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RESEARCH ARTICLE

PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *STRYCHNOSCAMPTONEURA* GILG & BUSSE (LOGANIACEAE) LEAVES

[1]Morabandza, C.J., [1]Amboyi, G.A.S., [2]Gouolali, T., [1]Elion Itou R. D.G., [1]Etou Ossibi, W.A., [3,4]Andzi Barhé, T., [3,4]Ongoka, P.R. and [1]Abena, A.A.

[1]Laboratory of Biochemistry and Pharmacology, Health Sciences Faculty, Marien Ngouabi University, P.O.Box 69, Brazzaville-CONGO
[2]Vegetable chemical and life Unity, Sciences and Technical Faculty, Marien Ngouabi University, P.O. Box 69, Brazzaville-CONGO
[3]Departments of Exact Sciences, E.N.S, Marien Ngouabi University, P.O. Box 69, Brazzaville-CONGO
[4]Natural substances and organics synthesis Laboratory, Faculty of Sciences USTM, P.O. Box 941, Franceville Gabon

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ABSTRACT

Phytochemical constituents and antioxidant activity of *Strychnoscamptoneura* (Loganiaceae) leaves were studied by using classical tests. The aqueous, hydroethanolic, ethanolic and chloroform extracts were prepared. The most important extraction yield was obtained withhydroethanolic extract. Qualitative analysis by colored tube reactions of aqueous extract revealed 11 major chemical families. Thin layer chromatography of the chloroform extract confirmed the presence of sterols and terpens; the one of hydro-ethanolic extract, flavonoids and phenolic acids. Quantitative evaluation showed 3.56 ± 0.21% alkaloids, a higher content in total phenols and flavonoids with the ethanolic extract compared to the aqueous and hydro-ethanolic extracts. The antioxidant activity by using the 1-1-diphenyl-2-picrylhydrazyl (DPPH) method was greater withethanolic extract than with aqueous and hydro-ethanolic extracts. These results offer good prospects for a possible use of leaves in place of the bark, stems and exudates of this plant by the rural populations.

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INTRODUCTION

Strychnoscamptoneura (Loganiaceae) is a big liana of the Congolese (Brazzaville) spontaneous flora, which possess numerous traditional therapeutic indications. Commonly called yindza in Mbéti; iyindza in Mbokô, Ngaré, Mbôchis and Makoua, this specieis widely usedagainst several pathologies and symptoms as malaria, inflammation, pain, diabetes, fever, microbial infections, hernia, parasites infections and sexual weakness (Bouquet, 1969; Leeuwenberg, 1969). Previous studies of stem bark have confirmed the antimicrobial and anti-inflammatory effects (Morabandza *et al.,* 2016); analgesic and antipyretic effects (Morabandza *et al.,* 2016) and the non-toxic character of the aqueous extract (Morabandza *et al.,* 2016). The phytochemical study of bark and stem revealed the presence of chemical compounds endowed with antioxidant potentialities (Morabandza *et al.,* 2016).

****Corresponding author: Morabandza,***
Laboratory of Biochemistry and Pharmacology, Health Sciences Faculty, MarienNgouabiUniversity, P.O.Box 69, Brazzaville-CONGO

Currently, the stem barks, stems and exudatesof *S. camptoneura* are subject of an abundant and frequent use which, in the near future, would contribute to the rarefaction and disappearance of the specie. This overexploitation leads to the hypothesis that the leaves of *S. camptoneura* could effectively replace barks, stems and exudates in traditional medicine. Indeed, the leaves of the plant are the seat of metabolic syntheses by the photosynthesis and alsomost used organs in traditional medicine.

Those of *S. camptoneura* were never be used in traditional pharmacopeia probably in the reason of their difficult access by the farming populations.Several previous studies have indicated that the pharmacological virtues of plants are intimately related to the presence of chemical compounds in these species (Haleem *et al.,* 2016; Zubaida *et al.,* 2016, Bairy*et al.,* 2016, Amal*et al.,* 2016; Mohammad *et al*., 2016; Neelamma *et al*., 2016). Thus this study aimed to determine the phytochemical constituents and antioxidant activity of the leaves of *S. camptoneura*.

MATERIEL ET METHODES

Vegetal materials

A vegetal material wasconstituted by the leaves of *S. camptoneura*. The specimen was collected at M'voula, a village of Itoumbi (Cuvette west of Congo) situated at 765 km from Brazzaville, in June 2015. The specimen was identified in the Institute of Research of Exact and Natural Sciences (I.R.E.N.S.) of Congo recorded under the N° 2271. They were previously washed, air dried during 10 days at laboratory temperature (25 ± 1°C) 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 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 (Bouquet, 1967) and thin layer chromatography (TLC) with chloroformic extract for sterols and terpenoides and withhydroethanolic extracts for flavonoids and phenolic acids (Wagner *et al.,* 2001).

