

Influence of the type of Organ, the Origin and Regeneration Method of *Euadenia Trifoliolata* (Capparaceae) Schum & Thonn. Oliv. on the Sexual Behavior of Rats

Monh Alice Fah¹, Sifolo Seydou Coulibaly^{2*}, Kouakou Laurent Kouakou¹, Founzégué Amadou Coulibaly³, Witabouna Mamidou Koné¹ and Arsène Irié Bi Zoro¹

¹Laboratory of Functional Genomics and Breeding, University Nangui Abrogoua, Abidjan, Côte d'Ivoire

²Department of Biological Sciences, University "Peleforo Gon Coulibaly", Korhogo, Côte d'Ivoire

³Laboratory of Biochemistry, Institut Pasteur, Abidjan, Côte d'Ivoire

*Corresponding author: Sifolo Seydou Coulibaly, Department of Biological Sciences, University "Peleforo Gon Coulibaly", Korhogo, BP 1328 Korhogo, Côte d'Ivoire, Tel: +22508658467, +15095921202; E-mail: coulsifsey@gmail.com

Abstract

Euadenia trifoliolata is a plant that roots are used to treat earache, headache, inflammation, otitis, anemia, respiratory failure but little is known about its efficacy in the treatment of erectile dysfunction (ED). The present study aims to evaluate the effectiveness of *Euadenia trifoliolata* in the treatment of ED and determine the optimum dose that can induced maximum penile erection. Different concentrations of extracts from leaves and roots of plant from different origins were injected *per os* to rats and the sexual parameters were recorded during the experiment. Results showed that *Euadenia trifoliolata* roots as well as leaves had aphrodisiac effect. The concentrations of leaves extracts (100 mg/kg, 250 mg/kg and 500 mg/kg b.w.) increased more penile erection than the controls. The aphrodisiac effect of *Euadenia trifoliolata* differed significantly in function of the origin of the plant. The regenerated plant showed better aphrodisiac activity on rats. Among the tested concentrations, 250 mg/kg b.w. showed higher positive impact on the sexual behavior of rats. *Euadenia trifoliolata* can be regenerated and used in the treatment of ED. It can be recommended to use leaves rather than roots for sustainable management of the plant.

Keywords: *Type of organ; Origin; Regeneration method; Euadenia trifoliolata; Sexual behavior Rats*

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Introduction

Nowadays, reproductive health remains a worrying situation worldwide due to several factors. Erectile dysfunction (ED), one of the main causes, is the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse

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[1]. In 1995, ED affected over 152 million men worldwide and the projection for 2025 is a prevalence of 322 million men worldwide with the largest projected increases in the developing world that is Africa, Asia, and South America [1]. ED is very widespread and affects men at all ages especially between 40 years and 70 years [1]. More than 30% of couples in developing countries are suffering from ED of male origin [2]. ED is considered as a sentinel of health status in males. It is involved in several diseases such as diabetes, cardiovascular insufficiency, urinary bladder, kidney insufficiency [3].

In Côte d'Ivoire, more than 2,128,691 and 1,334,784 persons respectively in rural and urban areas either 35% of the global population is suffering from ED [4-6]. Different modern treatments such as psychosexual, transurethral and oral therapies, penile prostheses, revascularization and extracavernous injections are available to treat ED [7]. But these treatments are out of reach of people with limited financial means. This situation has led people to traditional medicine which remains the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities and local therapy [8,9]. Plants have been used in traditional medicine for several thousand years [10]. According to the world health organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary health care needs [11].

Three plants (*Caesalpinia bonduc*, *Bridelia ferruginea* and *Palisota hirsuta*) have been identified scientifically for ED treatment in Côte d'Ivoire [12,13]. These plants are overexploited so that they are on the way to disappear. Thus, identification of new plants in ED treatment is needed for a sustainable use of these natural resources. In these recent years, a new plant namely *Euadenia trifoliolata* has been identified in rural areas and traditionally used to treat ED. *Euadenia trifoliolata* is a leafy shrub that grows up to 4 m high and is known for its effectiveness in the treatment of earache, headache, inflammation, otitis, anemia, and respiratory failure [14] and for water purification [15]. Traditionally, *Euadenia trifoliolata* leaves, roots and stems are used to improve sexual capacities in littoral area of Côte d'Ivoire. Despite its large use traditionally, there is no scientific evidence to prove its effectiveness in the treatment of ED. Investigating the potential of *Euadenia trifoliolata* to treat ED is a prerequisite to wide use this plant in the treatment of ED. The present study was carried out to investigate on the chemical composition of *Euadenia trifoliolata* and its effect on penile erection. We hypothesized that extracts from *Euadenia trifoliolata* are rich in compounds responsible of penile erection and increase penile erection.

