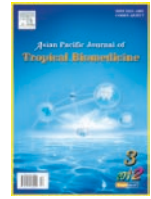




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Enhancement of erectile function of sexually naïve rats by β -sitosterol and α - β -amyrin acetate isolated from the hexane extract of *Mondia whitei*.

Pierre Watcho^{1*}, Fabien Zelefack², Silvere Ngouela², Telesphore Benoît Nguelefack¹,Pierre Kamtchouing³, Etienne Tsamo², Albert Kamanyi¹¹ Department of Animal Biology, Faculty of Science, University of Dschang, Box 67 Dschang, Cameroon.² Department of Chemistry, Faculty of Science, University of Yaoundé I, Box 812 Yaoundé, Cameroon.³ Department of Animal Physiology and Biology, Faculty of Science, University of Yaoundé I, Box 812 Yaoundé, Cameroon.

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ABSTRACT

Objective: The aim of the study was to investigate the prosexual activity of two compounds isolated from the hexane extract of the dried roots of *Mondia whitei* in rats. **Methods:** β -sitosterol and the mixture of α and β -amyrin acetate were isolated and characterised using several reagents, chromatography, mass spectrometry and physical analysis (RMN, ¹H and ¹³C). Sexually inexperienced adult male rats were orally treated with these compounds at doses of 0mg/kg (control), 10mg/kg or 50mg/kg. 1h after the treatment, mount and intromission latencies and frequencies, penile erection, ejaculation latency and post-ejaculatory interval were measured for 60 minutes. **Results:** β -sitosterol and the mixture of α and β -amyrin acetate significantly increased the mount frequency ($p < 0.05$), penile erection ($P < 0.001$) and the ejaculation latency when compared to control. The intromission frequency remained unchanged ($P > 0.05$) when compared to control. The two purified compounds were efficient at the lower dose (10 mg/kg b.w) with β -sitosterol being the most potent. **Conclusion:** Results of the present work give added value to the aphrodisiac property of *Mondia whitei* and further justify its popular use as a sex stimulant.

1. Introduction

Mondia whitei (Hook) Skeels (Periplocaceae) is a large woody liana with a pleasant vanilla smell. It's found in the Sudano-guinean zone. In the Southern and Western parts of Cameroon, it is known as « Limte », Nkang Bongo », or « Yang ». The roots are used as spices, aphrodisiac or to treat urinary tract infection, jaundice and headache while the whole plant is used to treat diarrhoea[1]. Chemical compounds already isolated in this plant include 2-hydroxy-4-methoxy-benzaldehyde, propacin, 5-chloropropacin and 5'-methoxypropacin[2, 3]. During our earlier studies of the reproductive function in male rats and guinea-pigs and by testing various extracts from this plant, we reported: 1- the androgenic effects of the aqueous and

hexane samples in rats[4,5]; 2- the relaxant properties of the methylene chloride:methanol (1:1), hexane and methanol extracts on KCl and adrenaline-induced rat vas deferens contractions[6]; 3- the expression of sexual behaviour induced by the aqueous and hexane extracts in sexually inactive rats[7] and, 4- the relaxant effects of the aqueous, hexane and methylene chloride extracts on guinea-pig isolated corpus cavernosum[8]. On the basis of the above mentioned findings, it was suggested that the bioactive principle(s) present in these extracts may be responsible for the reported biological activities with the hexane extract being the most efficient. The present investigation was therefore undertaken to examine the aphrodisiac activities of β -sitosterol and the mixture of α and β -amyrin acetate, two purified compounds, isolated from the hexane extract of *Mondia whitei* in sexually naïve albinos male rats.

2. Materials and Methods

*Corresponding author: Professor Pierre Watcho, Department of Animal Biology, Faculty of Science, University of Dschang, Box 67 Dschang, Cameroon
Tel: (237) 77 51 61 30 / 96 13 76 04
E-Mail: pwatcho@yahoo.fr.

