

Assessment of Sexual Stimulant Potential of Total Flavonoids Extracted from Leaves of *Palisota Hirsuta* Thunb. K. Schum (Commelinaceae)

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Abstract

Total flavonoids extracted from *Palisota hirsuta* leaves modify the sexual parameters such as the latent time of observation (LTO) and the number of tentatives of intromission (NTI) (vaginal penetration) among the male rats. Indeed, administration of unique dose (27.59 mg Kg⁻¹ of body weight (b. w.)) of flavonoids to male rats leads to a reduction of LTO and an increase of NTI in the time, this reveals the sexual stimulation. The present survey not only makes us understand but justifies the use of *Palisota hirsuta* leaves also as an aphrodisiac in traditional herbal medicine in Côte d'Ivoire.

Keywords: Sexual stimulation, aphrodisiac, *Palisota hirsuta*, traditional herbal medicine, Côte d'Ivoire.

Introduction

The erectile dysfunction is a problem of public health in our societies even though it remains taboo. This pathology is preoccupying because according to Hauri (2002), the erection symbolizes strength and great sexual male vigor.

From 1998, the event of some molecules of synthesis (sildenafil, tadalafil, vardenafil) brought a faint of hope not only in medicine but it also revived too much curiosity and interest for the medications which increase the erectile activity (Opsomer and Tombal, 2005). We must remember that these molecules don't induce a sexual desire.

The dearness of synthesis medicines and the undesirable effects that they can create a deep interest in the use of the plants in the therapy of the erectile dysfunction. Various plants have the reputation of be an aphrodisiac, which means that they are known to create and to stimulate sexual desire (Carpentier *et al.*, 2004). These plants contain secondary metabolites of which the flavonoids, occupy a place of choice in modern medicine in the treatment of the aforesaid pathology (Drewes *et al.*, 2002).

This study reports on the assessment of sexual stimulant properties among the male rats due to total flavonoids that we extracted from *Palisota hirsuta* leaves, a Commelinaceae spilled in Côte d'Ivoire and used extensively in traditional treatment of erectile dysfunction. (Brandao *et al.*, 1997).

Materials and Methods

Animals

Adult healthy Wistar rats (males and females, *Rattus norvegicus*, 200-300 g, b. w.) were used. They were fed with granules (5mm of diameter and 10 mm of length) to basis of cereals and received drinking water in beverage. They were maintained under standard laboratory conditions, (temperature $23 \pm 2^{\circ}\text{C}$).

Plant material and extraction of flavonoids

Palisota hirsuta leaves were collected in the forest of the University of Abobo-Adjamé (Abidjan / Côte d'Ivoire), identified and authenticated by Prof. L. AKE ASSI of the Centre National Floristique of the University of Abidjan-Cocody (Côte d'Ivoire). They were dried and pulverized to obtain a powder.

500 g of powder were treated previously with hexane and macerated in 2000 mL of absolute MeOH for 48 h. After filtration, the solvent was evaporated under reduced pressure in a rotary evaporator (temperature not exceeding 45°C) to give a residual to which was added hot water (400 mL). After some hours of agitation, the mixture was filtered. The filtrate underwent some successive extractions liquid-liquid with CHCl_3 , MeCOOEt and n-BuOH. The n-BuOH extract was dried over anhydrous Na_2SO_4 , filtered and evaporated to get a residual (Rn). Rn was kept to $+5^{\circ}\text{C}$. The phytochemical screening according to Békro *et al.*, (2007) and the thin layer chromatography (TLC) on Rn were achieved to confirm its contains only flavonoids.

Mounting behavior

In this experimentation, we used Rn. It uses non receptive females. Among the rats, the sexuality is governed by the female depending on whether it is receptive or not. With receptive females no male in the normal conditions can succeed an intromission. This experimentation permitted to qualify the sexual stimulation of the male (here the male is not stimulated by the vaginal broadcasts of the female) its insistence to pair off with the female against its will.

Three groups of five males were used. The first group (served as control) received by stuffing 1 mL of drinking water. The two other groups received respectively by stuffing, the unique doses of sildenafil (0.65 mg Kg⁻¹ of b. w.) and of Rn (27.59 mg Kg⁻¹ of b. w.). Then, we observed the sexual behavior of every rat lodged individually in a transparent cage, opposite a non receptive female during 3 h. After 15 min of acclimatization, a non receptive female is introduced in the cage. At the end of 15 min of observation, the NTI and the LTO are recorded. The female was separated a first time from the

male, was introduced then again in the cage 1 h after. After the second observation (15 min), the female was separated from the male during 2 h 45 min and introduced again (Lawler, 1984).

Statistical analysis

The values are expressed as average more the Standard mistake on the average ($m \pm S. E. M$). The statistical analysis of the data and the graphic representations was achieved respectively with GraphPad Instats and GraphPad Prism 4 (Microsoft, San Diego California, USA).

The results of the data between more than two experimental groups are analyzed by ANOVA. The meaningful differences are submitted to a post-hoc analysis with the test of Sheff. A value of $p < 0,05$ was considered like statistically meaningful.

