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ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES OF THE AQUEOUS EXTRACT OF THE STEMS BARK OF *STRYCHNOS CAMPTONEURA* GILG & BUSSE (LOGANIACEAE)

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ABSTRACT

The aqueous extract of *Strychnos camptoneura* (Loganiaceae), a medicinal plant of traditional Congolese pharmacopoeia widely used against several pathologies, was studied for antibacterial and anti-inflammatory activities by using classical tests. The results showed that aqueous extract inhibits the growth of all studied strains carried out by diffusion in solid medium method. The analysis revealed the IMC of 5 mg/ml for *Streptococcus sp* and *Candida albicans*; 6.25 mg/ml for *Staphylococcus aureus* and 12.25 mg/ml for *Escherichia coli*, *Enterobacter sp* and *Streptococcus β hemolytic*. Aqueous extract BMC was at 50 mg/ml for all strains. At the doses of 300, 600 and 1200 mg/kg aqueous extract of *S. camptoneura* inhibits significantly œdema induced by carrageenan and formation of granuloma induces by the cotton pellet. The present investigations established pharmacological evidence to support the traditional uses and may open up the possibility of finding the news antimicrobial and anti-inflammatory compounds.

KEY WORDS: *Strychnos camptoneura*, bark, antimicrobial, anti-inflammatory.

1. INTRODUCTION

The resistance to antibiotics reached critical point these last decades, especially in hospital environment (**Cushnie et al, 2011**). Indeed, the abusive consumption of antibiotics, coupled

to the bad management of the medical garbage dragged the apparition of the resistances strains and the emergence of non common infections that compromise nowadays the treatment by the existing antibiotics. These microbial infections often come with inflammatory illnesses to the non negligible consequences as the epidemiological data indicate: 61% in Burkina in 2008; 10.2 % in Democratic Republic of Congo and 3.5 % in 2005 in Congo-Brazzaville (**Bileckot et al, 1998**). This situation is especially giving cause for concern so that the values are in considerable increase whereas the present treatments basis on steroïdiens (glucocorticoïds) and no steroïdiens anti-inflammatory although being efficient, present the important undesirable secondary effects, notably gastro-intestinal and cardiovascular risks that limit their use during the long period (**Carrado et al, 2009; Zeilhofer, 2007**). Facing these pathologies, the treatment and the hospital fare of the patients become a real public health and a supplementary economic problem in the developing countries, one which is the Republic of Congo; because of the insufficiency and the inaccessibility to the conventional treatments. That is, the reason why there is urgent necessity to complete the present number of anti-infectious and anti-inflammatory agents (**Cushnie et al, 2011)**. That is the present interest research of the use of antimicrobial and anti-inflammatory virtues of several plant species accessible to all (**Morabandza et al, 2014; Elion Itou et al, 2014**; **Ghedabda et al, 2014**; **Bouterfas et al, 2014**). *S. campteneura* Gilg & Busse is a species belong to the Loganiaceae family, commonly named yindza in Mbétis or iyindza in Mbokô, Ngaré, Mbôchi and Makoua. The macerate of the stems bark is actively used again malaria, pain, fever, inflammation, stomach aches, hernia, microbial and parasitic infections (**Bouquet, 1972**). The leaves decoction and dry bark powder are applies on the injuries and the ulcers (**Leeuwenberg, 1980**). In spite of these assertions and its abundant used, this species was not subject to pharmacological investigations in order to confirm these claim virtues. So, the objective of the present work is to give a scientific basis to the uses of *S. camptoneura* in traditional medicine.

2. MATERIALS AND METHODS

2.1 Plant material

The stem bark of *Strychnos campteneura* was collected at Mvoula (Department of Cuvette West, around 740 km from Brazzaville-Congo) in June 2013. It was authenticated by National Research in Exacts and Naturals Sciences Institute (IRSEN) and unregistered under the number 2271.

2.2 Microbial strains: Six (6) microbial strains were used: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp*, *Enterobacter sp*, *Candida albicans* and *Streptococcus β hemolytic.* All strains were isolated from biologic withdrawals at Bacteriologic Laboratory of National Laboratory of Public Health of Brazzaville (Congo).

