

Research Article**Estrogenic activity of the aqueous extract of the roots of *Thonningia sanguinea* Valh (Balanophoraceae) in the wistar rat****Okassa Poma Chancelia Inès¹, Peneme Bonaventure Max Lazare^{1,2}, Wossolo Lingomo Berthrand Stephane^{1,2}, Bafounguila-Ngala Syntyche Maïna¹, Diamesso-Zola Kwa Nzambi Seigneur Gens¹, Etou Ossibi Arnaud Wilfrid^{1,2*}**¹Laboratory of Pharmacodynamics and Experimental Pathophysiology, Marien NGOUABI University²Laboratory of Biochemistry and Pharmacology, Faculty of Sciences and Health, Marien NGOUABI University

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Abstract

The objective of this work was to evaluate the estrogenic effect of the aqueous extract of the roots of *Thonningia sanguinea* in female wistar rats. The acute toxicity study was carried out according to OECD, 2022. Two batches of three mice each received respectively distilled water and the aqueous extract of the roots and *Thonningia sanguinea* at a single dose of 5000 mg/kg, then weight monitoring as well as food consumption and water intake were noted for duration of fourteen (14) days. We see that this extract is well tolerated in mice with an LD50 > 5000 mg/kg. To investigate the estrogenic effects of this plant, the animals were treated for 28 days with distilled water and the aqueous extract of the roots of *Thonningia sanguinea* at doses of 200 and 400 mg/kg. At a dose of 200 mg/kg, this extract causes an increase in the weight of the uterus, ovaries and adrenal glands, as well as an increase in estrogen and progesterone levels. In conclusion, chemical screening revealed the presence of tannins, alkaloids, flavonoids, free anthraquinones and saponosides. The estrogenic properties observed are due to the presence of these chemical families found in this extract.

Keywords: Estrogenic activity, *Thonningia sanguinea*, acute toxicity, anthraquinones, saponosides

Introduction

Infertility is defined as the absence of conception for at least one year in a couple desiring pregnancy and having regular sexual intercourse without the use of contraceptives (Khodja and Khodja, 2020). It is nowadays a real public health problem which concerns 60 to 80 million men and women (Azonbakin et al. 2021), because it is perceived as a negative social marker, even humiliating and stigmatizing for couples and for women in particular (Brochard, 2014), it is a source of marital frustration, violence, divorce or polygamy (Diame, 2010, Koman et al., 2019). It affects 60 to 80 million men and women (Azonbakin et al, 2021), or 15% of the world population according to the WHO, 186 million couples are affected in developing countries (WHO, 2000; Mbow and Diop, 2019). In women, infertility can be caused by age, polycystic ovary syndrome, endometriosis,

ovarian insufficiency, uterine anomalies (Nouhaud, 2015; Tolenado, 2019; Peyronel, 2021). Several treatments can overcome this problem such as: Intra Uterine insemination, In vitro fertilization, In vitro fertilization with microinjection (Thibault, 2012). Furthermore, these treatments offered by modern medicine are very expensive, so infertile people, especially those from poor areas of sub-Saharan Africa who represent the most affected sectors, are therefore unable to afford such treatments and the use of Medicinal plants become one of the alternatives to solve this problem because this technique is less expensive and accessible to all social levels (Assouma et al, 2018). This is why women have always used medicinal plants to resolve certain health problems (Dibong, 2015; Houmènou, 2018). It is in the context of promoting traditional medicine that *Thonningia sanguinea* was chosen with the aim of evaluating the estrogenic effects in rats, a plant used in traditional Congolese medicine for its numerous therapeutic virtues.

Material and Methods**Plant material**

The roots of *Thonningia sanguinea* were collected in the

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West Cuvette, precisely in Etoumbi (677.7km) of Brazzaville and identified, compared to a reference sample in the national herbarium of the national institute for research in exact sciences and natural.

Animal material:

Swiss mice and female Wistar rats respectively weighing between 20-25g and 150-160g were used for the study of acute toxicity and estrogenic tests. All these animals were provided by the animal facility of the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE) of the Faculty of Sciences and Technology of Marien NGOUABI University.

Preparation of the aqueous extract of *Thonningia sanguinea*

The roots of *Thonningia sanguinea* were prepared by 10% decoction. 100 g of the powder was dissolved in 1 L of distilled water and the solution brought to a boil for 30 minutes. After filtration with hydrophilic cotton, the decoction obtained was concentrated in an oven for 48 hours at 70°C and the dry extract obtained was preserved for experimentation.

Evolution of acute toxicity of the aqueous extract of *Thonningia sanguinea*

Acute toxicity was assessed according to OECD 2022 guideline no. 425. To do this, two batches of 3 mice each were formed and treated as follows:

- Batch 1 received distilled water at 0.5 mL/100 g
- Batch 2 received the aqueous extract at a single dose of 5000 mg/kg

Observations focused on behavior and general condition (mobility, stool status, tremor, sensitivity to pain, sleep, drowsiness, vocalization, vomiting). Mice mortality after 48 hours and food consumption as well as water intake were noted for 14 days.