Sterols and terpenoides identification

It has been achieved with the chloroformic extract of theleaves 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 usedsulphuricanisaldehyde. 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 leavesof *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 at 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 forthe flavonoids.

Quantitative evaluation of some chemical families

Total alkaloids

In order to determine the alkaloids content, 5g of powder of the leaves 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 (NH_4OH) 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 (Zorha *et al.,* 2012).

Total phenols (TP)

Total polyphenol content was determined by colorimetry, using the Folin-Ciocalteu's (F-C) method (Khacheba, 2008). 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 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 of2 ml, the extract is diluted with 0.9 ml ofdistilled 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 asolution 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/gMs).

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 510nm. 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 1ml 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 (Khacheba, 2008).

Antioxidant evaluation

The antioxidants potentiality of aqueous, hydroethanolic and ethanolic extracts of the leaves 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 atthe concentrations of 10; 5; 2,5; 1,25 and 0,625 mg/ml. The potentiality was measured at 517 nm safe from light after 30 minutes of incubation in obscurity with the help of avisible U.V spectrophotometer in comparison with the quercetin (Huang*et al,* 2005). The percentage of inhibition has been calculated by thefollowing relation:

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

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

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

RESULTS AND DISCUSSION

This work was initiated to research phytochemical constituents and antioxidant activity of the leaves of *S. camptoneura*. That is why the same methodology was used to compare the precedents analysis realized with the bark and stem of this plant (Morabandza *et al.,* 2016 b). The obtain results (Table 1) shows that thehydroethanolic extract presents the most important yield comparatively to aqueous, ethanolic and chloroformic extracts.

Table 1. Extraction Yield (%) of *S. camptoneura* leaves with different solvents

Extracts	Values
Aqueous	05.07 ± 0.06
Ethanolic	06.89 ± 0.03
hydroethanolic	10.65 ± 0.15
Chloroformic	03.24 ± 0.25

Table 2.Chemical screening of aqueous extract of *S. camptoneura*leaves

Chemical Families	Leaves
Alcaloids	++
Anthocyans	+++
Anthraquinons	+++
Carotenoids	-
Coumarins	+
Red. com.	-
Flavonoids	+++
Card. Het.	+++
Mucilages	+++
Quinons	+++
Saponins	++
Sterols/Terpens	+++
Tannins	++

Card. Het. :Cardiotonicheterosids Red. Com.: Reducing compound - : Absent; + : present ; + + : average; + + +:abundant,

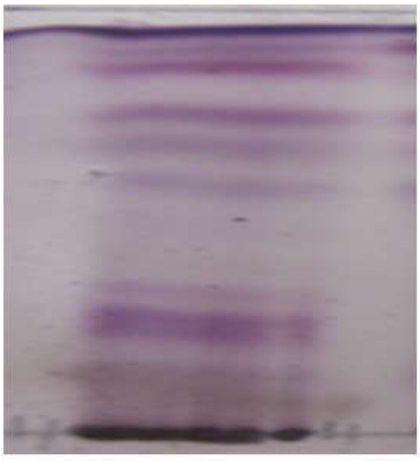

Figure 1. TLC of chloroformic extract of *S. camptoneura*, leaves Petrolether / ethylacetate(7: 3) revealed byanisaldehyde at visible light

The qualitative analysis with aqueous extractby colored reactions in tube method revealed the presence of 11 chemical families.Table-2indicates that theanthocyans, anthraquinons, flavonoids, mucilage, cardiotonicheterosids, quinons, sterols and terpens are more abundant and in the same proportionsthan other compounds.

Table 3. Frontal Ratio of chloroformic extract of *S. camptoneura*leaves

	F.R							
Spots	0,15	0,22	0,30	0,57	0,67	0,75	0,87	0,92

Figure 2.

Figure 3.

T.L.C of hydroethanolic extracts of*S.camptoneura*leaves Ethyl acetate / formic Acid / water (8:1:1), UV 254 and 365 nm, with Neu

These families are followed by tannins, alkaloids, saponins and coumarins at the last.We note however, the absence of the reducing compounds and carotenoids in the leaves. These results are similar with those obtains with the barks and the stems of the same plants with however some nuances (Morabandza *et al.,*2016 b).This result oriented the revelation of the sterols, terpens 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 anisaldeh yde after heating to 110°C, show the presence of the tasks of purple colors and chestnuts in the leaves. According to the literature, these different tasks could be assigned to the

presence of phytosterols and terpenoides (Wagner*et al.,*2001; Eleyinmi *et al.,* 2006). This result is confirmed by the values of the frontal ratio presented in Table-3, showing that the leaves of *S. camptoneura* contain more compounds that the barks and the stems.