2.1. Animals

In all, 50 Wistar rats (160–200g; > 90 days) of either sex (25 males and 25 females) were obtained from our colony. The animals were raised under standard conditions of temperature ($23 \pm 1^\circ\text{C}$) and light/dark cycle. They were fed a standard laboratory diet and water given ad libitum. Female rats were ovariectomized^[9] and brought one month later into oestrus by a sequential subcutaneous injection of $30 \mu\text{g}$ of estradiol benzoate (Sigma Chemicals, USA) and $600 \mu\text{g}$ of progesterone (Sigma Chemicals, USA), 48h and 6h respectively before testing. Furthermore, they were screened with non-experimental vigorous males and only females exhibiting good sexual receptivity and no rejection behaviour were employed in the tests. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established by the European Union on Animal Care and Experimentation (CEE Council 86/609).

2.2. Plant material

Fresh roots of *Mondia whitei* were collected in Dschang, Cameroon. Botanical identification was done at the Cameroon National Herbarium (HNC), Yaoundé, Cameroon. It has been deposited in the herbarium with a Herbarium Voucher Specimen No. 42920/HNC (collected by Westphal No.10050). The roots of *Mondia whitei* were cut into small pieces of about 1.5–2cm, air-dried and powdered using an electric grinder (Moulinex).

2.2.1. Isolation and characterisation of compounds

Air-dried and powdered roots (4 kg) of *Mondia whitei* was sequentially extracted with hexane, ethyl acetate and methanol at room temperature for 48h each and occasionally stirred. After filtration using Whatman paper N^o 3, the filtrate was evaporated under reduced pressure to yield 70g of hexane extract, 70g of ethyl acetate extract and 120g of methanol extract. The hexane extract (70g) was subjected to vacuum liquid chromatography over silica gel using a mixture of hexane–ethyl acetate and ethyl acetate–methanol of increasing polarity. 63 fractions of 500 ml were collected and combined into 6 main fractions [A (5g), B (15g), C (15g), D (10g), E (9g) and F (7g)] by thin layer chromatography examination. Fraction B (15g) obtained at the polarity of hexane–ethyl acetate (90–10) was subjected to column chromatography over silica gel, using a mixture of hexane–ethyl acetate of increasing polarity as eluent. 40 fractions of 100mL each were collected and combined into 5 main fractions (F1, F2, F3, F4, and F5) by the thin layer chromatography analysis. Fraction F2 (2g) obtained at a polarity of hexane–ethyl acetate (95–5) was crystallized with hexane to yield compound 1 (65 mg). Compound 2 (47 mg) was obtained from the crystallization with methanol of fraction F4 (3g) collected at the polarity of hexane–ethyl acetate

(80–20). The structures of compound 1 and compound 2 were elucidated by analysis of their spectroscopic data, especially by 1D and 2D NMR and by comparison with those obtained from the literature. Compound 1 was characterized as a mixture of α -amyirin acetate and β -amyirin acetate^[10,11] (MW1) and compound 2 as β -sitosterol^[11] (MW2) (Figure 1).

2.2.2. Preparation of the extracts

Two hundred and eighty mg (280 mg) of either β -sitosterol or the mixture of α and β -amyirin acetate were dissolved in 5mL of ethanol 95% and the final volume adjusted to 20mL with distilled water (15mL). The doses used in our study were 10 and 50mg/kg of body weight (b.w).

2.3. Treatment

Sexually naïve animals were randomly divided into five groups of 5 rats each. Group 1 received the vehicle (10mL/kg b.w of 5mL of ethanol 95% in 15mL of distilled water) and served as control. Groups 2 and 3 received 10 and 50mg/kg b.w of the mixture of α and β -amyirin acetate whereas Groups 4 and 5 were treated with 10 and 50mg/kg b.w of β -sitosterol respectively. The different doses of the pure compounds were orally given (single administration) to animals via a gastric tube 2h after the onset of darkness.