Evaluation of estrogenic effects

An estrogenic product stimulates endometrial proliferation and vascularization. It improves vaginal comfort by preventing vaginal dryness and allowing good vaginal trophicity. During the menstrual cycle, during the ovulatory period, it ensures the secretion and good quality of cervical mucus, which allows the sperm to reach the egg to fertilize it. During pregnancy, it works hand in hand with progesterone to ensure the baby's proper development. Any plant that acts like estrogen would therefore have estrogenic effects.

The estrogenic effects were evaluated according to the method of Coulibaly et al. (2022). The main parameters sought concerned changes in the weight of the uterus, ovaries and adrenal glands as well as the weight evolution of the animals. Thus, 15 female rats were divided into 3 groups of 5 animals and

treated for 28 days as follows:

- batch 1: Distilled water at 0.5 mL/100 g;
- batch 2: Aqueous extract at 200 mg/kg bw;
- batch 3: Aqueous extract at 400 mg/kg bw

At the end of the treatment, each animal anesthetized with diethyl ether is sacrificed by decapitation then the arteriovenous blood is collected and some organs removed.

Evaluation of the effects of the aqueous extract of *Thonningia sanguinea* on the fresh and dry weight of organs

In each sacrificed animal, after dissection at the abdomen, the uterus, ovaries and adrenal glands were removed and gently removed from the surrounding adipose tissue. These freshly removed organs were weighed and the fresh weights noted. Then, they were placed in an oven at 50 °C and weighed until the unchanged dry weights were recorded.

Evaluation of the effect of the aqueous extract of *Thonningia sanguinea* on the concentration of reproductive hormones

The collected blood was centrifuged at 4000 rpm for 15 minutes. The serum obtained on the surface was collected and stored in the freezer at -4°C for the determination of estrogens and progesterone using the FINECARE technique.

Phytochemical screening

The characterization tests are based in part on qualitative analysis, either on the formation of insoluble complexes using precipitation reactions, or on the formation of colored complexes, using specific coloring reactions Souley Kallo et al. (2018); Hamid et al. (2018). Seven (7) chemical families (alkaloids, flavonoids, mucilages, oses and holosides, saponosides, tannins and free anthraquinones) were searched using classic phytochemistry methods (Elion Itou et al., 2023; Tony et al., 2023).

Results

Acute toxicity

Effects of the aqueous extract of *Thonningia sanguinea* roots on the behavior and general condition of mice

The results of the effects of the aqueous extract of the roots of *Thonningia sanguinea* on the behavior and general condition of the mice are recorded in table I. It appears that, with the exception of the reduction in mobility, this extract does not modify the behavior of mice, 4 hours after its administration. No cases of mortality were recorded up to 48 hours after administration of the products, therefore a mortality rate of 0 %. The same result is obtained until the fourteenth day of the experiment.

Table 1: General condition of the mice after administration of the products

Settings Parameters	Treatments	
	ED (0,5ml/kg)	<i>T. sanguinea</i> (5000 mg/kg)
Number of animals	3	3
Mobility	N	N
Aggressiveness	HAS	HAS
Stool condition	C	C
Tremors	HAS	HAS
Sleep	HAS	HAS
Drowsiness	HAS	HAS
Vocalization	HAS	HAS
Vigilance	N	N
Vomiting	HAS	HAS
Ptosis	HAS	HAS
Fatigue	P	P
Reactions to external stimuli	N	N
Death toll	0	0

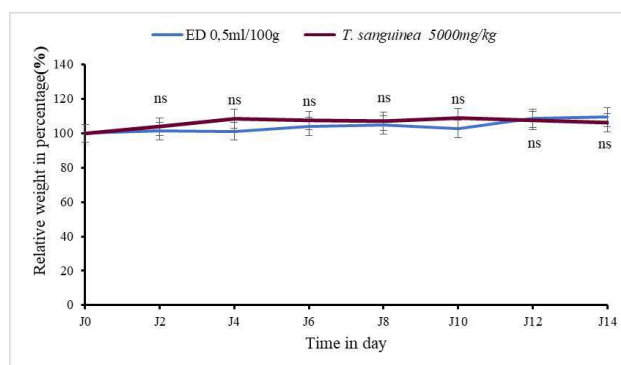
ED: Distilled water; A: Absence, C: Compact; N: Normal and P: Presence

Effects of the aqueous extract of *Thonningia sanguinea* roots on the weight evolution of mice

Figure 1 shows the effects of the aqueous extract of the roots of *Thonningia sanguinea* on the weight development of mice in mice. There was a non-significant weight variation from the start to the end of the experiment in the mice having received the aqueous extract of *Thonningia sanguinea* at 5000 mg/kg compared to the distilled water control mice 0.5 mL/100 g.

