Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/21737
DOI URL: http://dx.doi.org/10.21474/IJAR01/21737

RESEARCH ARTICLE

EVALUATION OF THE FOLLICLE-STIMULATINGACTIVITY OF THE AQUEOUS LEAF EXTRACT OF CISSUSARALIODESON THE VAGINAL EPITHELIUM OF THE DOE (FEMALE RABBIT)

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Manuscript Info

Manuscript History
Received: 09 July 2025
Final Accepted: 11 August 2025
Published: September 2025

Key words:-
Reproductive pharmacology, female fertility, follicular stimulation, herbal medicine, Cissusaraliodes, estrogenic index.

Abstract

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 follicle-stimulatingactivity of the aqueous leaf extract of Cissusaraliodeson the vaginal epithelium of the doe (female rabbit).

Methods: Phytochemical screening of this extract was first performed. Then, for the follicle-stimulating activity, twelve 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 administered increasing doses of 200, 300, and 600 mg/kg body weight (b.w.) of the aqueous leaf extract of C. araliodes, respectively. Vaginal smears were taken before and during treatment to observe changes in surface cells.

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 estrogenicindex (EI) were observed four days after treatment of the does with C. araliodes extract at 300 and 600 mg/kg b.w. The EI increased by over 60% in the does after eight 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. araliodescould contain bioactive metabolites that can stimulate follicular growth.

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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. In these countries, such as Cote 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) andMoyabiet 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, Cissusaraliodes,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(Balogunet al., 2021; Kouassiet al., 2021;Nagoet al., 2023) and pharmacological activities (Ezejaet al.,2015a; 2015b;Balogunet al., 2021; Nagoet al., 2023). Moreover, the leaves of this plant are traditionally used by women who are expecting to conceive(Gbaranoret al., 2021).This study aims to evaluate the follicle-stimulating activity of the aqueous leaf extractof Cissusaraliodes on the vaginal epithelium of the doe (female rabbit)in order to verify the validity of its traditional use as conception-expecting plant.

Materials and Methods:-

Plant material:

The plant material consists of the leaves of Cissusaraliodes. Fresh leaves of this plant were harvested in the Haut Sassandra classified forest, situated in the Centre-West of Cote d'Ivoire. Samples were sent to the National Floristic Center 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.araliodeswere 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 (3 mm). The filtrate was placed in an oven at 50°C for 48 hours.The resulting extract from this process constitutedthe aqueous leaf extract of C. araliodes.

Phytochemical screening:

Phytochemical screening was performed using the analytical techniques described in the works of Lazureskiet al. (2007)and Mea et al. (2017). A solution of the aqueous leaf extract of C. araliodeswas 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 analyzed.

Assessment of follicle-stimulatingactivity:

Treatment of animals:

Twelve (12) 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.araliodesat 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).The estrogenic index (EI), a cytological marker for estimating estrogenic impregnation, was calculated as the proportion of superficial cells within a sample of 100 cells.The EIis 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 Tukey'smultiple comparison test.The difference was considered significant when the p-value was less than 5% ($p < 0.05$).

Results:-

Phytochemical screening:
The results revealed the presence of the following secondary metabolites in the aqueous leaf extract of Cissusaraliodes: 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 Cissusaraliodes

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 Cissusaraliodesonthe body weight of does:
Table 2 shows the weights of does before and during the treatment with aqueous leaf extract of Cissusaraliodes. The results showed no significant difference ($p > 0.05$) 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 Cissusaraliodes

Treatment	Body weight (Kg)			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Group 1 (Distilled water)	1,82±0,141[a]	1,86±0,084[a]	1,88±0,084[a]	1,935±0,049[a]
Group 2 AECa (200 mg/Kg)	1,92±0,183[a]	1,88±0,183[a]	1,905±0,205[a]	1,925±0,205[a]
Group 3 AECa (300 mg/Kg)	1,835±0,148[a]	1,82±0,127[a]	1,815±0,134[a]	1,855±0,162[a]
Group 4 AECa (600 mg/Kg)	1,595±0,077[a]	1,745±0,035[a]	1,735±0,021[a]	1,805±0,007[a]

AECa:aqueous leaf extract of Cissusaraliodes.Values are expressed as means ± SEM (n = 3).Letters represent statistical significance. For each period (column), the mean weights with the same letter are not significantly different ($p > 0.05$).

