Evaluation of the folliculo-stimulant activity of the aqueous leaf extract of *Cissus araliodes* on the vaginal epithelium of the doe (female rabbit)

Background: Reproductive disorders representing 15-30% of the African population cause great concern for many couples of childbearing ages. Among them, more than 80% have recourse to medicinal plants for treatment. This study aims to evaluate the folliculo-stimulant activity of the aqueous leaf extract of *Cissus araliodes* on the vaginal epithelium of the doe(female rabbit).

Methods: Phytochemical screening of this extract was first performed. Then, for the folliculo-stimulant activity, eight does were divided into fourgroups of threeeach and treated daily for two weeks. Group 1, which served as the control group, received orally distilled water, while groups 2, 3, and 4 wereorally administrated increasing doses of 200, 300, and 600 mg/kg body weight (b.w.) of the aqueous leaf extract of *C. araliodes*, respectively. Vaginal smears stained using the Papanicolaou method were used to observe the evolution of superficial cells before and during treatment.

Results: Phytochemical screening revealed the presence of polyphenols, flavonoids, catechic tannins, quinones, alkaloids and saponins in the leaf extract of *C. araliodes*.Regarding follicular activity, significant increases in the oestrogen index (OI) were observed four days after treatment of the does with *C. araliodes* extract at 300 and 600 mg/kg b.w. The OI increased by over 60% in the does after eignt days of treatment with the 600 mg/kg b.w. extract (82.5%) and after 12 days with the 300 mg/kg b.w. extract (63.5%), compared to the control.

Conclusion: Theseresults suggest that the aqueous leaf extract of *C. araliodes* could contain bioactive metabolites that can stimulate follicular growth.

Key words: Follicular stimulation, vaginal smears, *Cissus araliodes,* oestrogen index.

Introduction

Infertility affects nearly 48 million couples in the world, 15-30% of whom are in Africa. The costly and difficult acquisition of certain gonadotropins for ovarian stimulation, growth, follicle maturation and ovulation induction **(WHO, 2021)**, as well as the need for surgical interventions, are prompting infertile populations in developing countries to turn to herbal treatments. This reproductive system disease is a real problem for many couples of childbearing ages in developed countries. In these countries, such as Côte d'Ivoire, the use of

medicinal plants is the most common way to solve public health problems, particularly in rural areas. Indeed, in addition to being less expensive, studies conducted by **Padashetty and Mishra (2007)** and**Moyabi *et al*. (2021)** have shown the interest of medicinal plants in the management of reproductive disorders by valuing the traditional medicine. To pursue this perfect initiative, *Cissus araliodes*,a plant of the Ivorian pharmacopoeia was used to conduct this study. According to the literature, this plant has been the subject of numerous studies investigating its phytomolecules **(Kouassi *et al.*, 2021)** and pharmacological activities **(Ezeja *et al.*,2015a; 2015b)**. This study aims to promote traditional Ivorian medicine by evaluating the folliculo-stimulant activity of *Cissus araliodes* on the vaginal epithelium of the doe (female rabbit).

Material and Methods

Plant material

The plant material consists of the leaves of *Cissus araliodes.* Fresh leaves of this plant were harvested in the Haut Sassandra classified forest, situated in the Centre-West of Côte d'Ivoire. Samples were sent to the National Floristic Centre of Felix Houphouët-Boigny University of Abidjan for identification.

Animals

Study was carried out using New Zealand female rabbits (does) aged 2 to 3 months and weighing between 1.7 and 2.1 kg. These animals were bred at the animal house of the Pharmaceutical and Biological Sciences Research Unit, Felix Houphouët-Boigny University. They were housed individually in cages and kept at room temperature under natural lighting conditions. They also had free access to water and food (pellets). All experimental procedures were examined and approved by the Health Sciences Ethical Committee of Felix Houphouët-Boigny University.

Extract preparation

The leaves of *C. araliodes*were dried in the open air and in the shade for two weeks. They were then pulverized into powder using a grinder. Thus, 300 g of powder were homogenized in 3 L of distilled water using blinder. After homogenization, the resulting homogenate was collected in a clean cloth square. The four edges of the cloth were firmly joined together to form a pouch to completely contain the homogenate. The pouch was finally squeezed out by hand with gloves. The homogenate was filtered successively twice on absorbent cotton and

once on Wattman filter paper. The filtrate was placed in an oven at 50°C for 48 hours.The resultant extract from this operation constitutes the aqueous leaf extract of *C. araliodes*.