2.3 Animals: Male and female Wistar rats weighing between 150-200g of The Faculty of Health Sciences of Marien Ngouabi University (Brazzaville-Congo), were used for anti-inflammatory activity. They were fed and maintained under standard lighting conditions (12 hours light and 12 hours dark). Animals were fasted for 24 hours before experimentation.

2.4 Preparation of aqueous extract: The collected stem barks were previously air dried at a temperature of 25° ± 1°C during 14 days in the laboratory and grounded into powder, thanks to a mortar. 25 g of powder was subjected to magnetic maceration extraction with 250 ml of water distilled during 48 hours. The macerate was filtered three (3) times to the absorbent cotton and the filtrate concentrated at 50°C in a steam room. The extract tightly closed and kept to 4^{o}C to the refrigerator for pharmacological tests.

2.5 Antimicrobial activity: To evaluate the antimicrobial effect of aqueous extract, decreasing concentrations of 50; 25; 12.5; 6.25 and 5 mg/ml was prepared corresponding to the dilutions of 1, 1/2, 1/4, 1/8 and 1/10 either a geometric continuation of reason 2 from the concentrate extract. The antimicrobial test was achieved by diffusion on solid medium technique (**Ouabonzi, 2004**) with two medium cultures: Mueller Hinton for *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp*, *Enterobacter sp*, *Candida albicans* and the chocolate medium (prepaid specific medium) for *Streptococcus β hemolytic*.

2.5.1 Preparation of Mueller-Hinton Agar medium

Mueller-Hinton Agar medium is a nourishing of the microbial strains environment. 38 g of powder are dissolved in 1 000 ml of distilled water heated then in a ball in 100 °C during 15 minutes until complete dissolution. The mixture is recovered in an erlenmeyer and is sterilized to the autoclave, flowed then in boxes of kneaded at the rate of 20 ml by box.

2.5.2 Preparation of Inoculums

A sample series of each species was taken with a shackle then introduced in 5 ml of distilled water. After agitation, one gets a suspension that is kept during 24 hours in the steam room in 37 °C to permit the growth of the germs. A millimeter of the inoculums prepared is poured in

boxes of kneaded and spread with the help of a pipette pastor. The solution is spread on the surface of the gels and is hatched in 37 °C in the steam room during 20 minutes.

2.5.3 Antibiogram and results reading: The cupules are prepared previously so that on the boxes of kneaded sowed, one achieves some wells with the tip unraveled of the pipette pastor of 5 mm of diameter. The extract are introduced then according to the concentration (5; 6.25; 12.5; 25 and 50 mg/ml) in the wells with the help of micropipette followed standard antibiotics used in the same conditions and distilled water like control. After extract diffusion, the boxes contain the mixture are hatched in 37 °C in the steam room during 24 hours. Reading achieves itself by the measure in millimeter (mm) of inhibition diameter. The sensitivity of the stocks towards extracts is classified according to the inhibition diameter halos (**Choi et al, 2006**) Ø < 8mm: non sensitive strains; 9 < Ø <14 mm: sensitive strains; 15 < Ø < 19 mm: very sensitive strains and Ø > 20mm: extremely sensitive. The inhibitory minimal concentrations (IMC) and bactericidal minimal concentration (BMC) were evalued by comparing inhibition diameters of the extract (5; 6.25; 12.5; 25 and 50 mg/ml) to the one of the control and in relation with dilutions.

2.6 Anti-inflammation activity

2.6.1 Acute Inflammation Induced by Carrageenan

Acute inflammation was induced by carrageenan (**Borgi et al, 2007**). 5 groups of 5 rats were used. Animals were treated as follow: group negative control, treated with distilled water at the dose of 0.5 ml/100g; group positive control treated with diclofenac as reference drug at the dose of 5 mg/kg; three groups tests treated with aqueous extract of *S. campteneura* at the doses of 300, 600 and 1200 mg/kg respectively. One (1) hour after oral administration of products. Animals received 0.05 ml of carrageenan 1% by sub-plantar administration at the right hind paw. The volumes *Vo* and *Vt* of paw oedema were measured using an Ugo Basile 7140 plethysmometer, Italy. The anti-inflammatory effect was evaluated by determining the percentage of inflammation inhibition (I %), using the following formula

$$V = Vt - V_0$$

$$\%\, I = \frac{(vt - vo)t - (vt - vo)tr}{(vt - vo)t} \times 100$$

With: *V* = volume of oedema; *Vt* = volume of the paw oedema at a time *t*; *Vo* = volume of the paw before induction of inflammation; $(Vt - Vo)t$ = volume of oedema of the control group of rats; $(Vo - Vt)tr$ = volume of oedema in the group of treated rats.

