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Ethnobotanical Survey of Medicinal Plants Used against Infertility in the Nyong and So'o Division (Cameroon) and Pro-fertilizing Activities of *Mammea africana* (Clusiaceae) Aqueous Extract Rats

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Authors' contributions

This work was carried out in collaboration among all authors All authors read and approved the final manuscript.

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ABSTRACT

Background: World-renowned as primary healthcare, traditional medicine often represents the only therapeutic resource for many communities with very low incomes, although it shows valuable benefits. The objective of this investigation was to evaluate the medicinal plants used to treat infertility in the Nyong & So'o Division (Cameroon) and the effect of *Mammea africana,* the most commonly used medicinal plant extract, on reproductive parameters in female rats.
Methods: First, a transversal and analytic investigation using semi-structured interviews was conducted on a sample of 22 traditional healers. Secondly, two pharmacological assays were carried out to evaluate M. *africana* aqueous extract activities in 8-10-week-old female Wistar rats. During 14 days, non-ovariectomized (Novx) mice were given distilled water (DW) at 10 mL/kg and M. *africana* extract at 40, 80, and 160 mg/kg. In the end, cervical smears were realized, animals were sacrificed, and extract activities were evaluated on reproductive parameters. The estrogenic activities of M. *africana* extract were assessed on estrogenic-dependent tissues *per os* in ovariectomized (Ovx) rats in the second assay. They received respectively distilled water (DW), estradiol valerate (E_2V) at 1 mg/kg, and the extract at the aforesaid doses, and 3 groups were co-treated with E_2V (0.75 mg/kg) and the extract. A sham-operated group received DW
Results: Ethnobotanical investigation revealed that 20 species of plant belonging to 13 different families are used to treat infertility. *Mammea africana* was the most used plant and its extract at 80 mg/kg aqueous extract induced a significant increase ($p < 0.001$) of FSH, LH, and estradiol in Novx rats compared to control. Furthermore, the extract induced the maturation of ovarian follicles. *M. africana* extract exhibited also estrogenic activities in Ovx rats. Indeed, *M. africana* aqueous extract induced the formation of *stratum corneum* in the vagina of Ovx rats. E_2V activities at 0.75 mg/kg were maximized by *M. africana* extract.
Conclusion: To sum up, many species are used in the Nyong & So'o Division to manage reproductive failure. Among them, *Mammea africana* is the most commonly used and possesses estrogenic-like activities.

Keywords: Infertility; estrogenic activities; Nyong & So'o division; Mammea africana.

1. INTRODUCTION

Infertility is the inability to get pregnant despite unprotected and carefully planned sex. It is defined by the World Health Organization (WHO) as the inability to be pregnant after 12 months or more of regular, unprotected coitus in couples of reproductive ages (women aged 18 to 45) [1]. Men and women are both affected. In a couple, pure male factors account for 20–35% of infertility, while 40-50% of factors are solely due to women, with the remaining being attributed to both or unknown causes [2]. Globally, 15% of couples are affected by infertility, amounting to 48.5 million couples who are confronted with this worldwide public health issue with personal, social, and economic consequences. In Africa, pregnancy disabilities are a particular concern because of the extent of the problem and the social stigma attached to them. In Cameroon, although the infertility rate among women aged 22 to 44 is 19.2%, the use of modern medical assistance techniques remains costly and not easily accessible [3]. The accessibility, availability, and affordability of medicinal plants ensure that 80% of the African population continues to use them to handle primary medical problems. Furthermore, the Nyong and So'o division is reported as key place for traditional medicine in Cameroon [4,5]. In these countries, a variety of plants are claimed to have fertility-regulating properties, and a few have been tested for such effects [2]. Among these plants, *Mammea africana* is most famous in the Nyong & So'o Division of Cameroon. Although this plant has been the subject of numerous studies [6-8], its activity on fertility has not yet been elucidated. Given the production of safe and cheap traditional medicine, it was necessary to carry out a survey of medicinal plants used by traditional healers to manage female infertility and verify scientifically the activity of the most commonly used. This study aimed to identify plants used to remedy infertility in the Nyong &

So'o Division of Cameroon and evaluate the activities of the most commonly used (50%) plant, named *Mammea africana*, on reproductive parameters in female rats.