Effect of the aqueous leaf extract of Cissusaraliodeson the vaginal epithelium of does:

Vaginal smears taken from the does before and during treatment with the aqueous leaf extract of Cissusaraliodes showed various well-distributed cell populations, including superficial cells(Figure 1c), intermediate cells (Figure 1a)and parabasal cells(Figure 1b).The variations of the estrogenic index (EI)in the treated and untreated groups are shown in table 3.Before treatment (Day 0, first sampling), the EIvalues of the different groups of does were statistically equal ($p > 0.05$) and ranged from 11.5 ± 2.12 to $17 \pm 2.82\%$.

Regarding the second sampling (Day 4), a significant increase ($p < 0.05$ and $p < 0.01$) in the EI was observed in group 3 and group 4, that were treated with C. araliodes aqueous leaf extract at 300 and 600 mg/kg b.w., respectively, compared to the control group. Only the EI of group 2, treated with the plant extract at 200 mg/kg bw, did not significantly increase.On the 8^{th} day (third sampling) and 12^{th} day (fourth sampling) of treatment, the EI 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 estrogenic index after eight days of treatment, whereas the 300 mg/kg b.w. dose induced maximum estrogenic indexafter 12 days.

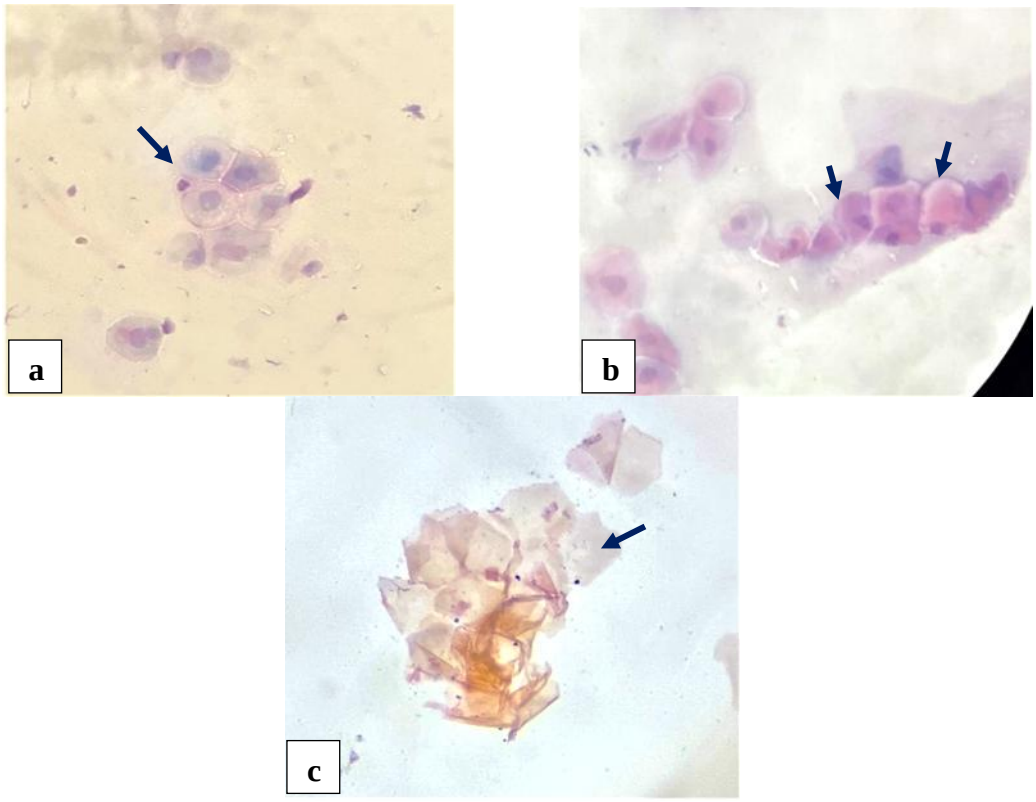

Figure 1:Cell populations of the vaginal epithelium of does

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

Table 3: Variations of the estrogenic index in does treated with aqueous leaf extract of *Cissusaraliodes*

Treatment	Estrogenic 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^{a}	17.5 ± 0.7^{a}	22 ± 1.41^{a}	25 ± 2.82^{a}
Group 2 AECa (200 mg/Kg)	11.5 ± 2.12^{a}	23 ± 0.1^{a}	35 ± 4.24^{b}	36 ± 4.24^{b}
Group 3 AECa (300 mg/Kg)	17 ± 2.82^{a}	31 ± 5.65^{b}	41.5 ± 2.12^{b}	63.5 ± 0.7^{c}
Group 4 AECa (600 mg/Kg)	15.5 ± 3.53^{a}	42.5 ± 3.53^{c}	82.5 ± 3.53^{c}	39.5 ± 3.53^{b}

AECa: aqueous leaf extract of Cissusaraliodes.Values are expressed as means ± SEM (n = 3). Letters represent statistical significance. For each period (column), the means ofestrogenic indexwith different letters are significantly different ($p < 0.05$).

Discussion:-

The phytochemical screening of the aqueous leaf extract ofCissusaraliodes revealed the presence of secondary metabolites including polyphenols, flavonoids, catechic tannins, quinones, alkaloids and saponins.Moyabi (2019) and Nwoguezeet 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 (Nazrullaevet 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. araliodesextract at these doses.