Results and Discussion

Chemical study

n-Butanolic extract was obtained with 1.26% of yield. It served for the phytochemical screening, the TLC and for the assessment of the sexual stimulation potential. The phytochemical screening achieved on Rn showed that it contains some flavonoids solely.

Rn was Chromatographed on an analytical silicagel 60 F₂₅₄ coated TLC plates and developed in n-butanol / ethylacetate / water (4: 1: 5; V / V / V). The chromatogram put in evidence of green fluorescent stains under an UV lamp to 366 nm with very distinct Rf. Under UV observation to 254 nm, the chromatogram revealed purple and brown stains clear. The green fluorescent stains would correspond to Flavonols, flavonones, and to auronones. Our results correspond to those obtained by Markham (1982) and Mohammadi (2006) which showed that the flavonols, the flavonones and the auronones appear as green fluorescent stains under UV 366 nm.

The administration of 27.59 mg Kg⁻¹ of b. w. Rn modified the two sexual parameters compared to the control rats crammed to the drinking water (Tableau 1). Indeed, we measured two parameters, LTO and NTI. With regard to LTO, to the first observation 15 min after the treatment of the males, the middle time of reaction of these at the time of their first contact with the non receptive female is of 180±20 s against 420.5±15 s for the control rats. At the second observation, 1 h after the treatment, LTO is of 50±10 s against 688.2±16 s for the control rats. At the third observation at 2 h 45 min, LTO occurred to 25±8 s against 830.6±10 s for the control rats (Fig. 1). We noticed in the time, a reduction of the LTO of the male rats treated with Rn. As for the NTI revealing the sexual stimulation of the males, we made three observations whose results are consigned in the Table 1. To the first observation, we noted an average of 35.8±6 of tentative of intromission of the non receptive female rats by the male rats treated with Rn against an average of 6.8±2 for the control rats. The second observation was made 1 h after during which an average of 50±10 against another of 2.3±1 was recorded. Finally, the third observation indicated an average of 60.3±7 tentative of intromission against an average of 4.4±1. From these observations, a report clears out. Indeed, the number of tentative of intromission increases considerably in the time among the rats treated with Rn. during the three observations, no meaningful difference was observed between the rats of the cage C and those of the cage A (fig. 1, 2).

Table 1: Summary of the sexual parameters of the rats

Animals	Sexual parameters	Middle Observations		
		15 min	1 h	2 h 45 min
Males and non receptive females				
Cage A	LTO	420.5±15	688.2±16	830.6±10
Control rats	NTI	6.8±2	2.3±1	4.4±1
Cage B	LTO	417.5±16	690.1±10	828.2±5
Rats crammed Sildenafil (0.65 ±0.5 mg Kg ⁻¹ of b. w.)	NTI	5.9±0.5	3.2±1	5.0±1
Cage C	LTO	180±20	50±10	25±8
Rats crammed Rn (27.59 ±3 mgKg ⁻¹ of b. w.)	NTI	35.8±6	53.1±4	60.3±7

Figure 1: Latent time of observation (LTO) of the control male rats, the rats treated with Rn (27.59 mg Kg⁻¹ of b. w.) and with Sildenafil (0.65 mg Kg⁻¹ of b. w.). LTO of the rats treated with Rn lowers considerably in the time. As for the rats treated with Sildenafil and to the control rats LTO increases.

