OPEN ACCESS
Scholars International Journal of Traditional and Complementary Medicine
Abbreviated Key Title: Sch Int J Tradit Complement Med
ISSN 2616-8634 (Print) |ISSN 2617-3891 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Oestrogenic Activity of the Methanolic Extract of the Stem Bark of *Cylicodiscus gabunensis* Harms (Fabaceae)

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DOI: 10.36348/sijtcm.2022.v05i08.003 | **Received:** 15.07.2022 | **Accepted:** 23.08.2022 | **Published:** 28.09.2022

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Abstract

The aim of this study is to evaluate the estrogenic effects of the methanolic extract of the stem bark of *Cylicodiscus gabunensis* in the female rat. The evaluation focused on the effect of the extract on the oestrous cycle, the evolution of the fresh weight of the uterus and ovaries, the total cholesterol level, and the total protein and oestradiol levels. The doses of extract administered to the female rats were 100, 200 and 400 mg/kg for 28 days. Administration of *C. gabunensis* extract at 400 mg/kg induced an increase in total protein levels. The administration of the extract at doses of 100 and 200 mg/kg gives the same results as the negative control which is distilled water. No significant variation of the *C. gabunensis* extract on the other parameters studied was observed compared to the negative control. This study shows that *C. gabunensis* would have anti-estrogenic effects, additional work should be carried out in order to validate the use of this extract in the treatment of female infertility.

Keywords: *Cylicodiscus gabunensis*, oestrous cycle, oestrogens, female fertility.

INTRODUCTION

Since antiquity, mankind has used various plants found in its environment, in order to treat and cure all kinds of diseases [1]. Even today, the treatment and control of diseases through the use of medicinal plants continue to play a decisive role in the implementation of medical care, particularly in developing countries and in the management of problems related to fertility [2, 3]. The latter is based on multiple factors that can be environmental, genetic, infectious and even hormonal. Oestrogen plays a crucial role in the normal physiology of various tissues, including the mammary glands, reproductive tract, central nervous system, and skeleton [4]. Oestrogen homeostasis in humans requires fine- tuning to maintain appropriate levels in the tissues to allow the performance of its intended functions; disruption of this balance can lead to the onset of various diseases [4]. Especially during menopause, in women, low serum levels of 17-β- oestradiol often lead to symptoms such as hot flushes, genitourinary atrophy, or degenerative processes such as osteoporosis [5].

It is estimated that 80% of the world's population uses medicinal plants in the treatment of diseases. This rate is also higher in African countries where up to 90% of the population depends on the use of medicinal plants to meet their primary health care needs [6]. Since traditional medicines have been used to regulate fertility since ancient times, a large number of plant species have been examined to demonstrate their effectiveness, including their use in female fertility relief and contraception. Studies have indeed demonstrated the involvement of many secondary metabolites from medicinal plants in the regulation of reproductive functions [7]. Several plants have been mentioned for this purpose by phytotherapists such as *Eremonastax speciosa*, *Ageratum conyzoides*, *Tetrapleura tetraptera*, *Mangifera indica*, *Mammea africana*, *Piper guineense* and *Cylicodicus gabunenesis* [8]. It is with a view to providing scientific validation to the uses of medicinal plants in the African

pharmacopoeia, particularly Cameroonian, in the treatment of female fertility problems that this work was carried out. The objective of this was to evaluate the estrogenic activity of the methanolic extract of the stem barks of *C. gabunenesis* on the female rat.

MATERIALS AND METHODS

Plant Material

The fresh stem bark of *Cylicodiscus gabunensis* was harvested in Nkong-Keni village, located in Bondjock district, Nyong-Ekelle department, (Centre, Cameroon).

Extraction

The freshly harvested bark was first cleared of all dead parts, then washed with water to remove any form of impurity. The fresh bark was weighed, then dried away from light and humidity for three weeks. The dried barks were crushed, then macerated in methanol for 72 hours. The solution obtained was filtered with Wattman paper and then concentrated using a rotary evaporator.

Phytochemical Screening

The detection tests for groups of chemical compounds were carried out by colouring and precipitation tests (sterols, coumarins, polyphenols, flavonoids, alkaloids, polyterpenes and saponins).

