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Phytochemical Study and Evaluation of the Effect of Aqueous Extract from the Roots of *Hymenocardia acida* (Euphorbiaceae) on Sexual Behaviour in Diabetic Wistar Rats

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Abstract | **Original Research Article**

Sexual behaviour plays a key role in personal fulfilment and emotional relationships. Erectile dysfunction, by disrupting this sphere, can have significant psychological consequences, hence the importance of understanding and treating it. This study aims to evaluate the effect of aqueous extract from the roots of *Hymenocardia acida* on sexual behaviour in diabetic rats. H. acida and Viagra were administered orally to 20 diabetic males divided into four groups. Group 1 received distilled water; group 2 received sildenafil citrate (Viagra), the reference substance, at a dose of 5 mg/kg; groups 3 and 4 received *H. acida* at doses of 500 and 1000 mg/kg BW, respectively. Ovariectomised females were induced into oestrus by administering oral ethinyl oestradiol at a dose of 100 µg/animal and subcutaneous progesterone at a dose of 1 mg/animal, 54 hours before the experiment. The parameters relating to the rats' sexual behaviour were recorded on the 1st and 8th days and, after gavage, the rat was placed in isolation for 1 hour in a cage, then a female rat in oestrus was introduced into the same cage. The test was negative if the latency times for intromission and ejaculation exceeded 20 minutes. The aqueous extract of *H. acida* roots increased sexual motivation in diabetic animals at doses of 500 and 1000 mg/kg bw, significantly increasing the number of mounts and intromissions. The extract has a sexually stimulating effect, confirming its traditional use as an aphrodisiac.

Keywords: Erectile dysfunction, diabetes, sexual behaviour, *Hymenocardia acida*, rat.

INTRODUCTION

Hymenocardia acida (H. Acida) is a plant species belonging to the Euphorbiaceae family. It is a dioecious, deciduous shrub that grows to a height of 6 to 10 metres and has smooth, pale brown or grey bark (Arbonnier, 2004) It is a savannah species, common in northern Côte d'Ivoire and Burkina Faso, extending as far as Uganda (Patrick Maundu, 2009). Ethnobotanical studies have shown that in Côte d'Ivoire, a decoction of the bark of the trunk and roots or an infusion of the leaves of *H. acida* is ingested to treat abdominal pain, diarrhoea, female infertility and painful swelling, and is used as an aphrodisiac ((Kamanzi Atindehou *et al.*, 2004); (Koné *et al.*, 2005). The presence of erectile dysfunction (ED) remains somewhat subjective and ambiguous. Indeed, ED often reflects a man's inability to perform sexually in line with his own expectations, which are in turn influenced by cultural and social factors (Mulhall *et al.*, 2001). The aetiology of diabetes-induced erectile dysfunction is multifactorial. It involves both nerve damage and vascular damage. Some studies show that neuropathy, rather than vasculopathy, contributes significantly to the development of erectile dysfunction through selective nitrergic degeneration, which leads to a decrease in nNOS (neuronal nitric oxide synthase) activity and, consequently, NO (nitric oxide) activity (Hecht *et al.*, 2001).

Pharmacological studies conducted on *H. acida* have shown that this plant has antibacterial, antioxidant, antidiabetic and anti-inflammatory properties (Nkemzi *et al.*, 2022), (Ogbunugafor *et al.*, 2010).

The present study aims to evaluate the effects of the aqueous extract of *H. acida* roots on sexual behaviour in Wistar rats.

MATERIALS AND METHODS

Plant material

The roots of H. acida were collected in Korhogo in the Poro region (Côte d'Ivoire). A sample of this plant was identified at the National Centre for Floristics at Félix Houphouët-Boigny University (Abidjan, Ivory Coast) on the basis of taxonomic characteristics and by direct comparison with herbarium specimen No. 4CJ006089. The roots of H. acida were cut up and then dried in a room at room temperature (28-30°C) for four weeks.