2.6.2 Chronic Inflammation Induced by Cotton Pellets

The effect of aqueous extract of *S. campteneura* bark was evaluated on the cotton pellet induced granuloma model (**Mossa et al, 1995**). 5 groups of 5 rats were used. Animals were treated as follow: group negative control, treated with distilled water at the dose of 0.5 ml/100g; group positive control treated with Diclofenac as reference drug at the dose of 5 mg/kg; three groups tests treated with aqueous extract of *S. campteneura* at the dose of 300, 600 and 1200 mg/kg respectively. 100 mg of cotton pellets were sterilized at 60˚C during 24 h and placed in the right interscapular region of animal after ether anesthesia and incision. The incision was closed by suture. Animals were then treated with drugs for seven (7) days. On the eighth day the granulation tissue with cotton pellet was removed, cleared of adhering tissue and placed at 60˚C for 24 h and weighed. The anti-inflammatory effect was given by the percentage inhibition (PI) of the granuloma:

$$PI = [(PG_{control} - PG_{animal})/PG_{control}] x\ 100$$

$$PG = B - A$$

PG: weight of the granuloma, *A* = weight of cotton pellet before implantation (100 mg);
B = weight of dried cotton pellet after implantation.

2.7 Statistical analysis

The results were expressed in terms of mean ± ESM. Analysis of variance followed by Student-Ficher *t*-test was performed. The significance level was set at $p < 0.05$.

3. RESULTS

3.1 Antimicrobial activity

Table 1 summarized the results of antimicrobial activity of the aqueous extract of *S. camptoneura*. It comes out that ofloxacin and Ceftriaxon inhibit the growth of *Escherichia coli*, *Enterobacter sp*, *Staphylococcus aureus* and no the one of *Streptococcus sp*, *Streptococcus β hemolytic* and *Candida albicans*. Distilled water remains without effect on all studied strains. Aqueous extract of *S. camptoneura*, inhibit in vitro growth of all stocks on the other hand according to the concentration and the type of germ. *Streptococcus sp* and *Candida albicans* were the most sensitive germs with the IMC of 5 mg/ml followed by *Staphylococcus aureus* with 6.25 mg/ml whereas *Escherichia coli*, *Enterobacter sp*, and *Streptococcus β hemolytic* 12.5 mg/ml. The extract is bactericidal at 50 mg/ml. The reports (IMC/BMC) of the inhibition parameters (IMC) and of bactericidal (BMC) are lower to 1 for

all studied stocks, either 0.25 for *Escherichia coli*, *Enterobacter sp*, *Staphylococcus aureus* and *Streptococcus β hémolytique* or 0.10 for *Streptococcus sp* and *Candida albicans*.

Table 1: Effect of aqueous extract of *S. camptoneura* at different concentrations on the different studied stocks

Products	Diameters (mm)					
	E. coli	***Ent. sp***	***S. aureus***	***Strep. sp***	***S.β hémo.***	***C. albicans***
Oflo. 5µg	22.86±0.28	14.66±0.57	17.33±1.15	0	0	0
Cef. 30µg	20.33±0.57	19.33±0.57	15.00±0.00	0	0	0
Dist. water	0	0	0	0	0	0
S. c (mg/ml)	-	-	-	-	-	-
50	21.66±1.52	19.00±0.00	19.00±0.00	20.00±0.00	17.00±0.00	19.32±0.67
25	14.50±0.50	12.01±0.23	13.50±0.57	16.10±0.23	12.00±0.00	17.00±0.00
12,5	10.00±0.00	09.50±0.45	11.80±1.04	13.20±0.35	10.10±0.11	14.45±0.45
6,25	08.00±0.00	08.00±0.00	09.50±0.50	10.67±0.75	08.03±0.05	11.00±0.00
5	05.13±0.15	05.00±0.00	05.50±0.50	09.10±0.21	05.50±0.57	09.50±0.57

Oflo: ofloxacin ; Cef : Ceftriaxon ; dist. water: distilled water ; *S. c*: *Strychnos camptoneura*

Table 2: Inhibition Grow Diameters (IGD), Inhibitory Minimal Concentration (IMC), Bactericidal Minimal Concentration (BMC), reports MIC/MBC of extract.