2.4. Sexual behavioural testing protocol

All sexual behaviour tests were conducted 2h after the onset of darkness (08:00 p.m local time) in a quiet room for 1h duration. After a 10 minutes adaptation period in the copulation cage, a stimulus-receptive female rat was gently introduced and the sexual behaviour recorded along 60 minutes. The following parameters were analyzed according to standard methods^[12–14]: mount (ML) and intromission latencies (IL), the time elapsed from the introduction of the female into a cage until the first mount and intromission respectively; mount (MF) and intromission frequencies (IF), the number of mounts and intromission preceding ejaculation respectively; ejaculation latency (EL), the time from the first intromission to ejaculation; post-ejaculatory interval (PEI), the time from the first ejaculation to a new first intromission; penile erection (PE), the number of times the rat bent down to lick the penis.

2.5. Statistics

Data are presented as means \pm SEM. The behavioural parameters were analyzed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. One way analysis of variance (ANOVA) was followed by Dunnet post-hoc test. The test was significant if $P < 0.05$.

3. Results

Figure 2 summarises the data obtained with the heterosexual behavioural study. Single dose administration of the purified compounds significantly ($P < 0.05$ – 0.001) improved the sexual behaviour of sexually inactive male rats and the effects were more expressed at the lower dose (10mg/kg b.w). The mount frequency (MF), penile erection (PE) and ejaculation latency (EL) were particularly increased ($P < 0.05$ – 0.001) in rats receiving 10 mg/kg b.w of β -sitosterol than in the

other groups. At all doses, the intromission frequency (IF), mount latency (ML) and intromission latency (IL) remained statistically unchanged although a trend toward an increase can be observed in the case of IF. As for the post-ejaculatory interval (PEI), only rats treated with 50mg/kg b.w of β -sitosterol showed a significant increase ($P < 0.001$) compared to control rats. For each compound, the lower dose was more potent than the highest one.

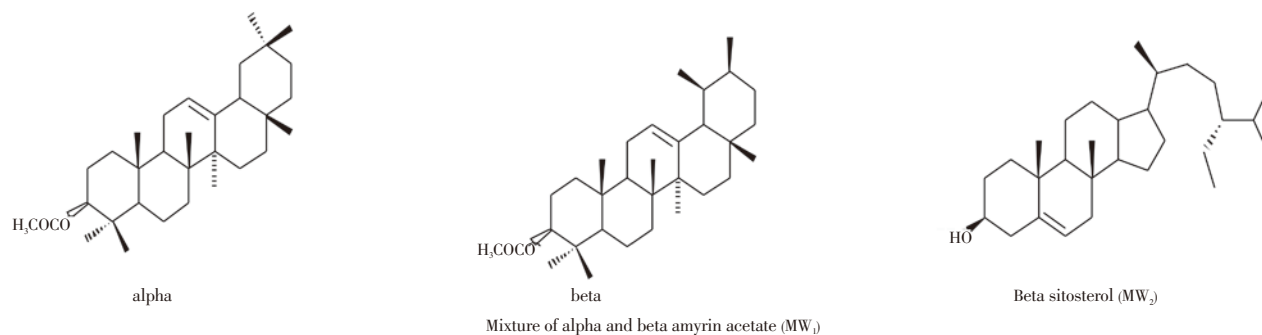


Figure 1. Chemical structures of the compounds isolated from the hexane extract of *Mondia whitei*.