Indeed, according to El Hilalyet 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 a LD_{50}>5000 mg/kg b.w. (Ezejaet al., 2015;Nwoguezeet al., 2018).

As for the effect of C. araliodesextract on the vaginal epithelium, smear analysis shows low estrogenic impregnation (25-36%) in both control does and those treated with 200 mg/kg b.w. of extract. This result could be indicative of a pro-estrus state.However, the high level of estrogenic impregnation (more than 60%) observed in does treated with 300 and 600 mg/kg b.w. of extract suggest an estrus state.

These results are in agreement with those of Okon (2015) and Malandain and Fontbonne (2006), who indicated that the pro-estrus state would be characterized by an estrogenic index (EI) of about 30%, and that of the estrus state by an EI of more than 60%, or even higher than 80%.This change in state would result from the discharge of estrogen in the vaginal epithelium of the does, leading to estrus.

Indeed, the ovarian cycle, marked by folliculogenesis and steroidogenesis, is influenced by follicle-stimulating hormone (FSH) and luteinizing hormone (LH)(Monniauxet al., 2009).During this cycle, the active principle in the aqueous leaf extract of C. araliodes could stimulate the pituitary gland to trigger the release of gonadotropins, which then stimulate the ovary to obtain an estrogenic peak.With regard to the vaginal epithelium, this high level of

estrogen 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 estrogen stimulates the growth and proliferation of superficial keratinized 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 Cissusaraliodes. Moreover, this extract was found to have follicle-stimulating properties on the vaginal epithelium does. Further studies are needed to isolate and characterize the active compounds responsible for this activity, and to determine their exact mechanism of action.

Acknowledgements:-

The authors thank the National Center of Floristic and the Pharmaceutical and Biological Sciences Research Unit of Felix Houphouët-Boigny University of Abidjan for identifying the plant and granting access to the animal house, respectively.

Competing interests:

The authors have no competing interests.

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