Material and Methods

Animals and *Euadenia trifoliolata*

Sexually inexperienced Wistar rats (*Rattus norvegicus*) were provided by Animal Resource Management Unit (ARGU) of the "Institut Pasteur" of Côte d'Ivoire to be used in this study. The rats were weaned for 30 days and isolated from females until the age of 90 days (age of maturity). They were free to food access (FACI) and water *ad libidum*.

Some samples of *Euadenia trifoliolata* were harvested in Adzopé, Banco, Ehotilé islands, Marahoué and in the University Nangui Abrogoua (UNA). Adzopé is situated between latitudes 6°06 N - 6°25 N and longitudes 3°51 W - 3°36 W. Banco is located between latitudes 5°23 N - 5°40 N and longitudes 4°03 W - 4°07 W. As for Éhotilé islands, they are situated between latitudes 5°10 N - 5°37 N and longitudes 3°13 W - 3°44 W. Marahoué is between latitudes 7°05 N - 7°49 N and longitudes 6°01 W - 6°32 W and the UNA is located between latitudes 5°23 N - 5°43 N and longitudes 4°17 W - 5°43 W. *Euadenia trifoliolata* was found in these zones after a prospection. The samples harvested were identified at the "Centre National Floristique de l'Université Felix Houphouët Boigny de Cocody".

Preparation of plant extracts

The samples of leaves and roots of *Euadenia trifoliolata* harvested in the different environments were dried at room temperature and then crushed with a grinder "Culatti typ MFC" brand. The different powders obtained were macerated in distilled water, methanol and dichloromethane (DCM) using 400 g of powder for 1 L during 24 hours and then filtered. The residues were macerated again for 24 hours twice always in different solvents. After filtration, the filtrates were dried in an oven at 40°C to obtain the extracts [16,17]. Mean yields of wild and cultivated extracts of *Euadenia trifoliolata* were calculated and aqueous extracts were selected for the study. Thus, 1 g of extract was dissolved in 10 ml of distilled water. The doses of plant extracts used were 100 mg/kg, 250 mg/kg and 500 mg/kg b.w.

Phytochemical analysis

Phytochemical studies consisted of qualitative and quantitative analysis of extracts from wild and cultivated plants. The presence of various compounds in the plant extract were detected by preliminary phytochemical screening [18-20]. The contents of total polyphenols, tannins and flavonoids were determined respectively in the different extracts [21,22].

Treatment of animals

Forty-five sexually inexperienced rats were randomly assigned to one of the following groups: Group 1 receiving distilled water (negative control or vehicle). Groups 2 and 3 were treated respectively with sildenafil citrate (Viagra®) at a dose of 100 mg/kg b.w. and Mestersonone (testosterone) at a dose of 25 mg/kg b.w. as positive controls. Groups 4, 5 and 6 were treated with aqueous extracts of wild leaves while groups 7, 8 and 9 received wild roots extract. Groups 10, 11 and 12 were crammed with the extracts of leaves of cultivated plants while groups 13, 14 and 15 received the extracts of cultivated roots. After subjecting the animals to the different plant extracts and observing their reaction, the leaves extracts were selected to continue the study with a goal of protecting the plant. The doses from leaves (DL_e) received by the test groups were 100 mg/kg (D100), 250 mg/kg (D250) and 500 mg/kg (D500) b.w. The experiment was carried out for 28 days [7,23-25].

Study of sexual behavior of rats

One hour after crammage, the male rats were placed in a copulation cage for 10 minutes of acclimation. Then a female receptor was introduced and the behavior of the male was observed for 30 minutes. The copulatory parameters as open-field was carried out with the aim of removing the hypothesis of a possible reduction of the motility induced by the plant extract which could influence the sexual behavior, mount latency (TLM) that is the time separating the introduction of a female into the cage from the first goes up, ejaculation latency (TLE) that is the time that separates the first intromission and the first ejaculation, frequency of goes up (FG) that is the number of goes up with or without intromission which precedes an ejaculation, ejaculation frequency (FDE) that is the number of ejaculation recorded in the course of the time of observation, and post-ejaculatory interval (PEI). Also the time separating an ejaculation from the very next non ejaculatory intromission was recorded [26-29].

