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Effects of Aqueous Extracts of *Sarcocephalus latifolius* (Rubiaceae) and *Cnestis ferruginea* (Connaraceae) on the Body Weight and Relative Weight of Certain Sex Organs of Male Rats

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Abstract

Sarcocephalus latifolius and *Cnestis ferruginea* are shrubs with heights of 5 to 8 m and 3 to 3.6 m, respectively, and are known to be powerful aphrodisiacs. Our aim was to evaluate the effect of aqueous extracts of their leaves on the body weight and relative weight of some androgen- dependent sex organs of male rats. For this purpose, the rats were subjected to an 8-day treatment. These extracts had no significant effect on either the body weight or the relative weight of the androgen-dependent secondary sex organs of the rats compared to the control over the 8-day treatment period. The leaves of *Sarcocephalus latifolius* and *Cnestis ferruginea did* not have any detectable androgenic properties during this period.

Keywords: *Sarcocephalus latifolius*, *Cnestis ferruginea*, androgen-dependent androgenic.

INTRODUCTION

Erectile dysfunction is defined as the persistent or recurrent inability of a man to achieve or maintain a penile erection sufficient for sexual activity (Lue *et al.*, 2004). Studies in several regions of Côte d'Ivoire have reported that over 90% of the populations rely on traditional medicine for their primary health care. Thus, to assist in the development of traditional medicine, the Ivorian state has established collaboration between traditional and modern medicine (Manda *et al.*, 2017). This collaboration was translated into priority in the National Health Development Plan, specifically the PNDS 1996 - 2005, by the creation in 2000 of a National Programme for the Promotion of Traditional Medicine and a traditional medicine unit within the University Hospital Centre of Treichville (Anonymous, 2017; Manda *et al.*, 2017). The evaluation of the effects of aqueous extracts of the leaves of *Sarcocephalus latifolius* and *Cnestis ferruginea* on the body weight and the relative weight of certain sexual organs of male rats is part of the scientific valorisation of plants used in therapy in the Ivory Coast.

Sarcocephalus latifolius is a shrub of 5 to 8 metres in height (Arbonnier, 2002). *Cnestis ferruginea* is also a shrub of 3 to 3.6 metres in height (Garon *et al.*, 2007). They occur in the SudanoGuinean and Sudano-Sahelian savannahs throughout intertropical Africa (Arbonnier, 2002; Garon *et al.*, 2007). These plants are known to create and stimulate sexual desire. To this end, we will evaluate the effect of aqueous extracts of the leaves of these two plants on the body weight and the relative weight of certain androgen-dependent organs, namely the testicles, the epididymis, the seminal vesicles, the prostate, the vas deferens, the penis, the lift muscle of the anus and Cooper's gland.

I- MATERIALS AND METHODS

I-1- Materials

I-1-1- Plant material

The leaves of *Sarcocephalus latifolius* (Rubiacea) and *Cnestis ferruginea* (Connaraceae) were collected in the Soubré department in the south-west of Côte d'Ivoire. The plants were identified by the Botany Laboratory of the UFR Biosciences of the Felix Houphouët BOIGNY University of Abidjan. The leaves were dried in the shade at room temperature. These dried leaves were ground with a Moulinex type electric grinder to obtain powders that we used to prepare our aqueous extracts. The macerates are obtained from 50 g

of powder put in 1 litre of distilled water and shaken for 15 minutes in a blinder then filtered with cotton wool and 3mm Watman paper. The filtrates obtained were evaporated at 55°C in an oven.

I-1-1-1- Systematic

***Sarcocephalus latifolius* (Rubiaceae)**

Kingdom: Plant
Subdomain: Metaphytes
Phylum: Spermaphytes
Sub-branch: Angiosperms
Class: Eudicots
Subclass: Asteridae
Order: Gentianales
Family: Rubiaceae
Genus: *Sarcocephalus*
Species: *Sarcocephalus latifolius* (Traoré, 2020)

***Cnestis ferruginea* (Connaraceae)**

Kingdom: Plant
Subdomain: Metaphytes
Phylum: Spermaphytes
Sub-branch: Angiosperms
Class: Eudicots
Subclass: Rosidae
Order: Oxalidales
Family: Connaraceae
Genre: *Cnestis*
Species: *Cnestis ferruginea Vahl ex DC* (HA, 2017)