Phytochemical screening

Phytochemical screening was performed using the analytical techniques described in the works of **Lazureski *et al.* (2007)**and **Mea *et al*. (2017)**. A solution of the aqueous leaf extract of *C. araliodes* was prepared by dissolving 5 g of the extract in 50 mL of distilled water and compounds such as sterols, polyterpenes, polyphenols, flavonoids, tannins, quinones, alkaloid and saponins were analysed.

Assessment of folliculo-stimulant activity

Treatment of animals

Eight (8) does were divided into four groups of three each and treated daily for two weeks. Group 1 served as control group and received orally distilled water. Groups 2, 3, and 4 were administered orally the aqueous leaf extract of *C.araliodes*at doses of 200, 300 and 600 mg/kg b.w., respectively. The animals were weighed before and during the treatment at four-day intervals. Vaginal smears were also performed.

Study of vaginal smears

The study of vaginal smears was carried out on an interval of four days. A cotton bud moistened with distilled water was inserted into the doe's vagina. Upon contact with the mucosa, it was rotated and then delicately extracted from the genital tract. The samples were spread on the slide and fixed with lacquer, then left to dry for 24 hours before staining. Staining was performed using the regressive method of Papanicolaou (1942).Theoestrogen index (OI), a cytological marker for estimating oestrogenic impregnation, was calculated as the proportion of superficial cells within a sample of 100 cells.The OI is also known as the percentage of superficial eosinophilic cells and is expressed as a percentage (%).

Data analysis

Statistical analysis of the data was performed using XLSTAT 2014 software. The results were expressed as means with standard Deviation (Mean ± SD).The difference between means was determined usingone-way analysis of variances (ANOVA)followed by Dunnett's multiple comparison test. Significant differences between means were determined at the theoretical α = 5% threshold.

Results

Phytochemical screening

The results revealed the presence of the following secondary metabolites in the aqueous leaf extract of *Cissus araliodes*: polyphenols, flavonoids, catechic tannins, quinones, alkaloids and saponins. However, sterols, polyterpenes and gallic tannins were absent**(Table 1)**.

Table 1: Phytochemical compounds of aqueous leaf extract of *Cissus araliodes*

Chemical groups		**Results**
Sterols and polyterpenes		-
Quinones		+
Polyphenols		+
Flavonoids		+
Tannins	**Gallic**	-
	Catechic	+
Alkaloids		+
Saponosides		+

(+) chemical group present, (-) chemical group absent.

Effect of the aqueousleaf **extract of *Cissus araliodes*onthe body weight of does**

Table 2 shows the weights of does before and during the treatment with aqueous leaf extract of *Cissus araliodes.* The results showed no significant difference in weight between the groups treated with increasing doses of the plant extract and the control group (Group 1). There was also no significant difference in weight between the groups treated with the extract.

Table 2: Weight changes in does treated with aqueous leaf extract of *Cissus araliodes*

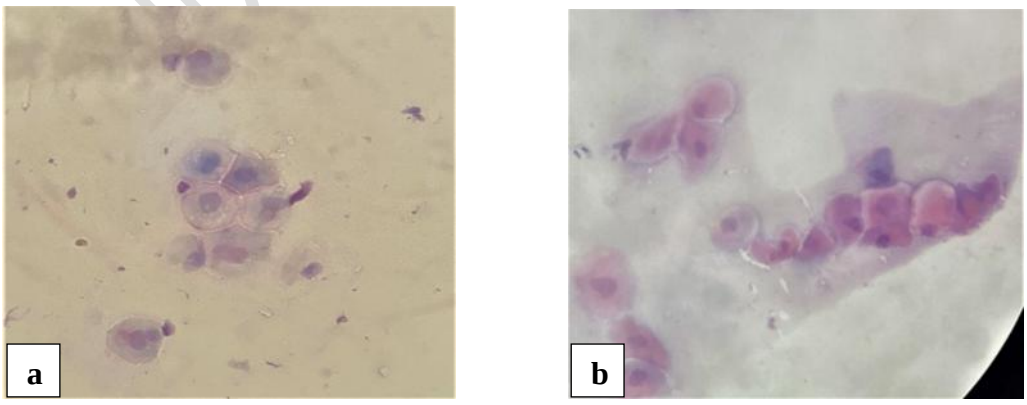
Treatment	**Body weight (Kg)**			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Group 1 (Distilled water)	1,82±0,141	1,86±0,084	1,88±0,084	1,935±0,049
Group 2 AECa (200 mg/Kg)	$1,92±0,183^{ns}$	$1,88±0,183^{ns}$	$1,905±0,205^{ns}$	$1,925±0,205^{ns}$
Group 3 AECa (300 mg/Kg)	$1,835±0,148^{ns}$	$1,82±0,127^{ns}$	$1,815±0,134^{ns}$	$1,855±0,162^{ns}$