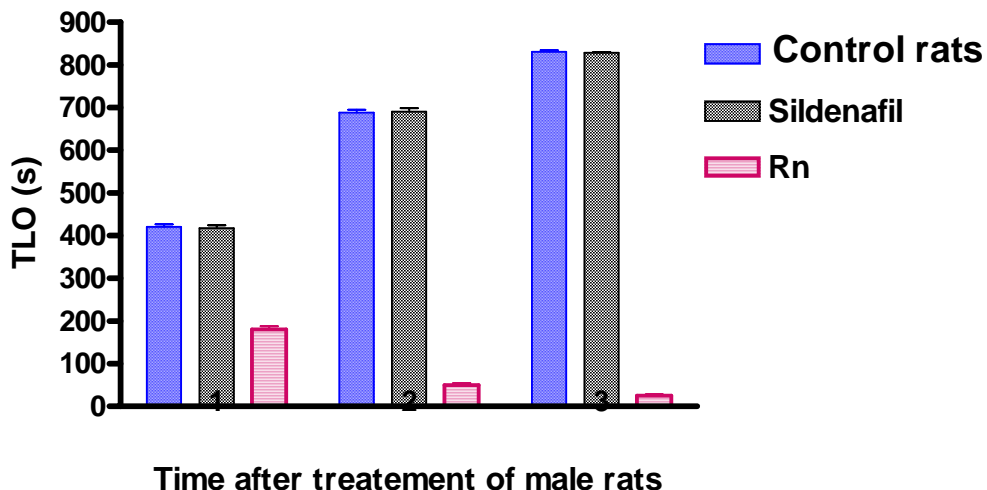
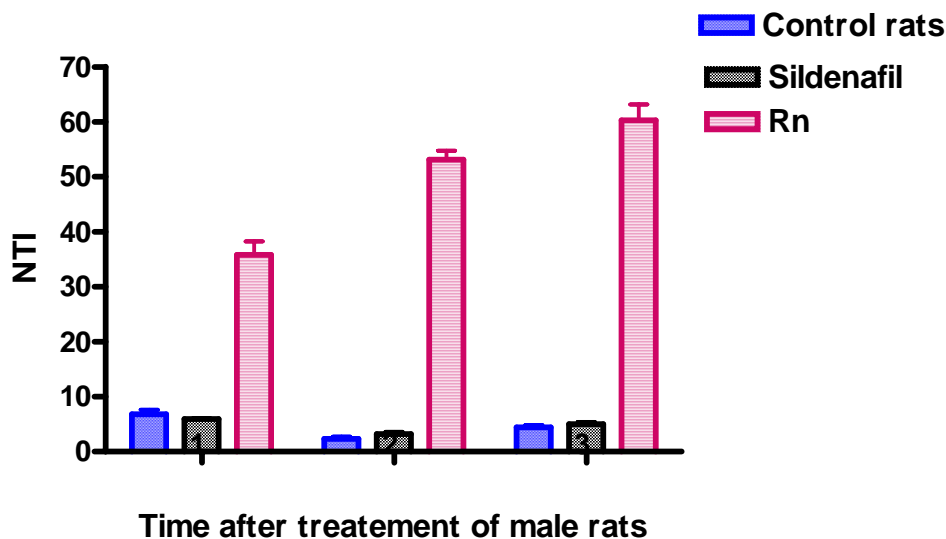


Figure 2: Number of tentative of intromission (NTI) of the control male rats and those treated with Rn (27.59 mg Kg⁻¹ of b. w.) and the Sildenafil (0.65 mg kg⁻¹ of b. w.) observed to 15 min, 1 h and 2 h 45 min. The number of tentatives of the rats treated with Rn increases in relation to the control rats in the time. As for the rats treated with Sildenafil, NTI doesn't vary in relation to the control rats.



This investigating permitted to value the potential of sexual stimulation of Rn. Indeed, the meaningful reduction of LTO and the increase of NTI of the male rats having received 27.59 mg kg⁻¹ of b. w. of Rn, bring evidence the real aphrodisiac properties (increase of the libido) of the total flavonoids that Rn contains because the visual and olfactory stimuli are lessened among the non receptive females. Our results agree with those obtained by Zamble (2006). Indeed, his works on plants (*Mezoneuron benthanianum*, *Microdesmis keayana*) used in Côte d'Ivoire to treat the erectile

dysfunction, showed that their aqueous extracts possess aphrodisiac properties (sexual stimulation and sexual performance).

The insistence of the male rats to want to mate with the non receptive females reveals an increased central stimulation of the sexual parameters. Indeed, the desire and the different stimuli are centralized, treated and integrated to the level of the central nervous system and they succeed, in the normal physiological conditions, in the erection. Several central neurotransmitters were identified and would play a primordial role as well in the central regulation of the sexual parameters among the animal that at the man. We can mention as central mediators γ -aminobutanoic acid (GABA), dopamine and oxide of nitrogen synthase (Bjorklund *et al.*, 1975; Pehek *et al.*, 1989; Melis *et al.*, 1989; Melis *et al.*, 2000; Anderson, 2001).

GABA inhibits the somatic and autonomous reflexes. The fibers and the gabaergic receptors were localized in the sacred parasympathic nucleus and in the bulbocavernous motor. The injection of gabaergic antagonists in the median preoptic area (APOM) creates an increase of the libido (Melis *et al.*, 2000). In the same way, the receptors D2 of dopamine are responsible for the increase of the libido and the seminal emissions (Pehek *et al.*, 1989).

Rn being an extract of total flavonoids, these could act on the pharmacological parameters integrating the synthesis or the action of the above stated neuromediators. This proved aphrodisiac property justifies the use of the leaves of *Palisota hirsuta* in African traditional medicine in the treatment of the erectile disease since Rn is derived from the methanolic extract of the leaves of this plant. The presence of these phytoactive molecules may perhaps account the pharmacological effects demonstrated by *Palisota hirsuta*.

Otherwise the unique dose of 0.65 mg Kg^{-1} of b. w. of Sildenafil administrated to a lot of 6 male rats (fig. 1, 2) didn't have an effect on the two studied sexual parameters (LTO and NTI) compared to the control rats. It confirms the mode of action of Sildenafil. Indeed, Sildenafil is not a sexual stimulant. It is a selective agonist of phosphodiesterase V. It acts in the peripheral regulation while reinforcing the normal mechanisms of the erection (Anonymous, 1997; Karydis *et al.*, 2001; Hanoune, 2005).

In conclusion, the results of this study showed that the leaves of *Palisota hirsuta* possess aphrodisiac property.

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