Statistical analysis

Data were expressed as mean ± deviation (ESM). The composition of plant's extract and the effect of factors (organ type, origin and regeneration method) on each copulatory parameter were analyzed using the repeated measurement Anova test. The test of turkey was applied for pair wise comparison of the plant's composition. Comparison of the control and tested groups was made using one way-Anova followed by the non-parametric test of Dunnett. All analysis was performed using the Graph Pad Prism software V5.01 software (Washington, USA).

Results

Phytochemical analysis

Table 1 contains the quantitative results of *Euadenia trifoliolata*. It appeared that the concentrations of polyphenols, tannins and flavonoids in the raw leaves and root extract differed in function of the origin of the plants. Concerning leaves, content of polyphenols extracted from Adzopé was lower than that of Banco, Éhotilé Islands, Marahoué and UNA that were equal statistically. The highest content in tannins (42.88 ± 0.87 mg/g) was observed in the leaves from Banco and the lowest (20.19 ± 6.34 mg/g) in the leaves from Marahoué. According to the test of Turkey, there was no significant difference between tannins content in leaves from Ehotilée and from Marahoué. Tannins content in leaves from Adzopé and UNA were also equal. Flavonoids content in leaves from Banco and Adzopé were not statistically different but lower than those from Ehotilé, Marahoué and UNA.

In roots, the highest content of polyphenols (0.18 ± 0.11 mg/g) was recorded from Marahoué and the lowest (0.07 ± 0.03 mg/g) from Adzopé. Tannins content varied from 6.72 ± 5.90 mg/g in roots from Ehotilé to 23.63 ± 13.08 mg/g in roots from Adzopé. Flavonoids content was similar in roots from Adzopé, Ehotilé, Marahoué and UNA (BLCR).

Aphrodisiac activity of *Euadenia trifoliolata*

Comparative effect of organs

There was a variation in the motility time of rats after injection of different concentrations of extracts from leaves and roots (Figure 1). Extracts from roots and leaves at (100, and 500) mg/kg b.w. induced a motility of 4 minutes when that at 250 mg/kg provoked 6 minutes of motility. According to Turkey test, the motility time at 250 mg/kg b.w. (6 minutes) was higher than the 4 minutes obtained at 100 and 500 mg/kg b.w. These results showed better open field test with extracts at 250 mg/kg b.w. compared to 500 mg/kg and 100 mg/kg b.w.

The number of erections differed significantly after injected to rats leaves extracts from different origins (Table 2). With an extract concentration of 500 mg/kg, 250 mg/kg and 100 mg/kg b.w., the number of erections varied from 1 to 4. The highest number of erections for each extract concentration (4) was obtained with leaves from BLCR and the lowest was obtained with leave extracts from Banco and Ehotilé Island. However, according to the test of Dunett, these latter were statistically equal to that of the control (1). At a dose of 250 mg/kg b.w., there was no significant difference between the number of erections observed with extract from Adzopé and that from BLCR (UNA). The decreasing order in number of erections after extract injection in rats was obtained with the following origins; BLCR > Adzopé > Marahoué > Banco \geq Ehotilé \geq Vehicle.

Organs	Compounds	Origin				
	Content (mg/g)	Adzopé	Banco	Ehotilé Island	Marahoué	UNA
Leaves	Polyphenols	$0.07 \pm 0.04^*$	$0.09 \pm 0.03^{**}$	$0.09 \pm 0.02^{**}$	$0.09 \pm 0.02^{**}$	$0.09 \pm 0.02^{**}$
	Tannins	$33.09 \pm 13.16^{**}$	$42.88 \pm 0.87^{***}$	$22.51 \pm 7.57^*$	$20.19 \pm 6.34^*$	$32.92 \pm 6.49^{**}$
	Flavonoids	$0.27 \pm 0.06^*$	$0.29 \pm 0.03^*$	$0.32 \pm 0.04^{**}$	$0.35 \pm 0.01^{**}$	$0.31 \pm 0.09^{**}$
Roots	Polyphenols	$0.07 \pm 0.03^*$	$0.08 \pm 0.03^{**}$	$0.10 \pm 0.02^{**}$	$0.18 \pm 0.11^{***}$	$0.09 \pm 0.02^{**}$
	Tannins	$23.63 \pm 13.08^{***}$	$23.02 \pm 2.34^{**}$	$6.72 \pm 5.90^*$	$7.99 \pm 2.09^*$	$21.90 \pm 0.18^{**}$
	Flavonoids	$0.34 \pm 0.03^{**}$	$0.28 \pm 0.06^*$	$0.35 \pm 0.00^{**}$	$0.33 \pm 0.03^{**}$	$0.31 \pm 0.09^{**}$