Animals and Treatments

Nulliparous, non-pregnant albino rats of the Wistar strain, 02 months old, weighing between 115 g and 160 g were used in this study. They were raised in the animal facility of the Department of Biology of Animal Organisms of the University of Douala. They were subjected to a 14-day acclimatization period in cages lined with shavings. They received tap water and a standard diet ad libitum throughout the experiment. Twenty-five rats were divided into 05 batches of 05 rats each. The treatments were as follows:

- Batch 1: control receives distilled water
- Batch 2: 1 mg Clomiphene per kg
- Batch 3: 100 mg *C. gabunensis* per kg
- Batch 4: 200 mg *C. gabunensis* per kg
- Batch 5: 400 mg *C. gabunensis* per kg

Evaluation of the Effect of the Extract on the Phases of the Oestrous Cycle of Animals

Every morning between 8 a.m. and 9 a.m. for 28 days, each batch of rats was transported to the experiment room in order to perform vaginal smears. Vaginal secretion from each rat was collected with a plastic micropipette filled with 10 µl of normal saline (0.9% NaCl), inserting the tip into the animal's vagina, but not deeply. Vaginal fluid was placed on glass slides. A different glass slide was used for each animal. The coverslip-covered slides were observed under the light microscope without the use of the condenser lens, with 10x and 40x objective lenses. Three types of cells can be recognized: round and nucleated cells (epithelial cells), irregular cells without a nucleus (cornified cells), and small round cells (leukocytes) were sought for the determination of the effect of the extract on the phases of the oestrous cycle of female rats [9].

Evolution of the Fresh Weight of the Uterus and Ovaries of Animals

The rats were sacrificed 24 hours after the last treatment, after the last weighing. The uterus and ovaries were carefully removed, separated from fatty adhesions and weighed separately.

Evaluation of Total Cholesterol, Total Protein and Oestradiol Level of Animals

The blood was collected after the sacrifice, in dry tubes previously labelled, then centrifuged and finally aliquoted. The right ovary of each rat was stripped of its tunica albuginea and ground to 15% with Tris buffer in the mortar of the homogenizer. The ground material in solution is centrifuged at 3000 rpm for 15 min. The supernatant was recovered and distributed in 25 Eppendorf tubes then stored at -20°C. The same protocol was done on the uterus of each rat. Total cholesterol was determined by colourimetry and total protein by spectrophotometry [10]. Oestradiol was assayed by the ELISA method using the BIOREX ELISA kit.

Statistical Analyzes

Data were analyzed statistically using Graphpad Prism 8.1 software and results expressed as mean ± standard deviation. The one-factor analysis of variance (ANOVA) applied to the results obtained made it possible to assess the effects of the different treatments and doses of products with a significance level of 5%. Dunett and Bonferroni tests were used to locate the origin of significance. The mean values of the parameters of the test groups were compared with those of the various controls.

RESULTS

Qualitative Phytochemical Study

Phytochemical screening revealed the presence of phenols, saponins, flavonoids, terpenes, sterols and coumarins in the methanolic extract of *Cylicodiscus gabunensis* (Table 1).

Table 1: Secondary metabolites of *Cylicodiscus gabunensis* stem bark extract

Secondary metabolites	Extract
Alkaloids	-
Saponins	+
Flavonoids	+
Anthraquinones	-
Free and combined quinones	-
Terpenes	+
Sterols	+
Coumarins	+
Phenols	+

(+): presence; (-): absence

Effect of Methanolic Extract from the Stem Bark of *Cylicodiscus Gabunensis* on the Oestrous Cycle

No significant change ($p > 0.05$) is observed between the batches treated with *Cylicodiscus gabunensis* extract and batch 1 which received distilled water. On the other hand, a significant increase ($p < 0.001$) in the frequency of oestrus is observed in batch 2 having received clomiphene compared to batch 1, negative control. A significant increase ($p < 0.05$) of the metoetrus in batches 3 (100 mg/kg of extract) and 5 (400 mg/kg of extract) is observed compared to batch 2, positive control. Similarly, a significant decrease ($p < 0.001$) in oestrus is observed in treated batches 3, 4 and 5 compared to batch 2.