Animal material

Rats of the species Rattus norvegicus (Muridae), of the Wistar strain, aged 8 weeks and weighing between 110 and 120 g were used. These animals, all male, came from the vivarium of the Ecole Normale Supérieure (Abidjan, Ivory Coast) . In this vivarium, the average temperature is 28 ± 2 °C with a relative humidity of 60% and a photoperiod of 12/12. The animals were fed a standard diet for experimental animals and given water ad libitum.

METHOD

Preparation of the aqueous extract from H. acida roots

Three hundred grams (300 g) of H. acida root are boiled in 1 L of distilled water for 30 minutes in a glass container. After cooling and filtering through cotton wool and Wattman paper, the resulting decoction is freeze-dried (N'guessan *et al.*, 2012). The powder obtained after freeze-drying constitutes the aqueous extract of H. acida roots.

Phytochemical study of the aqueous extract of Hymenocardia acida roots

The identification of metabolites with therapeutic potential involves testing to characterise the major groups of chemical compounds contained in the aqueous extract. The detection of these compounds is based on the principle that they induce chemical reactions in the presence of appropriate reagents (Wagner & Bladt, 1996). These tests were carried out using the analytical techniques described in the works of (Ogbuagu *et al.*, 2022) and (Riaz *et al.*, 2023). For these tests, an aqueous extract solution is prepared by dissolving 5 g of the extract in 50 mL of distilled water.

Study of the effects of aqueous extract of Hymenocardia acida on sexual behaviour in diabetic rats.

The aqueous extract of *Hymenocardia acida* and the reference products were administered orally using an oesophageal probe. Administration took place between 3 p.m. and 4 p.m. to avoid interference with the natural increase in libido observed in rats in the morning.

The aqueous extract of *Hymenocardia acida* roots and the substances were administered once a day for eight consecutive days.

Preparation of substances to be administered

Aqueous extract solution from Hymenocardia acida roots

The extract solution administered to rats was prepared by dissolving the aqueous extract from H. acida roots in distilled water to obtain a stock solution.

The stock solution thus obtained was stored in a cool place (4 °C) for subsequent treatment of the animals. Knowing the dose D (mg/kg), the weight P (kg) of the animal and the concentration C (mg/mL) of the extract, the volume V (mL) to be administered to a rat was determined using the following formula.

Sildenafil citrate solution (Viagra)

Sildenafil citrate from Micron Pharmaceuticals (India) was administered at a dose of 50 mg/kg. To prepare this solution, a 100 mg tablet of sildenafil citrate was dissolved in 10 mL of distilled water, and the mixture was then homogenised using a magnetic stirrer. The volume of solution administered to each rat was determined as previously described for the aqueous extract of Hymenocardia acida, considering the dose of 50 mg/kg and the concentration of 10 mg/mL.

Induction of diabetes

The streptozotocin solution was prepared at a concentration of 55 mg/mL (C) so that each animal could be administered a dose of 55 mg/kg (D) for the induction of diabetes. In order to prevent the oxidation of streptozotocin that occurs during prolonged exposure to air, the solution was prepared extemporaneously and in small quantities.

Induction of oestrus in ovariectomised females

The females were artificially induced into oestrus using the method described by Szechtman *et al.* (1981). Female rats only allow mating during the oestrus phase. A suspension of ethinyl oestradiol (Lynoral tablets, Organon Pharma) was administered orally at a dose of 100 µg/animal 48 hours before progesterone (Dubaget tablets, Glenmark Pharma) was injected subcutaneously at a dose of 1 mg/animal 6 hours before the experiment. The receptivity of the females was confirmed before the test by exposing them to male animals other than the control, experimental and normal animals.

Evaluation of the effects of aqueous extract of *Hymenocardia acida* roots on sexual behaviour in diabetic rats

The evaluation of the aphrodisiac properties of aqueous extract of *Hymenocardia acida* roots on the sexual behaviour of rats, after 8 days of treatment, was conducted according to the protocol of (Ide Ngaha Njila *et al.,* 2018)**.**

The copulation parameters used to characterise the effects of Hymenocardia acida on the sexual behaviour of diabetic rats were recorded on the 1st and 8th days of the experiment. Immediately after force-feeding, the rat was placed in isolation for one hour in a cage, then a female rat selected in oestrus was introduced into the same cage.