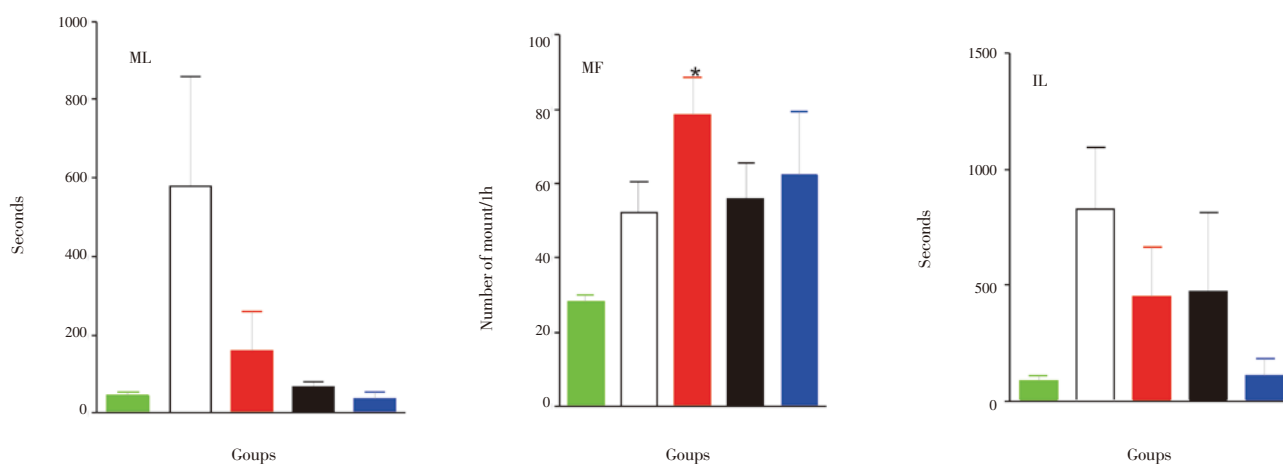


Figure 2. Effects of the mixture of α and β -amyirin acetate (MW₁) and β -sitosterol (MW₂) isolated from the hexane extract of *Mondia whitei* on sexual performance of naive rat.

Values are Mean \pm SEM; number of rats per dose = 5

*: $P < 0.05$ and **: $P < 0.001$ significantly different compared to control rats.

ML: mount latency; MF: mount frequency; IL: intromission latency; IF: intromission frequency; PE: penile erection; EL: ejaculation latency; PEI: post-ejaculatory interval.

4. Discussion

Results of the present study revealed the ability of the purified *Mondia whitei* products to enhance the erectile function in sexually inexperienced male rats. The sexual facilitatory action of these compounds was more expressed at lower dose (10mg/kg b.w) and the effect of β -sitosterol was comparatively greater than that of the mixture of α and β -amyirin acetate. The significance of this finding may relate to 1) the inhibitory effect at high dose and 2) the chemical classes of the compounds. Indeed, phytochemical characterisation of the hexane extract of *Mondia whitei* showed that the mixture of α and β -amyirin acetate is a

triterpen in nature whereas β -sitosterol belongs to the steroid group. It has been demonstrated that administration of triterpen such as forskoline or steroid such as testosterone stimulates male sexual activity[15,16]. However, data from the literature also reveal the antifertility effect of α -amyirin acetate in rat[17] and the safe or detrimental properties of β -sitosterol on the reproductive profile of the American mink (*Neovison vison*)[18], in male fighting fish *Betta splendens* [19] and mouse[20]. In the present study, the significant increases in MF, PE, EL as well as the trend in the increase of IF denote the ability of the isolated compounds to induce pro-sexual performance of naive rats and further support the aphrodisiac potential of *Mondia whitei*. According to

Sandroni^[21], aphrodisiacs imply substances that increase libido, potency and sexual pleasure. In line with this notion, it could be suggested that the two purified principles extracted from the hexane extract of *Mondia whitei* act by inducing changes in the levels of neurotransmitters or modulating the action of these neurotransmitters at the level of their target cells, or by increasing the androgen levels^[22, 23]. In a previous work, we reported the androgenic effect of the hexane extract of *Mondia whitei* in adult rats^[5] and which may be of great support as for the possible androgen-dependent pathway of *Mondia whitei* in the activation of the sexual performance of animals^[24,25]. Results of the present work give added value to the aphrodisiac property of *Mondia whitei* and further justify its popular use as a sex stimulant.

Conflict of interest statement

We declare that we have no conflict of interest.

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