Table 1: Phenolic compound in leaves and roots of *Euadenia trifoliolata* according to origin.

Note: Values are mean \pm SEM (n = 5). ***P < 0.0001; **P < 0.001; *P < 0.05 compared to group in the same line (One-way ANOVA followed by Turkey's post hoc test).

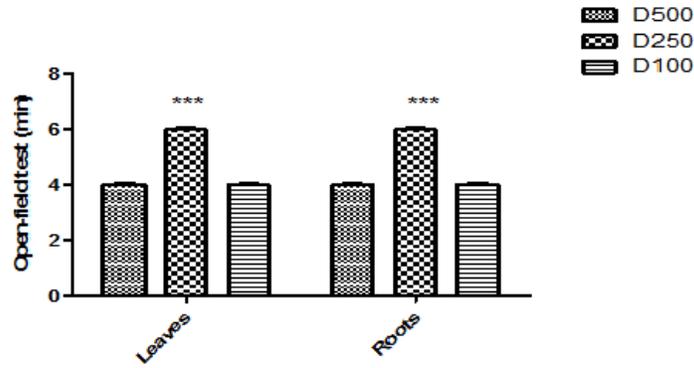


Figure 1: Comparative effect of organs on open-field test.

Note: Values are means \pm SEM. (n =5). ***showed that the better dose to obtain highest copulatory parameters.

Origins	Doses (mg/Kg)		
	DLe500	DLe250	DLe100
Vehicle	1 \pm 0.00	1 \pm 0.00	1 \pm 0.00
Adzopé	3 \pm 0.00**	4 \pm 0.00***	3 \pm 0.00**
Banco	2 \pm 0.00	2 \pm 0.00	2 \pm 0.00
Ehotilé Island	2 \pm 0.00	2 \pm 0.00	1 \pm 0.00
Marahoué	3 \pm 0.00**	3 \pm 0.00**	3 \pm 0.00**
BLCR	4 \pm 0.00***	4 \pm 0.00***	4 \pm 0.00***

Table 2: Effect of leaves origin on number of erections.

Note: Value are mean \pm SEM (n=5). Data were analyzed by one-way ANOVA, followed by Dunett post hoc test. **p < 0.01 < ***p<0.001 significantly different compared to the vehicle in the same column.

Sexual behavior of rats differed in function of the origin of leaves. There was significant difference between the latency time of goes up that varied in function of leaves origin and the doses (Figure 2a). The shortest latency time was obtained with DLe 250 from Adzopé, Marahoué and BLCR. Frequencies of goes up also varied in function of the leave origin and the doses (Figure 2b). The test of ANOVA showed that whatever the origin the highest frequency of goes up was observed with the DLe 250. According to the test of Turkey, the increasing order of the frequency of goes up with DLe 250 was obtained respectively with the origin BLCR, Marahoué, Adzopé, Banco and Ehotilé Island.

Relatively to the ejaculation time (Figure 2c), there was no significant difference between the doses effect from extracts of a same origin. The highest ejaculation latency times were observed with the origin BLCR followed in decreasing order by Marahoué, Ehotilé Island, Banco and Adzopé.

The frequency of ejaculation increased in the groups crammed by leaves extracts from medium BLCR, Marahoué and Adzopé compared to vehicle (Table 3). Results showed that with different doses, the frequency of ejaculation in rats varied from 1 to 8. The test of ANOVA showed that there was a significant difference ($P<0.05$) between the frequencies of ejaculation in the rats in function of leaves origin. The highest frequency of ejaculation was obtained with the leaves from BLCR and the lowest (1) was got with the leaves from vehicle. DLe 250 registered the highest frequency of ejaculation.