	DIC	IMC	BMC	IMC/BMC	Strains
S. camptoneura	21.66	12.5	50	0.25	*E. coli*
	19.00	12.5	50	0.25	*Ent. sp*
	19.00	12.5	50	0.25	*S. aureus*
	20.00	5	50	0.10	*Strep. sp*
	20.00	12.5	50	0.25	*S.β hémo*
	21.32	5	50	0.10	*C. albicans*

E. coli : Escherichia coli; *Ent. sp : Enterobacter sp; S. aureus : Staphylococcus aureus; Strep. sp: Streptococcus sp; S.β hémo: Streptococcus β hémolytique; C. albicans: Candida albicans.*

3.2 Anti-inflammation activity

3.2.1 Acute inflammation induced by carrageenan.

Figure 1 and table 3 summarized the results of the anti-inflammatory activity of the aqueous extract of *S. campteneura*. At the doses of 300, 600 and 1200 mg/kg the aqueous extract and diclofenac inhibit very significantly (***p<0.001) the volume of œdema induced by carrageenan comparatively to the control. The inhibition is marked since the first half-hour (**p <0.01) after injection of carrageenan until the sixth hour or it reaches maximal values, and is translated by important values of percentage that are dose depending (table 3).

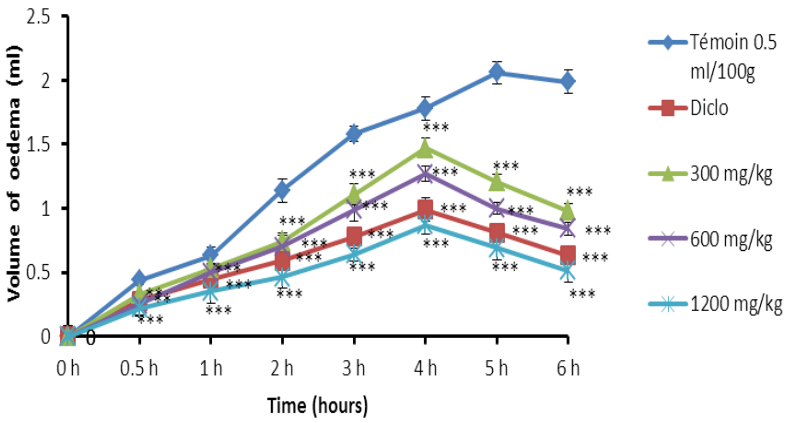

Figure 1: Effect of aqueous extract of *S. campteneura* on inflammation induced by 1 % carrageenan in Wistar rat. Each value represents the mean ± ESM, versus control group, with n = 5

Table 3: Effect of diclofenac and aqueous extract on percentages (%) of inhibition of the volume of oedema

Time (hours)	Diclo (5mg/kg)	S. Campteneura (mg/kg)		
		300	600	1200
0.5	34.36	25.81	33.63	37.36
1	38.57	35.08	38.59	40.62
2	48.24	41.74	45.95	51.35
3	52.63	49.08	51.58	56.57
4	56.38	51.74	55.10	60.71
5	60.67	58.26	60.10	66.28
6	68.34	60.75	68.01	70.01

3.2.2 Chronic inflammation induced by cotton pellet

Table 4 showed the results of chronic inflammation. It indicates that the aqueous extract of *S. camptoneura* at the doses of 300, 600 and 1200 mg/kg inhibits very significantly (***p <0,001) the formation of granuloma with percentages of 22.83 ± 2.96; 42.47 ± 1.71 and 49.54 ± 0.23 respectively against 28.29 ± 0.27 for diclofenac.

Table 4: Effect of aqueous extract of *S. campteneura* on the evolution of the weight of granuloma and percentages of inhibition of inflammation to Wistar rat

Products ((mg/kg)		Weight of granuloma (g)	Inhibition (%)
control	5ml/kg	0.121 ± 0.003	-
Diclofénac	5	0.071 ± 0.003***	28.29 ± 0.27
S. camptoneura	300	0.090 ± 0.020***	22.83 ± 2.96
	600	0.069 ± 0.002***	42.47 ± 1.71
	1200	0.053 ± 0.002***	49.54 ± 0.23

Each value represents the mean ± ESM, versus control group, *** $p < 0.001$ (n = 5).