For the study, there were 20 male diabetic animals and 10 ovariectomised female animals, and the 20 males were divided into four groups of five animals each, as follows:

- Lot 1: rats given distilled water at a rate of (control)
- Lot 2: rats given sildenafil citrate at a dose of 50 mg/kg body weight (positive control)
- Group 3: rats receiving aqueous extract of Hymenocardia acida at a dose of 500 mg/kg body weight -Group 4: rats receiving aqueous extract of Hymenocardia acida at a dose of 1000 mg/kg body weight

The male rats were placed in a cage for 10 minutes to acclimatise, then a receptive female was introduced into the cage for 30 minutes. During this time, the following parameters were recorded:

- the mounting latency time, which corresponded to the time interval between the introduction of the female rat into the cage and the first mounting;
- the intromission latency time, corresponding to the time interval between the introduction of the female rat into the cage and the first intromission;
- the ejaculation latency time, corresponding to the time interval between the introduction of the female rat into the cage and the first ejaculation;
- the average copulation interval, corresponding to the time between the first intromission in a series and the ejaculation marking the end of that series;

The test was considered negative if the latency times for intromission and ejaculation exceeded 20 minutes. After 8 days of experimentation, the rats were sacrificed under ether anaesthesia between 7am and 10am in order to minimise hormonal variations due to the circadian rhythm.

RESULTS

Chemical composition of the aqueous extract of *Hymenocardia acida* roots Phytochemical sorting carried out on the extract *of Hymenocardia acida* roots enabled the chemical constituents to be identified. The aqueous extract of *Hymenocardia acida* roots revealed the presence of polyphenols, flavonoids, catechin tannins, saponosides, alkaloids, sterols and polyterpenes, and the absence of gallic tannins and quinone substances.

Effects of aqueous extract of *Hymenocardia acida* roots on diabetic animals

In Figure 1, streptozotocin used at a dose of 55 mg/kg bw caused a very significant increase ($P < 0.001$) in the basal blood glucose levels of rats after three (3) days. The animals had an average basal blood glucose level of 94 ± 4 mg/dl before the injection. This blood glucose level rose to 249 ± 19 mg/dl three days after the streptozotocin injection. It remained almost constant one week later, at 233 ± 18 mg/dl. Treatment of these diabetic animals for 8 days with doses of 500 and 1000 mg/kg PC of the extract caused a very significant decrease in their basal blood glucose levels to 110 ± 10 mg/dl ($P < 0.01$) and 113 ± 15 mg/dl ($P < 0.01$). In contrast, Viagra did not cause any variation in blood glucose levels in diabetic rats. Thus, no significant difference was observed between the blood glucose levels of animals treated with Viagra and those of animals in the control group.

Evaluation of the aqueous extract of Hymenocardia acida roots on the sexual behaviour of diabetic rats Effects of the aqueous extract of Hymenocardia acida roots on mount latency time.

On the first day, a significant decrease ($p<0.001$) in mount latency was observed in diabetic animals treated with Viagra and doses of 500 and 1000 mg/kg BW of the aqueous extract of Hymenocardia acida roots. This decrease was 47.17% (275.6 ± 2.00 seconds vs. 520.6 ± 2.01 seconds), 41.34% (305.6 ± 0.92 seconds vs. 520.6 ± 2.01 seconds) and 45.92% (281.8 ± 4.90 seconds vs. 520.6 ± 2.01 seconds) compared to controls.

On day 8, a significant decrease ($p<0.001$) in mounting latency was observed in diabetic animals treated with Viagra and extract at doses of 500 and 1000 mg/kg BW and Viagra, respectively, of 55.39% (280.0 ± 3.00 seconds versus 626.8 ± 44.47 seconds), 49.64% (315.6 ± 20.95 seconds vs. 626.8 ± 44.47 seconds) and 54.30% (286.4 ± 4.22 seconds vs. 626.8 ± 44.47 seconds) compared to animals receiving distilled water.