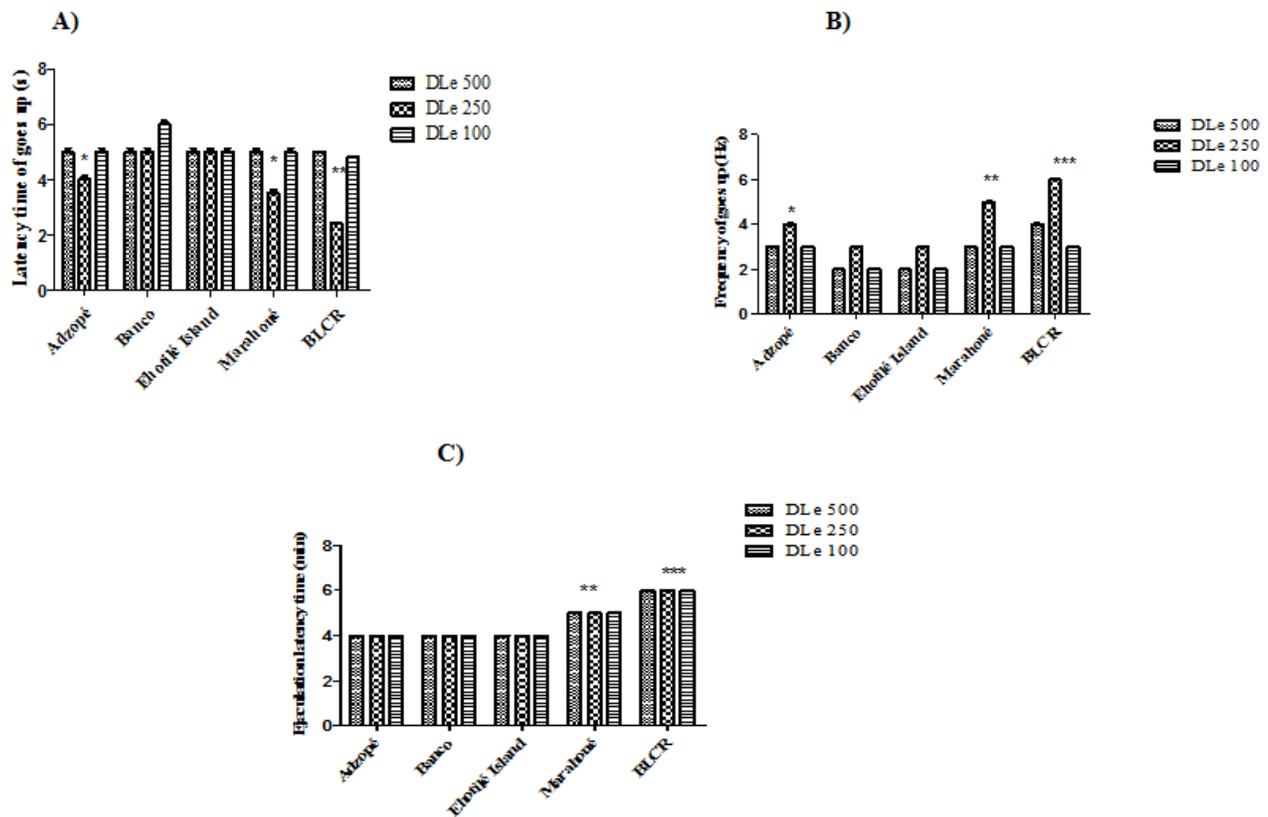


Figure 2: Effect of leaves origin on sexual behavior of rats, (A) latency time of goes up, (B) frequency of goes up, and (C) latency time of ejaculation.

Note: Value are mean ± SEM (n = 5). ***P < 0.0001; **P < 0.001; *P < 0.05 compared to group (One-way ANOVA followed by Turkey’s post hoc test).

These results suggested that the extracts from different origins increased differently the frequency of ejaculation. This parameter was highly influenced by the leaves extracts from the medium BLCR.