Estrogenic activity of the aqueous extract of the roots of *T. sanguinea* in rats

Table II presents the effect of the aqueous extract of the roots of *T. sanguinea* on the fresh and dry weights as well as the water contents of the reproductive organs. It appears from this table that at a dose of 200 mg/kg, this extract causes a significant increase in the organs and the water content and when the dose is doubled, we observe a reduction in the weight of these organs and the water contents per compared to animals from the control group (distilled water).

**Figure 1:** Effect of aqueous extract of *T. sanguinea* roots on the weight evolution of mice

Effects of the aqueous extract of *Thonningia sanguinea* roots on the level of reproductive hormones

Estrogen levels

Figure 2 shows the serum estradiol level under the effect of the aqueous extract of *Thonningia sanguinea* roots. It appears from this figure that this extract causes a significant increase ($p < 0.001$) in estradiol at a dose of 200 mg/kg, i.e. 160.86 pg/mL, and a decrease ($p < 0.001$) at a dose of 400 mg/kg. kg or 149.83 pg/mL compared to control animals having received distilled water (153.47 pg/mL).

Progesterone levels

Figure 3 shows the serum progesterone concentration in rats treated with aqueous extract of *Thonningia sanguinea*. This shows a very significant increase and decrease in the concentration of progesterone in the rats treated with the aqueous extract respectively at doses of 200 and 400 mg/kg compared to the controls receiving distilled water (0.5 mL/100g).

Chemical screening

Table III shows the different chemical families sought in the aqueous extract of the roots of *Thonningia sanguinea*

Table 2: Effect of the aqueous extract of *T. sanguinea* on the fresh and dry weights of the reproductive organs

Doses (mg/kg)	Adrenal glands (mg/100g)		Report (Pf/Ps)	Uterus (mg/100g)		Report (Pf/ps)	Ovaries (mg/100g)		Report (Pf/Ps)
	Fresh weight	Dry weight		Fresh weight	Dry weight		Fresh weight	Dry Weight	
0.5mL/100g	20.50±1.11	7.17 ±0.25	2,85	377.62± 1.36	71.22±0.56	5,30	33.54±0.53	6.51±0.03	5,15
Ts 200	39.32±2.21***	9.07± 0.16***	4,33	429.72± 0.55***	73.43±1.55 ^{ns}	5,85	47.81± 2.90***	6.57±0.004 ^{ns}	7,27
Ts 400	37.08±3.18***	7.44±0.008***	4,98	239.57± 4.51***	51.14±2.32**	4,68	29.60± 0.67***	6.51±0.004 ^{ns}	4,54

Ts: *Thonningia sanguinea*; ns: not significant; * $p < 0.05$: significant difference; ** $p < 0.01$: very significant difference and *** $p < 0.001$: highly significant difference

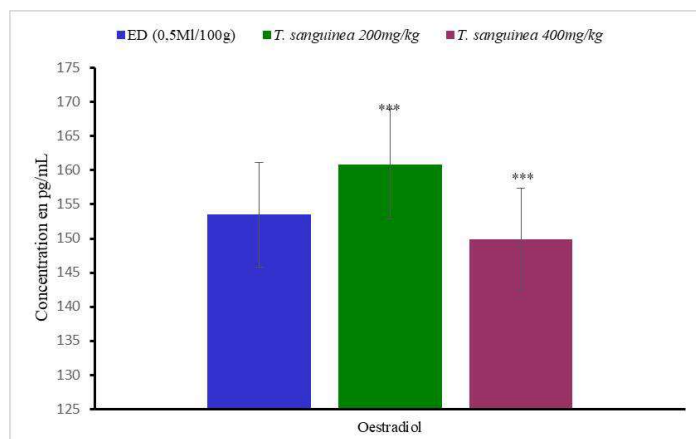


Figure 2: Effects of the aqueous extract of the roots of *T. sanguinea* on the serum level of estradiol in rats. The values are the means \pm SEM with $n=5$, *** $p<0.001$: very significant difference compared to the controls

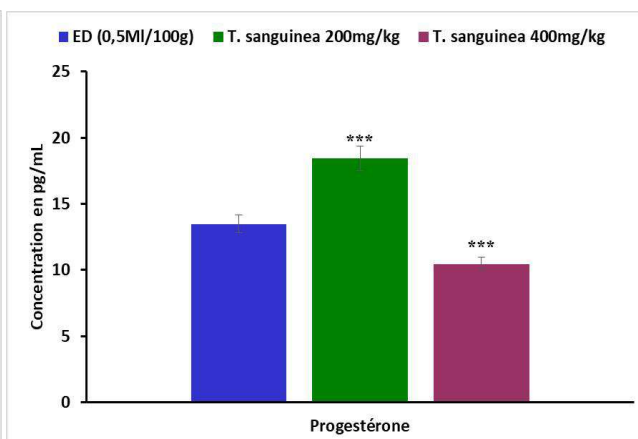


Figure 3: Effects of the aqueous extract of the roots of *T. sanguinea* on the serum level of progesterone in rats. The values are the means \pm SEM with $n=5$, *** $p<0.001$: very significant difference compared to the controls