Group 4 AECa (600 mg/Kg)	1,595±0,077^{ns}	1,745±0,035^{ns}	1,735±0,021^{ns}	1,805±0,007^{ns}

AECa:aqueous leaf extract of *Cissus araliodes.*
Values are expressed as means ± SEM (n = 3). ns: not significant.

Effect of the aqueous leaf extract of *Cissus araliodes* on the vaginal epithelium of does

Vaginal smears taken from the does before and during treatment with the aqueous leaf extract of *Cissus araliodes* showed various well-distributed cell populations, including superficial cells**(Figure 1c)**, intermediate cells **(Figure 1a)**and parabasal cells**(Figure 1b)**.The variations of the oestrogen index (OI) in the treated and untreated groups are shown in **table 3**.Before treatment (Day 0, first sampling), the OIvalues of the different groups of does were statistically equal ($p > 0.05$) and ranged from 9.5 ± 2.12 to 17 ± 2.82%.Regarding the second sampling (Day 4), a significant increase ($p < 0.05$ and $p < 0.01$) in the OI was observed in group 3 (31 ± 5.65%) and group 4 (42.5 ± 3.53%), that were treated with *C. araliodes* aqueous leaf extract at 300 and 600 mg/kg b.w., respectively, compared to the control group (17.5 ± 0.7%). Only the OI of group 2 (23 ± 0.1%), treated with the plant extract at 200 mg/kg bw, did not significantly increase.On the 8th day (third sampling) and 12th day (fourth sampling) of treatment, the OI significantly increased ($p < 0.05$ and $p < 0.01$) in all the groups of does treated with *C. araliodes* aqueous leaf extract.The 600 mg/kg b.w. dose reached peak oestrogen index (82.5±3.53%) after eight days of treatment, whereas the 300 mg/kg b.w. dose induced maximum oestrogen index (63.5 ± 0.7%) after 12 days.



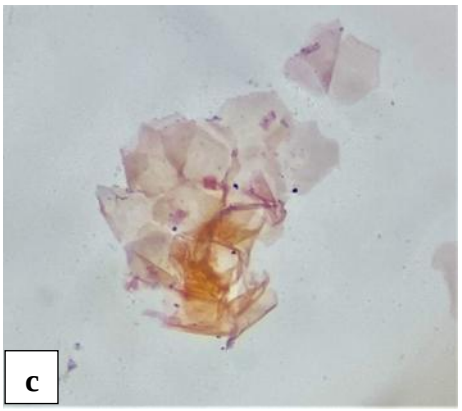


Figure 1: Cell populations of the vaginal epithelium of does

(a) basal cell clusters;(b) parabasal cell clusters; (c) superficial cell clusters.