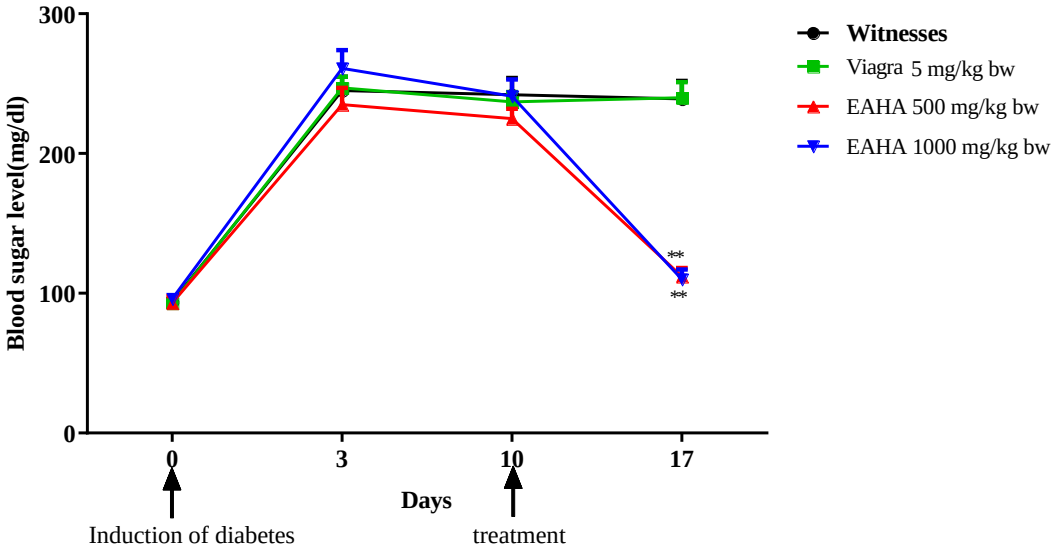

Figure 1: Curves for diabetic rats after administration of aqueous extract of *Hymenocardia acida* roots and Viagra

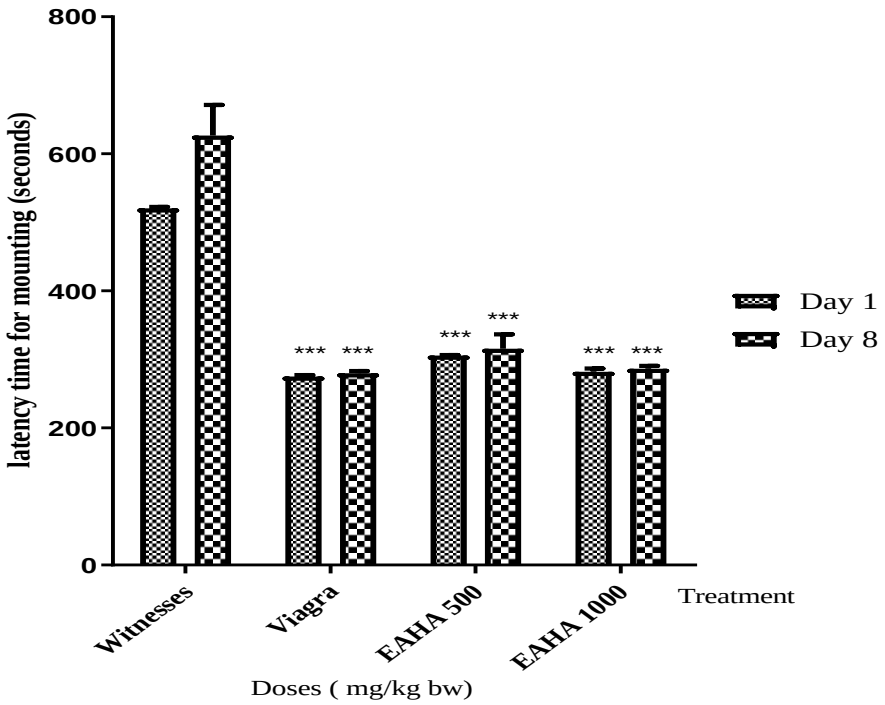

Figure 2: Latency time for mounting in diabetic rats

Effects of aqueous extract of Hymen cardia acida roots on intromission latency time