Origins	Doses (mg/Kg)		
	DLe500	DLe250	DLe100
Vehicle	1 ± 0.00	1 ± 0.00	1 ± 0.00
Adzopé	3 ± 0.03**	4 ± 0.03**	3 ± 0.03**
Banco	3 ± 0.03**	3 ± 0.03**	1,8 ± 0.03
Ehotilé Island	2 ± 0.03	2 ± 0.03	1 ± 0.03
Marahoué	3 ± 0.03**	4 ± 0.03**	2 ± 0.03
BLCR	6 ± 0.03***	8 ± 0.03***	6 ± 0.03***

Table 3: Effect of leaves origin on frequency of ejaculation.

Note: Value are mean ± SEM (n=5). Data were analyzed by one-way ANOVA, followed by Dunett post hoc test. **p < 0.01; ***p<0.001 significantly different compared to the vehicle in the same column.

Influence of regeneration methods on aphrodisiac effect

Table 4 presents the number of erections after administrating to rats’ leaves extracts from BLCR medium compared to controls. The number of erections varied significantly from one dose to another in function of the type of treatment. For each treatment, the lowest number of erections (1) was obtained with vehicle and the highest number of erections with DLe 250. According to the test of Dunett, the number of erections obtained with DLe 500 (6), DLe 250 (8) and DLe 100 (5) were

statistically equal and significantly higher than those recorded with the controls as vehicle (1), Mes 25 (2), Via 100 (3). These results showed that the different concentrations of extract (DLe 500, DLe 250 and DLe 100) influenced more the number of erections than the controls.

Doses (mg/Kg)	Treatment			
	Vehicle	Mesteronone	Sildenafil	BLCR
Vehicle	1± 0.00	1± 0.00	1± 0.00	1± 0.00
Mes 25	2± 0.00	2± 0.00	2± 0.00	2± 0.00
Via 100	3± 0.00	3± 0.00	3± 0.00	3± 0.00
DLe500	6± 0.00***	6± 0.00***	6± 0.00***	6± 0.00***
DLe250	8± 0.00***	8± 0.00***	8± 0.00***	8± 0.00***
DLe100	5± 0.00***	5± 0.00***	5± 0.00***	5± 0.00***

Table 4: Effect of BLCR on number of erections.

Note: Value are mean ± SEM (n=5). Data were analyzed by one-way ANOVA, followed by Dunett post hoc test.

***p<0.001 significantly different compared to all control groups in the same column.

The analysis of the results showed that the extract of cultivated leaves from different plants growth medium had a significant effect on the sexual behavior of rats (Figure 2).

The latency time of goes up differed significantly from one treatment to another (Figure 2a). The latency time obtained with vehicle was 6 seconds for each treatment. With Via100 and DLe 100, the latency time was constant (5 seconds) whatever the treatment. The latency time was 4 seconds when injected Mes 25 and DLe 500 to rats for each treatment. The shortest latency time (3 seconds) was obtained with DLe 250 whatever the type of treatment.

The frequency of goes up of rats varied significantly in function of the type of treatment (Figure 2b). For each treatment, the frequency of goes up was 2 Hz with vehicle, 3 Hz with Mes 25 and Via 100, 4 Hz with DLe 100 and DLe 500. The highest frequency of goes up was 6 Hz and that was registered with DLe 250. According to the test of Dunett, the frequency of goes up registered with DLe250 on one hand and those obtained with DLe 500 and DLe 100 on the other hand, were significantly higher than the ones recorded with the controls (vehicle, Mes 25 and Via 100).

The ejaculation latency time of the rats in function of the treatments is presented on Figure 2c. It appeared that the ejaculation latency time was 2 seconds with vehicle whatever the treatment with Mes 25 and Via 100, the ejaculation latency time was 3 seconds. After using, DLe 100, DLe 250 and DLe 500, the ejaculation latency time increased to 5 seconds. Statistically, the ejaculation latency time obtained with DLe 100, DLe 250 and DLe 500 were higher than the others.

Table 5 encapsulates the effect of leaves extracts from BLCR on the frequency of ejaculation. It appeared that the frequency of ejaculation varied significantly from one treatment to another. The frequency of ejaculation varied from 1 Hz to 5 Hz. Whatever the treatment, the frequency of ejaculation was 1 Hz with vehicle, 2 Hz with Mes 25 and Via 100, 3 Hz with DLe 500 and DLe 100 and 5 Hz with DLe 250. According to the test of Dunett, the frequency of ejaculation obtained with DLe 250 was the highest followed by those recorded with DLe 500 and DLe 100 which were statistically similar. These latter two were significantly higher than those obtained with the controls (Vehicle, Mes 25 and Via 100). However, there was no significant difference between the frequencies of ejaculation recorded with the controls.