Table 3: Variations of the oestrogen index in does treated with aqueous leaf extract of *Cissus araliodes*

Treatment	**Oestrogen index (%)**			
	Vaginal smears on day 0	**Vaginal smears on day 4**	**Vaginal smears on day 8**	**Vaginal smears on day 12**
Group 1 (Distilled water)	12 ± 1.41	17.5 ± 0.70	22 ± 1.41	25 ± 2.82c
Group 2 AECa (200 mg/Kg)	9.5 ± 2.12^{ns}	23 ± 0.1^{ns}	35 ± 4.24*	36 ± 4.24*
Group 3 AECa (300 mg/Kg)	17 ± 2.82^{ns}	31 ± 5.65*	41.5 ± 2.12*	63.5 ± 0.7**
Group 4 AECa (600 mg/Kg)	15.5 ± 3.53^{ns}	42.5 ± 3.53**	82.5 ± 3.53**	39.5 ± 3.53*

AECa:aqueous extract of *Cissus araliodes.*

Values are expressed as means ± SEM (n = 3). Symbol (*) indicates statistical significance. *Significant difference ($p < 0.05$); **Very significant difference ($p < 0.05$); ns: not significant.

Discussion

The phytochemical screening of the aqueous extract of*Cissus araliodes* revealed the presence of secondary metabolites including polyphenols, flavonoids, catechic tannins, quinones, alkaloids and saponins.**Moyabi (2019)** and **Nwogueze *et al.* (2018)** also demonstrated the presenceof alkaloids, saponins and flavonoids in the aqueous extract of *C. araliodes*.These compounds are knownfor their beneficial pharmacological properties, which can restore fertility. Indeed, alkaloids have been shown to exert estrogenic effects on the mammalian

reproductive system **(Nazrullaev *et al*., 2001)**. Flavonoids and saponins have both estrogenic and androgenic properties **(Padashetty and Mishra, 2007)**.

The aqueous leaf extract of *C. araliodes* was administered at different doses to the does in order to evaluate its effect on their body weight and vaginal epithelium.Regarding the body weight, the non-significant variation suggests that this extract does not affect weight gain in does.This finding could testify to the non-toxicity of *C. araliodes*extract at these doses. Indeed, according to **El Hilaly *et al.* (2004)**, the loss of weight is an index of toxicity of a drug. **Moyabi (2019)** demonstrated this in a subacute toxicity study of *C. araliodes* aqueous extract, in which he found no significant change in the body weight of rats that received 300 and 600 mg/kg b.w. of the extract. Moreover, acute toxicity studies of *C. araliodes* aqueous extract have shown an LD_{50}>5000 mg/kg b.w. (**Nwogueze *et al.*, 2018; Ezeja *et al.*, 2015)**.

As for the effect of *C. araliodes* extract on the vaginal epithelium, smear analysis shows low oestrogen impregnation (25-36%) in both control does and those treated with 200 mg/kg b.w. of extract. This result is characteristic of a proestrus state.However, the high level of oestrogen impregnation (more than 60%) observed in does treated with 300 and 600 mg/kg b.w. of extract suggest an oestrus state.These results are in agreement with those of **Okon (2015)** and**Malandain and Fontbonne (2006)**, who indicated that the proestrus state would be characterized by an oestrogen index (OI) of about 30%, and that of the oestrus state by an OI of more than 60%, or even higher than 80%.

This change in state would result from the discharge of oestrogen in the vaginal epithelium of the does, leading to oestrus. Indeed, the ovarian cycle, marked by folliculogenesis and steroidogenesis, is influenced by follicle-stimulating hormone (FSH) and luteinizing hormone (LH)**(Monniaux *et al.*, 2009)**.During this cycle, the active principle in the aqueous leaf extract of *C. araliodes* would have acted in one of two ways. Either it would stimulate directly the follicles through binding to specific receptors located in the granulosa and theca cells, or indirectly the pituitary gland to trigger the release of gonadotropins, which then stimulate the ovary to obtain an oestrogenic peak.With regard to the vaginal epithelium, this high level of oestrogen stimulates the differentiation of intermediate cells into superficial cells, which are subsequently desquamate**(Moyabi, 2019)**.This is confirmed by **Fitz and Dinan (2008)**, who state that oestrogen stimulates the growth and proliferation of superficial keratinised cells, as well as the development of the reproductive organs.

Conclusion

This study revealed the presence of several bioactive metabolites in the aqueous leaf extract of *Cissus araliodes*. Moreover, this extract was found to have follicle-stimulating properties on the vaginal epithelium does. Further studies are needed to isolate and characterise the active compounds responsible for this activity, and to determine their exact mechanism of action.

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Competing interests

The authors have no competing interests.

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