Furthermore, Figure 36 shows a significant decrease ($p<0.001$) in intromission latency on day 1 in diabetic animals treated with Viagra and the extract at doses of 500 and 1000 mg/kg PC, respectively, of 64.12% (240. 6±10.08 seconds vs. 669.0±22.62 seconds), 48.04% (347.6±7.60 seconds vs. 669.0±22.62 seconds) and 59.85% (268±8.6 seconds vs. 669.0±22.62 seconds) compared to the controls (distilled water). The effects of the aqueous extract of *Hymenocardi acida* roots on the latency time of intromission on day 8 showed a significant decrease ($p<0.001$) in diabetic animals treated with Viagra and the extract at doses of 500 and 1000 mg/kg PC, respectively, of 60% (260±8.00 seconds vs 650.0±24.00 seconds), 57.67% (275.12± 4.65 seconds vs 650.0±24.00 seconds) and 49.93% (268.6± 8.00 seconds vs 650.0±24.00 seconds) compared to the controls (distilled water).

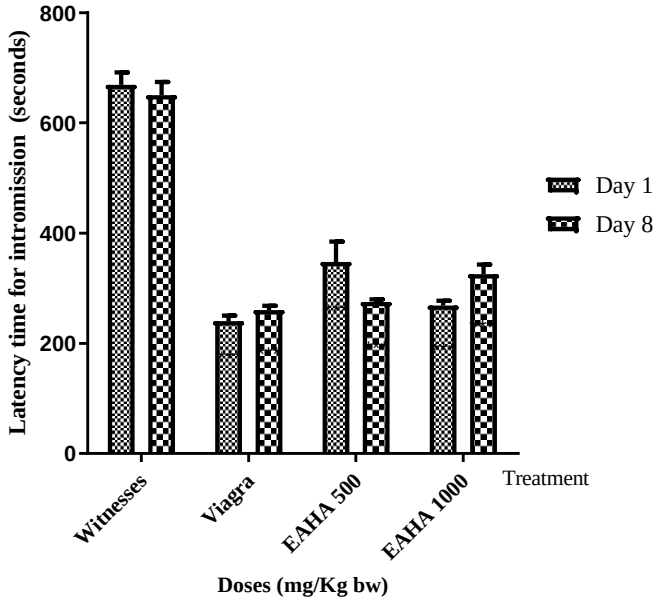

Figure 3: Latency time for intromission in diabetic rats

Effects of aqueous extract of *Hymenocardia acida* roots on ejaculation latency time

In diabetic animals on the first day of treatment, a significant decrease (P<0.001 and p<0.05) in ejaculation latency time was observed in animals treated with Viagra and aqueous extract of Hymenocardia acida roots at doses of 500 and 1000 mg/kg, respectively, of 53.36% (90.0±3.14 seconds vs 193.0±10.63 seconds), by 19.68% (155.4±9.84 seconds vs 193.0±10.63 seconds) and by 29.01% (137.20±6.92 seconds vs 193.0±10.63 seconds) compared to the controls (distilled water).

On day 8, a significant decrease (P<0.001 and p<0.05) in ejaculation latency time was also observed in diabetic animals a significant decrease (P<0.001 and p<0.05) in ejaculation latency time in diabetic animals treated with Viagra and aqueous extract of *Hymenocardia acida* roots at doses of 500 and 1000 mg/kg, respectively, of 57.89% (80.0±4.42 seconds vs 190.8±8.48 seconds), by 18.94% (154.6±11.20 seconds vs 190.8±8.48 seconds), and by 22.63% (147.6±4.27 seconds vs 190.8±8.48 seconds) compared to the controls (distilled water).