Doses (mg/Kg)	Treatment			
	Vehicle	Mesteronone	Sildenafil	BLCR
Vehicle	1± 0.00	1± 0.00	1± 0.00	1± 0.00
Mes 25	2± 0.00	2± 0.00	2± 0.00	2± 0.00
Via 100	2± 0.00	2± 0.00	2± 0.00	2± 0.00
DLe 500	3± 0.01**	3± 0.01**	3± 0.01**	3± 0.01**
DLe 250	5± 0.01***	5± 0.01***	5± 0.01***	5± 0.01***
DLe 100	3± 0.01**	3± 0.01**	3± 0.01**	3± 0.01**

Table 5: Effect of BLCR on frequency of ejaculation.

Note: Value are mean ± SEM (n=5). Data were analyzed by one-way ANOVA, followed by Dunett post hoc test. **p<0.01 significantly different compared to the vehicle, ***p<0.001 significantly different compared to all control groups in the same column.

Discussion

Aphrodisiac activity of *Euadenia trifoliolata*

Results showed that *Euadenia trifoliolata* leave, and roots contained flavonoids, tannins and polyphenols but at different concentrations in function of the origin of organs. The presence of these compounds in leave and roots of *Euadenia trifoliolata* testified the aphrodisiac effect of the plant. Phytochemicals such as flavonoids, tannins and polyphenols are known for their erectile potential [14]. The difference in the concentrations of the phytochemical compounds may depend on the growing environment of the plant. In fact, it has been showed that biotic stresses due to insects or abiotic factors such as light and water can affect the concentrations of phytochemical elements [14].

The treatment of rats with aqueous extracts of leaves and roots would have induced a change in their sexual behavior. This was observed by the changes made in the open field test, the number of erection, in the sessions of goes up, intromission and the frequencies of goes up, intromission and ejaculation. The change in the sexual behavior of rats could be induced by *Euadenia trifoliolata* extracts. Extracts from *Euadenia trifoliolata* might provoke a massive production of testosterone that could increase the frequency of goes up, the frequency of intromission and the frequency of ejaculation. In fact, testosterone is an androgen involved in the expression of secondary sex characteristics in males [30,31]. The extracts would have stimulated the hypothalamic-pituitary-testicular axis which in turn would induce abundant secretion of gonadotropins LH (luteinizing hormone) and FSH (follicle stimulating hormone) in rats. Similarly, Kpomah et al. [32] noted a reduction of goes up latency, and a reduction in the latency time of intromission after injected to rats extracts from two plant species *Zanthoxylum leprieurii* (Rutaceae) and *Piper guineense* (Piperaceae). They attributed that change in rat’s behavior to the aphrodisiac effect of the plants. According to authors, administration of the extracts from *Zanthoxylum leprieurii* (Rutaceae) and *Piper guineense* (Piperaceae) increases the production of testosterone, luteinizing hormone and follicle stimulating hormone that affect positively the sexual behavior of rats [32].

Oral administration of aqueous extracts of leaves and roots of *Euadenia trifoliolata* influenced the open-field test that increased in rats. Among the different concentrations tested, 250 mg/kg got the higher positive influence on sexual behavior of rats compared to others. This result suggested that all organs of *Euadenia trifoliolata* stimulated the sexual activity of the rats. The highest sexual activity observed with the dose of 250 mg /kg bw showed that that concentration of extracts from *Euadenia trifoliolata* leaves and roots is the most suitable to induce the best penile erection. However, our most suitable

concentration for highest penile erection was lower than the 600 mg/kg indicated as the best concentration of *Zingiber officinale* (Zingiberaceae) and *Pentadiplandra brazzeana* (Pentadiplandraceae) [33]. These differences in concentrations of extracts could be explained by the difference in the plant species and their chemical characteristics.