Table 4. Frontal ratio of hydroethanolic extracts of*S. Camptoneura*leaves

F .R						
Spots	0,38	0,56	0,64	0,72	0,82	0,93

Table 5. Weight (g) and contains (%) in alkaloids of *S. camptoneura*leaves

	Weight (g)	Contain (%)
Leaves	0 ,084±0,004	3,560 ± 0,210

Table 6.Total phenol contains of *S.camptoneura*leaves extracts

Total phenol of extracts (mg EAG/g.M.S)			
Leaves	Aqueous	ethanolic ethanolic	Hydroethanolic
values	13.23 ± 0.22	26.34 ± 0.33	19.22 ± 0.24

Table 7.Total flavonoid contains of S. camptoneura leaves extracts

Total flavonoids of extracts(mg ERt/g.MS).			
Leaves	Aqueous	ethanolic	Hydroethanolic
Values	7.35 ± 0.61	14.25 ± 0.31	11.24 ± 0.21

Table 8. Antioxidants activity (C.I_{50}mg/ml) of *S. camptoneura* leaves extracts

I.C_{50} (mg/ml)				
Leaves	Aqueous	ethanolic	Hydroethanolic	Quercétine
Values	05.01 ± 0.02	02.98 ± 0.04	03.56 ± 0.03	0.12 ± 0.05

Whereas the chromatogram (Figure-2 and 3) of hydroethanolic extract remains dominated by yellow orange, fluorescents and blue tasks that orient toward the flavonoids and phenolic acid (Wagner *et al.,* 2001;Eleyinmi *et al.,* 2006) as indicated in the Table-4 of frontal ratio; these results confirm those of the colored reactions in tubes. The leaves of *S. camptoneura* are rich of secondarymetabolites. The quantitative evaluation of some chemical families showed that the leaves are as rich as the barks and stems. Table 5 shows3,560 ± 0,210 % of alkaloids in the leaves, less important value than those of the barks and the stems (Morabandza *et al.,*2016*b*).Contrary, to the barks and the stems, the contents in 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 in the leaves are higher. Table 6 shows that the contents of phenol are 26.34 ± 0.33mg EqAG/g. M.S with the ethanolic extracts against 13.23 ± 0.22 and 19.22 ± 0.24 mg EqAG/g.M.S respectively with aqueous and hydroethanolic extracts. On the other hand, table-7 reveals that the contents in flavonoids are14.25 ± 0.31 mg EqRt/g.M.S against 7.35 ± 0.61 and 11.24 ± 0.21 mg EqRt/g.M.S with aqueous and hydroethanolic extracts respectively. This fact certainly explains itself by the polarity of the solvents; indeed, the ethanol is a more polar solvent than the two other solvents.Several authors revealed that the chemical families identified in our analysis present important pharmacological properties notably the antioxidant potentialities (Eleyinmi *et al,* 2006; Bruneton, 1999). Their setting in evidence in this study lets think that the leaves of *S. camptoneura*would present antioxidant activity. Other studies proved that aqueous, ethanolic and hydroethanolic extracts were the seat of phenolic compounds sensors of the free radicals (Braide, 1993; Hayashi *et al.,*2008).According to the obtained results, the ethanolic extract of the leaves, presents more important antioxidant activity thanaqueous and hydroethanolic extracts. Indeed, the values of IC_{50} are 02.98 ± 0.04; 05.01 ± 0.02 and 03.56 ± 0.03mg/mlrespectively with ethanolic, aqueous and hydroethanolic extracts, comparedto the quercetin 0.12 ± 0.05 mg/ml (control). More is theinhibitory concentration capable to trap 50% of free radicals (IC50) is raised, weaker is the antioxidant effect; least is thisconcentration more important is the antioxidant effect. Thesesuggest that the leaves of *S. camptoneura* have a betterantioxidant activity than the barks and the stems. All our results are in agreement with qualitative and quantitative analysis obtains with the other organs of this plant. Indeed, a study showed that the antioxidant plants would be appropriated to treat malaria and oxidative stress generating other serious pathologies.

Conclusion

The present study showed that the leaves of *S. camptoneura*are so rich in secondary metabolites endowed pharmacological activities than the barks and the stems. The results revealed interesting antioxidant activity and permit to certify the hypothesis that the leaves can be used in replacement of other organs in order to protect this species of the rarefaction and a possible disappearance.

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