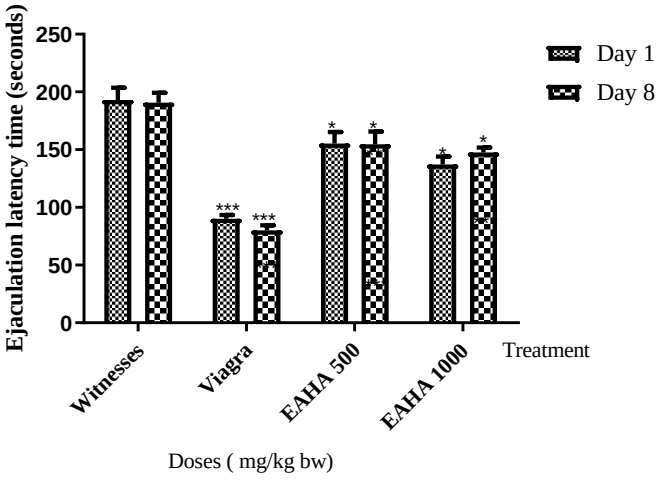

Figure 4: Ejaculation latency time in diabetic rats

Effects of aqueous extract of *Hymenocardia acida* roots on the average copulation interval

This interval could be determined in diabetic rats on day 1, as there was a significant decrease (P<0.001) in rats treated with Viagra and doses of 500 and 1000 mg/kg PC of aqueous extract of *Hymenocardia acida* roots, respectively, of 51.91% (62.8 ± 0.65 vs. 30.2 ± 2.70 seconds), 56.98% (70.2 ± 1.70 vs. 30.2 ± 2.70 seconds) and 58.05% (72.0 ± 1.20 vs. 30.2 ± 2.70 seconds) compared to the control group (distilled water).

On day 8, a significant decrease (P<0.001) was also observed in rats treated with Viagra and doses of 500 and 1000 mg/kg PC of aqueous extract of Hymenocardia acida roots, respectively, of 30.10% (57.8 ± 1.70 vs. 40.4 ± 1.38 seconds), 47.39% (76.8 ± 3.87 vs. 40.4 ± 1.38 seconds) and 39.52% (66.8 ± 2.31 vs. 40.4 ± 1.38 seconds) compared to the controls (distilled water).