I- 1-1-2- Botanical Description

Sarcocephalus latifolius is a bushy, stunted shrub 5-6 metres high, sometimes up to 8-9 metres, with a tortuous shaft 30 cm in diameter. The branches are flexible, lianascent, intertwined, upright and then drooping. The bark is very fibrous, the wood is white or yellow and fairly soft. The leaves are simple, opposite, oval, sometimes suborbicular, hairless, 10 to 17 cm long and 6 to 14 cm wide. They have deltoid stipules 3-5 mm long, single or biapiculate (which ends in a short, sharp point at the top), glabrous and more or less ornamented with a thin carina. The petiole is purple and 8-20 mm long. The foliage is green and shiny on top. The venation is pinnate, with 8-9 pairs of secondary veins, the midrib often a little micropuberulent on the inner side, the secondary veins having very small pubescent domatia in their axils. The shrub keeps its foliage green all year round (Traoré, 2020).

Cnestis ferruginea is a 2-5 m high sarmenting shrub or vine. The branches are tomentose- ferruginous. Leaves with petioles and rachis up to 35 cm long, tomentose-ferruginous, with 41 leaflets; The terminal elliptic or lanceolate- elliptic, cuneate to rounded at the base, acute or shortly acuminate at the apex, 1-15 cm long and 0.5-8 cm wide, the laterals slightly asymmetrical, oblong-lanceolate, mostly cordate at the base, all ± cactaceous, shiny and glabrous on the upper surface, dull and ± densely ferruginous-tomentose to indubitably slightly shiny on the underside; secondary veins 5-7 on each side. Panicles terminal to subterminal, ample; stalk and rachis 6-18 cm long, ferruginous-tomentose; bracts linear- oblong 4 mm long, ferruginous-tomentose. Flowers with lanceolate sepals, acuminate at the apex, 2-3 mm long and 0.5-1 mm wide, ferruginous-tomatose on the outer surface, tomatose- pubescent lighter on the inner surface; petals suborbicular, ± 1 mm long and wide, glabrous, whitish. The follicles pyriform, 4.5 cm long and 1.8-2.4 cm thick, velvety bright red. Seeds ± 2.5-3 cm long; testa shiny black; aril golden yellow (WFO, 2023).

I-1-2- Animal Material

Nulliparous, non-pregnant female mice of *Mus musculus* species, Swiss strain, with a mass of 24-30 g were used for acute toxicity testing. Virgin male rats, at least 9 weeks old and weighing between 160 and 200 g, of the species *Rattus norvegicus*, strain Wistar, were used for the tests. The animals were reared in the vivarium of the Ecole Normale Supérieure in Abidjan. The average temperature of the room was 28°C ± 3°C with a relative humidity of 70%. The photoperiod was 12/24 hours. The animals have free access to food and water.

I-2- Method

I-2-1- Characterisation of the main Chemical constituents

The characterisation of the different chemical groups with therapeutic potential was done according to the techniques described in the work of Alilou *et al.*, (2014), Mburu *et al.*, (2016). Sterols and polyterpenes were identified by the Liebermann reaction, polyphenols by the reaction with ferric chloride of chemical formula FeCl3, flavonoids by the reaction with cyanidine, quinone substances by the Bornstraëgen reagent, catechic tannins by the Stiasny reagent and gall tannins by ferric chloride. As for the saponosides, they are determined by the foams obtained after stirring the aqueous extracts.

I-2-2 Method of Studying Acute Toxicity by Gavage

It was conducted according to OECD (2001) guideline 423. Twelve nulliparous, non-pregnant female mice, ranging in weight from 24 to 30 g, were divided into four (4) batches of three (3) mice. The batches were numbered from 1 to 4. Animals in the same batch received the same dose. The doses of 2000 and 5000 mg/kg body weight of aqueous extract of *Sarcocephalus latifolius were* administered by means of a gastric tube to mice in batches 1 and 2 respectively. Mice in batches 3 and 4 received 2000 and 5000 mg/kg body weight of aqueous extract of *Cnestis ferruginea*, respectively. The mice were fasted for 13 hours before administration of the extracts and then after administration of the extracts for a period of three hours with free access to water. They were observed individually for the first 30 minutes, then for the first 4

hours and regularly for 24 hours after treatment. Thereafter, they were observed daily for 14 days. The mass of the mice was taken on days 1er, 7ème and 14ème.