Table 3: Results of chemical screening of aqueous extract of *Thonningia sanguinea* roots

Chemical families sought	Results
Tannins	+++
Alkaloids	+++
Flavonoids	++
Dare	+
Mucilages	-
Free anthraquinones	+++
Saponins	++

+++ : Very strong presence; ++ : Strong presence; + : Weak presence; - : Absence

revealed the presence of several chemical families such as tannins, alkaloids, flavonoids, oses, free anthraquinones and saponosides.

Discussion

The general objective of this work was to evaluate the estrogenic activity of the aqueous extract of the roots of *Thonningia sanguinea* in rats, particularly on the fresh and dry weight of the reproductive organs (uterus, ovaries and adrenal glands) as well as the hormonal dosage (estrogen and progesterone). The study of acute toxicity showed that with the exception of the reduction in mobility, this extract does not show signs of toxicity and does not modify the general behavior and does not cause the death of the mice; it is therefore well tolerated by mice up to a dose of 5000 mg/kg with an LD₅₀ > 5000 mg/kg. Similar results were obtained with certain plants such as *Tetracera potatoria* (Mikolo et al., 2020); Nature remedy (Manda et al., 2017). Oral administration in rats after 28 days of experimentation showed the very significant increase and decrease in the weights of the

uterus, ovaries and adrenal glands in the animals having received the aqueous extract of *Thonningia sanguinea* respectively at doses of 200 and 400 mg/kg, an increase which would be due to the presence in this extract of bioactive substances with estrogenic activity (Rimoldi et al, 2007). Indeed, some authors have proven that the administration of estrogens or estrogens like induces an increase in weight and water imbibition (Pinilla et al, 2001; Shivalingappa, et al., 2002) of the uterus, estrogen stimulates the proliferation of uterine endometrial cells (Kouakou, 2000; Clarke et al., 2003). Similar results were obtained by Eyi Mitsa et al. (2021) as well as Coulibaly et al., (2022). The decrease in organ weight at 400 mg/kg could be explained by the high dose. The aqueous extract of *T. sanguinea* induces an increase and a decrease in estrogens and progesterone at doses of 200 and 400 mg/kg, respectively. At a dose of 200 mg/kg, the increase in these two hormones could be explained by the fact that this extract acts at the level of the hypothalamus which

stimulates the pituitary gland and in turn secretes FSH and LH to stimulate the production of large quantities of estrogen and progesterone. Indeed, estrogens induce the secretion of large quantities of stringy cervical mucus which promotes the passage of spermatozoa to fertilize the egg (Vandenheede, 2024) and it is known that estrogens contribute to the release of GnRH and pituitary hormones by receptors located in the hypothalamus and pituitary gland of rats (Shupnik and Rosenzweig, 1991; Coulibaly et al., 2022). This explains the increase in the weight of the uterus, ovaries and adrenal glands. Progesterone, for its part, acts on the endometrium and promotes the maintenance of pregnancy. This means that at this dose, this extract would have estrogenic and progestogenic activities. When you double the dose, you see a decrease in these hormones. This reduction would be due to negative feedback which therefore caused a drop in these reproductive hormones, in addition administration of high doses of phytoestrogens can cause a hormonal imbalance (Ngoule et al., 2022). The chemical screening of the aqueous extract of *Thonningia sanguinea* roots revealed the presence of several chemical families such as polyphenols, flavonoids, anthraquinones, tannins, saponosides. These results are similar to those of Thomford, (2018) as well as Ouattara et al. (2013) who worked respectively on the chemical study of the Ghanaian parasitic plant: *Thonningia sanguinea* and on the in vitro antibacterial activity of *Thonningia sanguinea* flower extracts. Indeed, flavonoids, saponosides and tannins are known for their estrogenic properties and (Qian et al., 2006; Chan et al., 2002; Rimoldi et al., 2007) and therefore these families would be responsible for the pharmacological effects observed.

Conclusion

The evaluation of the estrogenic activity of the aqueous extract of the roots of *Thonningia sanguinea* showed that this extract is not toxic up to 5000 mg/kg in mice. It increases the weights of reproductive organs as well as serum levels of female sex hormones. This extract would therefore have estrogenic properties at 200 mg/kg, thus justifying its use in traditional medicine.

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