Table 2: Effect of *Cylicodiscus gabunensis* stem bark extract on oestrous cycle

Frequency	distilled water	Clomiphene	Cg 100	Cg 200	Cg 400
Proestrus	0.4 ± 0.2	0.1 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
Œstrus	1.4 ± 0.2	3.1 ± 0.1$^{\alpha 3}$	1.3 ± 0.2$^{\beta 3}$	1.4 ± 0.2$^{\beta 3}$	1.1 ± 0.1$^{\beta 3}$
Metoestrus	0.9 ± 0.1	0.3 ± 0.2	1.0 ± 0.0$^{\beta 1}$	0.7 ± 0.2	1.1 ± 0.1$^{\beta 2}$
Dioestrus	1.1 ± 0.3	0.3 ± 0.2	1.0 ± 0.2	0.9 ± 0.3	1.1 ± 0.1

Cg: *Cylicodiscus gabunensis*. α3: $p < 0.001$ significant difference compared to the negative control group; β1: $p < 0.05$; β2: $p < 0.01$; β3: $p < 0.001$ significant difference compared to clomiphene.

Effect of the Methanolic Extract of *Cylicodiscus Gabunensis* Stem Bark on the Weight Change of the Uterus and Ovary

No significant variation ($p > 0.05$) in the mass of the ovaries was observed between the batches treated with *Cylicodiscus gabunensis* extract and the control batches 1 and 2 which received distilled water and Clomiphene respectively. Regarding the mass of the uterus, a significant increase ($p<0.01$) at the dose of 400 mg/kg of 55.38% compared to batch 1 and a significant decrease ($p<0.05$) in batch 2 positive controls compared to batch 1 are observed. At a dose of 100 mg/kg, the mass of the uterus increased significantly ($p<0.05$) compared to group 2, as well as at a dose of 400 mg/kg. At 200 mg/kg no significant variation is observed.

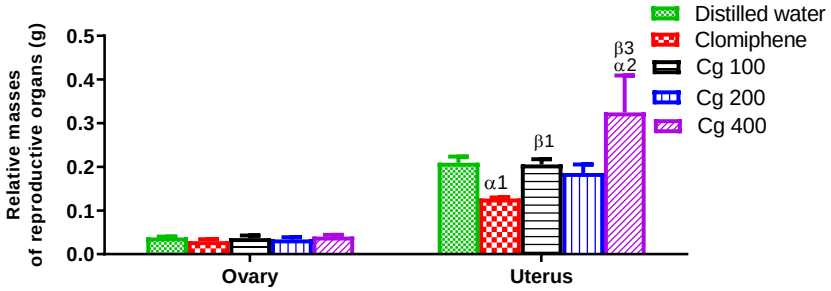

Figure 1: Effect of *Cylicodiscus gabunensis* stem bark extract on the weight change of the sexual organs

Cg: *Cylicodiscus gabunensis* α1: $p < 0.05$ significant difference compared to the negative control; α2: $p<0.01$; β1: $p< 0.05$ significant difference compared to the positive control β3: $p<0.001$.

Effect of *Cylicodiscus Gabunensis* Stem Bark Extract on Serum Oestrogenic Concentration

The serum oestrogenic concentration reveals a decrease in all batches compared to the control batch, but this drop is only significant ($p<0.05$) at 100 mg/kg with a raté of 70.57%.

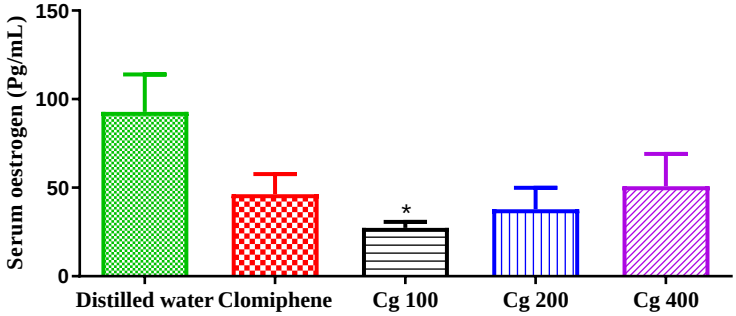

Figure 2: Effect of *Cylicodiscus gabunensis* stem bark extract on serum oestrogenic levels

Cg: *Cylicodiscus gabunensis*: $p < 0.05$ (significant difference compared to the negative control group)

Effect of *Cylicodiscus Gabunensis* Stem Bark Extract on Total Ovarian Cholesterol and Uterine Proteins

Total cholesterol did not undergo any significant variation (p>0.05) in the batches receiving the *Cylicodiscus gabunensis* extract compared to control batches 1 and 2 which received distilled water and Clomiphene respectively (Figure 3). At the level of the uterus, a significant increase in total proteins (p<0.01) at the dose of 400 mg/kg compared to control batches 1 and 2 is observed with a percentage increase of 218.18% for the control lot 1 and 136.18% for control lot 2 (Figure 4).