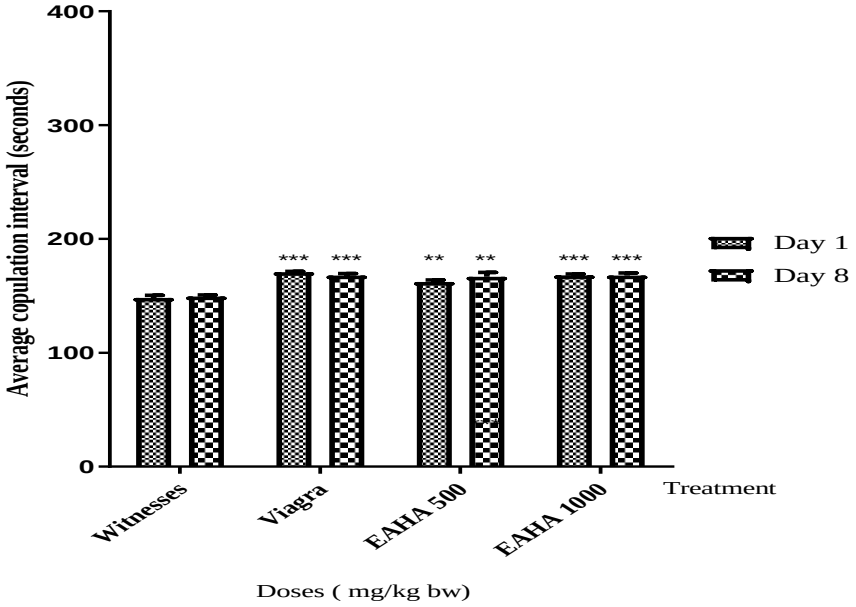

Figure 5: Average copulation interval in diabetic rats

DISCUSSION

Phytochemical analysis of the aqueous extract of *Hymenocardia acida* roots revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, catechin tannins, alkaloids and saponosides. However, quinonic substances and gallic tannins were not found in this plant. Nevertheless, these compounds were identified by (Amom *et al.*, 2013) in the aqueous extract of *Hymenocardia acida* roots harvested in Nigeria. Among the compounds identified in the aqueous extract of *Hymenocardia acida* roots, several have pharmacological activities that are beneficial to the functioning of the mammalian organism. Saponins have oestrogenic effects (Chan *et al.*, 2002). They have the ability to boost testosterone levels and trigger libido (Gauthaman & Ganesan, 2008). They therefore have aphrodisiac effects (Singh & Gupta, 2011) Catechin tannins have antibacterial, antifungal and antiviral properties (Liu *et al.*, 1999)**.** In addition to their strong oestrogenic actions (Diel *et al.*, 2004), flavonoids regulate the production of oestrogens and androgens (Padashetty & Mishra, 2007) in men. Treatment of diabetic animals with aqueous extract of *Hymenocardia acida* roots induced a significant decrease in blood glucose levels after 10 days. Aqueous extract of *Hymenocardia acida* roots therefore appears to have antidiabetic properties. Secondary metabolites such as alkaloids and flavonoids contained in the aqueous extract of *Hymenocardia acida* roots are believed to be responsible for this antidiabetic activity. The antidiabetic activity of *Hymenocardia acida* roots could be explained by the presence of flavonoids, alkaloids and tannins. Indeed, (Dimo *et al.*, n.d.) showed that certain plants have hypoglycaemic activity when they contain polyphenols, flavonoids, alkaloids, tannins and terpenes. Phenolic compounds such as flavonoids exert their antidiabetic effect through the liver by influencing gluconeogenesis, glycogenesis and glycogenolysis. They promote glucose storage in the liver and reduce glycogen breakdown. According to (Sarkhail *et al.*, 2007), flavonoids influence the beta cells of the islets of Langerhans in the pancreas and stimulate insulin secretion.

This suggestion is supported by (Palsamy & Pharmacotherapy, 2006), who demonstrated the same effect in diabetic rats treated with resveratrol, a flavonoid. Flavonoids may contribute to reducing intestinal glucose absorption in diabetic rats induced by streptozotocin (Pendashteh *et al.*, 2010) After 8 days of treatment, the aqueous extract of *Hymenocardia acida* roots led to an increase in the frequency of mounting and intromission in diabetic rats. Similarly, a reduction in the latency time for mounting and intromission was also noted in diabetic animals compared to diabetic control rats (distilled water). The decrease in mounting and intromission latency observed in this study shows that *Hymenocardia acida* root extract increases the libido of test animals. Furthermore, it is well documented that long-term experimental diabetes affects reproductive function in male rats. Indeed, after four weeks of streptozotocin-induced diabetes, the results of (Asuquo *et al.*, 2009) showed alteration and distortion of the germinal epithelium and seminiferous tubules in diabetic

Wistar rats (distilled water). The peritubular tissue surrounding the seminiferous tubules and interstitial cells was also altered. All structural and biochemical changes in the testicles and accessory organs are thought to be secondary to hormonal disorders such as the decrease in plasma FSH and LH levels observed in diabetic rats according to other authors (King & Kang, 1989)**.** Furthermore, studies (Isidori *et al.,* 2005)**)** emphasise that testosterone improves libido more consistently than erectile function. These latest observations could indicate a dependence of the nitric oxide (NO)-cGMP pro-erectile pathway on testosterone; hence the slight erection and ejaculation observed in diabetic rats. Indeed, the aqueous extract of *Hymenocardia acida* roots, due to its androgenic properties, would have contributed to the increase in libido observed in treated diabetic animals. In addition, the slight erection and ejaculation observed could also be due to an imbalance between antioxidant capacity and free radicals induced by diabetes. This imbalance may have affected intratesticular microcirculation and altered nitric oxide, a neurotransmitter responsible for smooth muscle relaxation during erection (Jones *et al.,* 2002)**.**

CONCLUSION

The phytochemical study conducted on the aqueous extract of the roots of this taxon revealed that the plant contains polyphenols, flavonoids, catechin tannins, saponosides, alkaloids, sterols and polyterpenes, and no gallic tannins or quinone substances.

In the study of sexual behaviour in rats, the aqueous extract of *Hymenocardia acida r*oots increased sexual motivation in both short-term diabetic animals, significantly increasing the number of mounts and intromissions.These various results justify the empirical use of *Hymenocardia acida* to combat erectile dysfunction.

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