I-2-3- Method for Studying the Effects of Aqueous Extracts of *Sarcocephalus Latifolius* and *Cnestis ferruginea*

Twenty rats were used in this study. Four batches of five rats were formed (batches A, B, C and D). The rats in the different batches were given distilled water at a rate of 10 ml/kg body weight; the doses of 5 mg/kg body weight of viagra and 1000 mg/kg body weight of aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea by* means of a gastric tube during the eight (8) days of treatment. Rats were weighed on day 1er before treatment and on day 9ème. After weight gain on day 9ème, the rats were anaesthetised and euthanised. The testes, epididymis, seminal vesicles, prostate, vas deferens, penis, anus lift muscle and Cooper's gland were removed and weighed. The relative weight was determined from the following formula:

Relative weight = $\frac{Weight\ of\ the\ organ\ (g)}{Weight\ of\ the\ animal\ (g)}$ x 100 (Etame *et al.*, 2017; Ehoussou *et al.*, 2022)

I-3- Statistical Analysis

The results were processed with GraphPadPrism 8.4.3 (686) software. Differences are considered significant when p is less than 0.05.

II- RESULT

The phytochemical study revealed the presence of polyphenols, flavonoids, catechic tannins, saponosides, alkaloids, sterols and polyterpenes, and an absence of gall tannins in the aqueous extract of *Sarcocephalus latifolius* and *Cnestis ferruginea* leaves.

Administration by gavage of the 2000 mg/kg body weight doses of the aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* did not result in any deaths of the mice, but we observed difficulty in locomotion of the mice within one hour of administration of the extracts before returning to normal for both extracts at the 5000 mg/kg body weight dose. These extracts have an LD50 greater than 5000 mg/kg body weight. They cause a small increase in body weight of the animals. At 2000 and 5000 mg/kg body weight of *Sarcocephalus latifolius*, the body weight of the animals increased from 25.33 g ± 1 to 27.08 g ± 0.6, which is equivalent to an increase of 1.75g, and from 27.67 g ± 1.45 to 29.10 g ± 1.67, respectively, an increase of 1.41g. No signs of mortality or behavioural changes were observed. When *Cnestis ferruginea was* administered at doses of 2000 and 5000 mg/kg body weight, the body weight of the animals increased from 26.88 g ± 0.56 to 28.35 g ± 1.47 g and from 28.33 g ± 0.23 to 28.83 g ± 0.73 g respectively, an increase of 0.50 g. No signs of mortality were observed. No signs of mortality or behavioural changes were observed.

The change in body weight and relative weights of the testes, epididymis, seminal vesicles, prostate, vas deferens, penis, levator ani muscle and Cooper's gland of rats given distilled water, Viagra, aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* are recorded in Tables 1 and 2 respectively.

Table 1: Change in weight of rats given distilled water, viagra, aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* after 8 days of treatment

Solutions given	Distilled water (10ml/kg)	Viagra (5 g/kg)	*Sarcocephalus latifolius* (1000 mg/kg	*Cnestis ferruginea* (1000 mg/kg)
Day 1: Body weight (g)	162.26 ± 0,41	165.84 ± 0,31	183.50 ± 0,29	191.32 ± 0.4
Day 9: Body weight (g)	164.36±0,33	167.99 ± 0,28	185.85 ± 0,33	193.99 ± 0.38
Percentage increase (%)	1.3	1.3	1.5	1.4

Table 2: **Relative weights of testes, epididymis, seminal vesicles, prostate, vas deferens, penis, anus lift muscle and Cooper's gland of rats given distilled water, viagra, aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea***