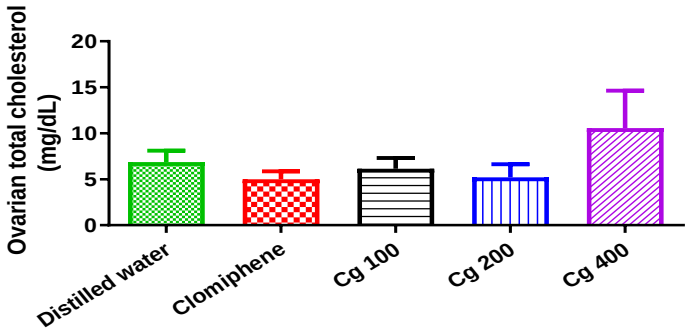

Figure 3: Effect of *Cylicodiscus gabunensis* stem bark extract on ovarian cholesterol levels

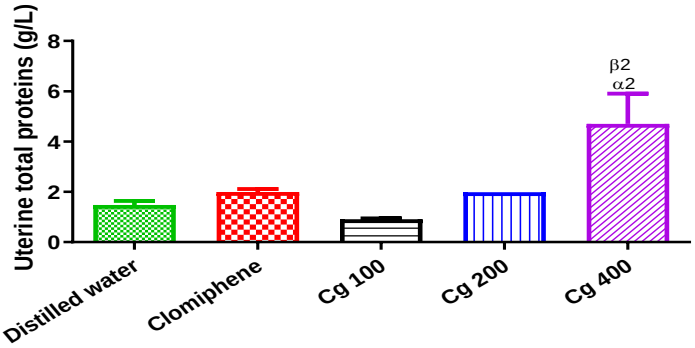

Figure 4: Effect of *Cylicodiscus gabunensis* stem bark extract on uterine protein levels

Cg: *Cylicodiscus gabunensis*; α2: ($p < 0.01$) significant difference compared to the neutral control group; β2: ($p < 0.01$) significant difference compared to batch 2 positive control.

DISCUSSION

Phytoestrogens are substances naturally present in plants with demonstrated oestrogenic activity *in vivo* (uterotrophy, vaginal cornification) [11]. The *in vivo* oestrogenic activity of *Cylicodiscus gabunensis* stem bark extract caused no significant changes in animal body weight during this study, demonstrating maintenance of standard metabolism and energy balance. The oestrogenic effects of the methanolic extracts of *C. gabunensis* observed in this study could be explained by the presence of flavonoids, in particular isoflavones [11]. Several studies have shown the action of flavonoids as substances with oestrogenic activities [12, 13]. This can cause inhibition of oestrogen synthesis and secretion by ovarian follicles as well as reduction in serum concentration by mimicking the effect of the hormone oestrogen [14]. Studies have shown that high doses of flavonoids lead to a reduction in oestrogenic effect through interaction with cytochrome P450 or by blocking CYP19, an enzyme very important in oestrogen biosynthesis [15] Uterotrophic assays are very often used as standard assays for the demonstration of oestrogenic activity *in vivo*. Administration of *C. gabunensis* extract induced a significant increase in total uterine protein. Similar results were obtained by some authors after administration respectively of the extract of *Holarrhena floribunda* (Apocynaceae) and the aqueous extract of the leaves of *Cissus aralioides* to rats [16, 17]. The administration of oestrogenic or oestrogen-like substances to rats induces an increase in mass and weight as well as aqueous imbibition of the uterus. The effects thus obtained can be explained by the fact that oestrogen very strongly stimulates the proliferation of cells in the uterine endometrium [18, 19]. Phytoestrogens, when administered in high doses, can cause a hormonal imbalance that can even put them in competition with oestrogens and behave like anti-oestrogens [20].

CONCLUSION

The study of the *in vivo* oestrogenic activity of *Cylicodiscus gabunensis* showed that the extract of the stem barks could exert anti-estrogenic effects in individuals normally secreting physiological oestrogens. Additional studies should be conducted to

scientifically validate the use of this extract in the treatment of female fertility.

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