. *Pharmacol. and Pharm*, 5, 1113-1118.

9. Bouquet A. (1967). Inventaires des plantes médicinales et toxiques du Congo Mémoire O.R.S.T.O.M.. Brazzaville-Congo, 34.

10. Wagner H. and Bladt S. (2001). Plant drug analysis. A thin layer chromatography Atlas. 2nd Ed, Springer New-York, USA.

11. Zorha B. et al. (2012). Toxicité aigüe des alcaloïdes totaux des graines de Datura Stramonium chez les souris femelle. 313-314.

12. Khacheba I. (2008). Effet des extraits de quelques plantes médicinales locales sur l'alpha amylase. 75, *http//www.memoire online.com/10/08/1554*, 29/06/2016, 12 h 00

13. Huang D.B. and Prior R.L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53, 1841-1856.

14. Eleyinmi A.F., Bressler D.C., Amoo I.A., Sporns P. and Oshodi A.A. (2006). Chemical composition of bitter cola (*Garcinia kola*) seed and ulls. *Polish Journal of food and nutrition sciences*, 15, 395-400**.**

15. Braide V.B. (1989). Antispamodic extracts from seeds of *Garcinia kola*. *Fitoterapia*, 60(2), 123-12910.

16. Bruneton J. (1999). Pharmacognosie, Phytochimie, plantes médicinales. Tec et Doc. 2ème Ed Lavoisier**.**

17. Braide V.B. (1993). Anti inflammatory effect of kolaviron, a biflavonoid extract of *Garcinia kola*. *Fitoterapia*, 64(5), 433-436.

18. Hennebelle T. (2006). Investigation chimique, chimiotaxonomique et pharmacologique de Lamiales productrices d'antioxydants». Université de sciences et technologiques de Lille-I, 304.

19. Hayashi T., Cottam H.B. and Chan M. et al. (2008). Mast cell-dependent anorexia and hypothermia induced by mucosal activation of toll-like receptor 7. *Am J Physiol Regul Integr Comp Physiology*, 295, 123-32**.**

20. Koudouvo K., Kavengue A., Agbonon A., Kodjo M., Aklikokou K., Kokou K., Essien K. and Gbeassor M. (2006). Enquête ethnobotanique sur les plantes à activité antiplasmodiale, antioxydante et immunostimulante dans la région maritime du Togo. *Revue Togolaise des sciences,* 1(2), 145-155**.**

21. Zhang J., Krugliak M. and Ginsburg H. (1999). The fate of ferriprotoporphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. *Molecular and Biochemical Parasitology*, 99, 129-141.

22. Becker K., Tilley L., Vennerstrom J.L., Roberts D., Rogerson S. and Ginsburg H. (2004). Oxydative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *International Journal of Parasitology*, 34, 163-189.

23. Urban B.C. and Roberts D.J. (2002). Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches of host cells. *Current Opinion in immunology*, 14, 458-465.