4. DISCUSSION

The present work was initiated to evaluate antimicrobial and anti-inflammatory activities of the aqueous extract of the stem bark of *S. campteneura*. It appears that, the aqueous extract of *S. campteneura* has a significant inhibitory effect on the growth of strains compared to the antibiotics or, some strains are resistant. Indeed, with the aqueous extract, *Streptococcus sp* and *Candida albicans* are more sensitive with a IMC of 5 mg/ml follow by *Staphylococcus aureus* with 6,25 mg/ml whereas *Escherichia coli*, *Enterobacter sp* and *Streptococcus β hemolytic* is least sensitive with a IMC of 12,5 mg/ml. The antimicrobial power and the tests of sensitivity achieved show that the aqueous extract of *S. camptoneura* is more active on the studied stocks than the reference antibiotics while comparing the diameters of inhibition and the specter of action. Otherwise, does one notice that the extract is more active on the Gram+ that on the Gram- bacteria outside of *Streptococcus β hemolytic* whose resistance is recognized in surroundings hospitable. It, probably explain himself by the presence in the Gram- bacteria of an external layer in relation to the Gram+ bacteria. Indeed, the Gram- bacteria possess an additional layer to the external membrane, composed by phospholipids, proteins and lipopolysaccharides, forming an impervious gate to most hydrophobic molecules (**Georgantelis et al, 2007**). Besides several authors demonstrated the big sensitivity of the Gram+ bacteria in relation to the Gram- bacreria (**Hayouni et al, 2007; Shan et al, 2007**). Thus, the determination of the inhibition parameters (IMC) and bactericidal (BMC) permitted us not only to confirm, to quantify and to compare the effects, but also to characterize the nature of the effect revealed by the extract on the tested stocks. The reports of the parameters (IMC/BMC) of inhibition (IMC) and bactericidal (BMC) are lower to 1 for all studied stumps, what confirms the bactericidal effect of the aqueous extract of *S. camptoneura*.

Microbial infections are often responsible of inflammatory reactions that drag other serious pathologies. Indeed, the multiplication of the microorganisms in the organism provokes the liberation of pro-inflammatory substances as histamin, bradykinin and prostaglandins that provoke inflammation, pain and fever in their turn (**Cilag et al, 2002; Larraud, 2003; Korganov, 2002; Rousselet et al, 2005**). It is why, the anti-inflammatory power of the aqueous extract preceded of a sharp toxicity study (non presented results) among the mice has been achieved (**OCDE/OECD, 2001**). This study has permitted to choose the dose of 300 mg/kg that we doubled to 600 mg/kg and 1200 mg/kg to search for the effect. The result shows that at 300, 600 and 1200 mg/kg aqueous extract of *S. camptoneura* inhibits inflammation induced by carrageenan. The inhibition is observed from the first half-hour

until the sixth hour. This extract also inhibits the formation of the granuloma induced by pellet of cotton with percentages of 22.83 ± 2.96; 42.47 ± 1.71 and 49.54 ± 0.23 respectively against 28.29 ± 0.27 for diclofenac (5mg/kg). At 1200 mg/kg of extract, the effect is more important than those observed at the doses of 300 and 600 mg/kg and, with diclofenac. Carrageenan is a mucosaccharide which provokes a sharp inflammation leading a œdema under the influence of vasoactives mediators (**Laurent et al, 1987**). The granuloma induced by pellet of cotton is a chronic inflammation model. It clearly appears that the aqueous extract of the stems bark of *S. camptoneura* inhibits the formation of œdema and granuloma measures out dependent. This important effect suggests that the extract could have an antagonistic action against histamin, bradykinin, and serotonin and against prostaglandins biosynthesis.

CONCLUSION

The results of the present study showed that aqueous extract of the stems bark of *S. camptoneura* have a large specter antimicrobial activity comparatively to the used antibiotics. It possesses an important anti-inflammatory effect. These results could explain the important traditional use of this species in part and, suggest the presence in this plant of the active principles responsible of the effects observed.

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