	Distilled water (10 ml/kg)	Viagra(5 mg/kg)	S. latifolius (1000mg/kg)	C. ferruginea (1000 mg/kg)
Testicles	0.99 ± 0.02	1.1 ± 0.01	1.1 ± 0.04	1.04 ± 0.02
Epididymis	0.29 ± 0.08	0.3 ± 0.00	0.3 ± 0.00	0.29 ± 0.01
Seminal vesicles	0.43 ± 0,02	0.36 ± 0.01	0.38 ± 0.04	O.34 ± 0.02
Prostate	0.17 ± 0.00	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.00
Deferential canals	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
Penis	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.09 ± 0.01
Lift muscle of the anus	0.36 ± 0.01	0.33 ± 0.01	0.35 ± 0.00	0.28 ± 0.04
Cooper's gland	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

Viagra, aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea*, had no significant effect on the body weight of the rats compared to the control over the 8 days of treatment. They also had no significant effect on the relative weight of the androgen-dependent adnexal organs compared to the control.

III- DISCUSSION

The phytochemical study of the aqueous extract of *Sarcocephalus latifolius* leaves revealed the presence of polyphenols, flavonoids, catechic tannins, saponosides as revealed in the work of (Plassart, 2015) and an absence of gall tannins in our aqueous extract unlike that of (Plassart, 2015) in which gall tannins are present (Plassart, 2015). Our sample has the same composition as that of Bahi *et al.,* (Bahi *et al.,* 2021).

As for the aqueous extract of *Cnestis ferruginea* leaves, phytochemical sorting revealed the presence of flavonoids, tannins, alkaloids and saponosides as highlighted by Ajala *et al., (*Ajala *et al.,* 2021).

Acute toxicity by gavage showed that at 5000 mg/kg body weight no deaths were recorded, no signs of mortality were observed. At this dose, aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* caused locomotion difficulties in mice as revealed by the work of Soro *et al.,* (Soro *et al.,* 2022). Motor activity is a measure of the level of excitability of the central nervous system (CNS). This decrease in spontaneous motor activity could be attributed to the depressant effect of plant extracts on the CNS (Rakotonirina *et al.,* 2001). Gamma-amino butyric acid (GABA) is the main inhibitory neuromediator of the CNS. Extracts could act by potentiating the inhibitory activity of GABA in the CNS through membrane hyperpolarisation leading to a reduction in the propagation rate of neuronal action potentials in the brain or through direct activation of GABA receptors (Gahlot *et al.,* 2013). Perez *et al.,* (1998) showed that the reduction in spontaneous locomotor activity and tachypnea could be due to saponosides and alkaloids. These results are similar to those obtained by studies conducted by Zihiri (2006) and Fahmida (2012) which showed that saponosides, flavonoids, and alkaloids have depressive activity on the nervous system, disruption of the respiratory system and reduced motor activity in rats. The 50% lethal dose or LD50 is greater than 5000 mg/kg body weight. Based on these results, the aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* were classified as category 5 or unclassified under the Globally Harmonised System of Classification of Chemical Substances. This category identifies substances with low oral toxicity. The aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* in this category would be a low toxicity extract (OECD, 2001). This lack of toxicity by this route of administration of the aqueous extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* has also been observed with the leaves of *Chrysophyllum welwitschii* (Agnéro, 2019) and with the root bark of *Cassia sieberiana* (Soro *et al.,* 2022).

The aqueous extracts of the leaves of *Sarcocephalus latifolius* and *Cnestis ferruginea* had no significant effect on the body weight or androgen-dependent sexual organs of the rats compared to the control, suggesting that the aqueous extracts of the leaves of these plants either did not have androgenic properties or that they were not detectable for the 8- day treatment. Indeed, it is known that androgens are steroids with anabolic activity in various target tissues (Johnson and Everitt, 1988). It has therefore been shown in rats that any increase in testosterone levels or androgenic treatment induces an increase in secretory activity and sex organ mass (Gonzales *et al.,* 2001).

IV- CONCLUSION

Our study showed that the 8-day oral administration of the aqueous leaf extracts of *Sarcocephalus latifolius* and *Cnestis ferruginea* in male rats revealed no androgenic activity of its aqueous leaf extracts despite their reputation as potent aphrodisiacs.

Aqueous extracts of the leaves of *Sarcocephalus latifolius* and *Cnestis ferruginea* would not have any detectable androgenic properties in this period of time.

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