Influence of *E. trifoliolata* origin on aphrodisiac effect

The aqueous extracts of the leaves of various origins were subjected to the various copulatory tests that are the number of erection, the frequency of mounting, latency time of goes up, ejaculation latency, and frequency of ejaculation. The latency time of goes up decreased in the rats that received extracts from BLCR growth medium, Marahoué and Adzopé compared to Banco, and Éhotilé Island. The frequency of goes up increased in the rats crammed by extracts from BLCR growth medium, Marahoué and Adzopé compared to Banco and Éhotilé Island. The latency time of ejaculation and the frequency of ejaculation increased in rats that received extracts from BLCR medium, Marahoué followed by Adzopé, Banco and Éhotilé Island. Sexual stimulating effect of the plant's origin could be attributed to the favorable environment (biotic and abiotic) which would have favored plant transpiration and absorbed the elements like flavonoids, sterol and polyterpenes compounds necessary for the synthesis of the compounds. BLCR medium might favor the biosynthesis of the nitrite oxide (NO) and hormones involved in the erectile function in the rats. The erectile stimulating factors would act either at the neurotransmitter level or at the target cell level [34]. These results also showed that the stimulating effect of extracts of *Euadenia trifoliolata* depends on the zone of harvest of the plants. Since the harvesting zone has an effect on the biological activity of *Euadenia trifoliolata*, the *Ex situ* regeneration was carried out in order to envisage a domestication of the species after having tested the aphrodisiac effect of the regenerated plants.

Influence of the regeneration methods on aphrodisiac effect

The rats that received extracts from leaves harvested in the BLCR medium showed the most pronounced aphrodisiac effect. This reaction could be attributed to the growing environment. In fact, plants, during their growth would have undergone stresses which could be at the origin of the production of the compounds such as saponins, alkaloids in addition to the flavonoids, sterols and polyterpenes. The growing soil might made available more nutrients to plants necessary for the synthesis of compounds in sufficient quantity and quality. Also, this would have increased the nutrient exchange capacity at the root level involved in the synthesis of these sex-based plant's compounds. The compounds squeezed phyto-alexines produced by plants to defend themselves against bio-aggressors. Sexually-active compounds would also increase the diameter of the blood vessels in erection-promoting sexual organs [35]. In this study, it appeared that the leaves extracts from the cultivated plants stimulate more sexual activity in rats than that of wild environment. This could be linked to competition for nutrients in wild environment. In contrast, in a growing medium, competition is less as nutrients are led to the growing plant. Young plants would have a high photosynthesis at the leaf surface, which would have allowed an accumulation of compounds having an aphrodisiac activity. Roots of those plants being in contact with the ground would have accumulated an abundant amount of bioactive compounds involved in the mechanism of erection. The compounds induced an increase in serum levels of androgens [34,36] whose stimulate the expression of the neuronal isoform of nitric oxide synthase [37,38]. The effect of the organs was more appreciated at the dose of 250 mg/kg b.w. that showed that the noradrenalin would have been sufficient to cause contraction of the smooth muscles of the cavernous body. Also, the physiology of erection that requires synergistic vascular and tissue intervention under neuropsychic and hormonal control (reflex phenomenon) could be triggered [39]. Administration of plant extracts at 250 mg/kg b.w. would have acted on the erectory regulating center which would activate

the parasympathetic system by cholinergic or non-cholinergic chemical mediator (nitric oxide (NO)) and inhibit sympathetic system [38]. At this dose the phosphodiesterase is modulated by androgens and gonadal steroids [40,41].

Locally in the penis, nerve impulses would have permitted the release of pro-erectile neurotransmitters by the nerve endings of the cavernous bodies and the emission of relaxation factors from the endothelial cells at this dose. The synergistic action of myorelaxation and arterial vasodilation would have contributed to the filling of the sinusoidal spaces by the arterial blood and induced the variation of the volume and the rigidity of the penis. The dose of 250 mg/kg b.w. would have promoted the diffusion of NO in membrane cells to activate guanylate cyclase and transformed guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) [13].

Conclusion

Based on results obtained in this study, we conclude that *Euadenia trifoliolata* (Capparaceae) contains phytochemicals such as tannins, flavonoids, and sterols. *Euadenia trifoliolata* leaves and roots have the same effect on penile erection. The aphrodisiac activity was better performed at a concentration of 250 mg/kg of leaves extracts. The leaves from BLCR medium induce the better aphrodisiac activity. Overall, our results suggest that *Euadenia trifoliolata* has an aphrodisiac activity and hence could be recommended in the treatment of erectile dysfunction while using leaves extracts at a dose of 250 mg/kg b.w.

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Conflict of interest

There is no conflict of interest among authors.

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