2. MATERIALS AND METHODS

2.1 Ethnobotanic Investigation

An investigation of traditional healers in Nyong and So'o Division, Centre Region (Cameroon) (3°31'0"N and 11°30'0"E), was undertaken from December 2019 to January 2020. Nyong & So'o Division has an area of 3581 km^2 with 1452,907 inhabitants, i.e., a density of 40 inhabitants/km^2. It comprises six communes, namely *Akoeman*, *Dzeng*, *Mengueme*, *Ngomezap*, *Nkolmetet,* and *Mbalmayo* (Fig. 1). Data sought from the questions included sociodemographic characterization of the informants, such as education level, age, family situation, and academic level. The names of the plants were recorded according to the pronunciation of the interviewees. The use of local plant species for medicinal purposes and information on methods of preparation and organs used, as well as the name, directions for uses of the plant, and diseases treated by the plant. All plants registered were authenticated at the National Herbarium of Cameroon by comparison with the botanical collection.

2.2 Plant Materials

2.2.1. Collection

Mammea africana Sabine (Clusiaceae) was harvested in Mbalmayo (Center, Cameroon) in the village of NGOCK in January 2020. Its name has been certified at the National Herbarium of Cameroon by comparison with the botanical collection of Leuwenberg N° 9786 registered under N° 43678/HNC.

2.2.2 Extraction

Fresh barks of M. *africana* were harvested, cleaned, cut into small pieces, and shade dried at room temperature. The dried material was reduced to powder form using an electric grinder. 500 g of M. *africana* bark were macerated in 10 liters of water for 24 hours before being boiled for 30 minutes. The decoction was filtered through *Whatman* N°3 paper, and the filtrate was lyophilized to yield 35.41 g of the crude extract with a yield of 7.08%.

2.2.3 Determination of study doses

Following the traditional healer's advice, 600 mL of macerate and 0.90 g of crude extract were obtained after drying. This mass of extract was divided by 70kg and then multiplied by 1000 to obtain a human equivalent dose (HED) equal to 16mg/kg. The dose used in rats was determined by multiplying the HED by 6.2 according to the method described in [9], resulting in a dose of approximately 80mg/kg. This dose was divided by 2 and then multiplied by 2 to obtain the doses of 40, 80, and 160mg/kg.

2.3 Animal Materials

Eight to ten-week-old female Wistar rats, weighing between 120 and 130g were housed at the Animal Facility of the Laboratory of Animal Physiology of the University of Yaoundé I. These animals were lodged in plastic cages with a diameter of 360mm and a height of 130mm at the rate of six animals per cage, kept at room temperature under a natural day/night light cycle with access to a soy-free standard diet and tap water *ad libitum*.

2.4 Effects of *Mammea africana* extract on Some Parameters of the Reproductive System

Through vaginal smears, a regular check of three estrous cycles was carried out before starting the assay. Rats were divided into four groups of five. A control group received distilled water, and three groups were treated with *M. africana* extract at doses of 40, 80, and 160mg/kg. During the 14 days of administration *per os*, the body weight of the animals was recorded daily, and vaginal smears were taken at 10 a.m. In the end, rats were sacrificed under light anesthesia with diazepam and ketamine after a 12-hour non-water fast. Arterio-venous blood was collected after decapitation. Blood samples were centrifuged at 3500rpm (15 minutes at 4°C) to obtain serum samples, which were kept at -15°C. The resulting serum was used for biochemical analysis of LH, FSH, estradiol, and cholesterol. The vagina, uterus, and ovaries were dissected. The left ovary, vagina, and uterus were fixed in 10% buffered formaldehyde for routine histological analysis with hematoxylin-eosin staining. The right ovary was cleaned with 0.9% saline, weighed, and homogenized with a phosphate buffer (0.1M, pH 7.4). The homogenate was stored at -15°C for subsequent cholesterol determination.

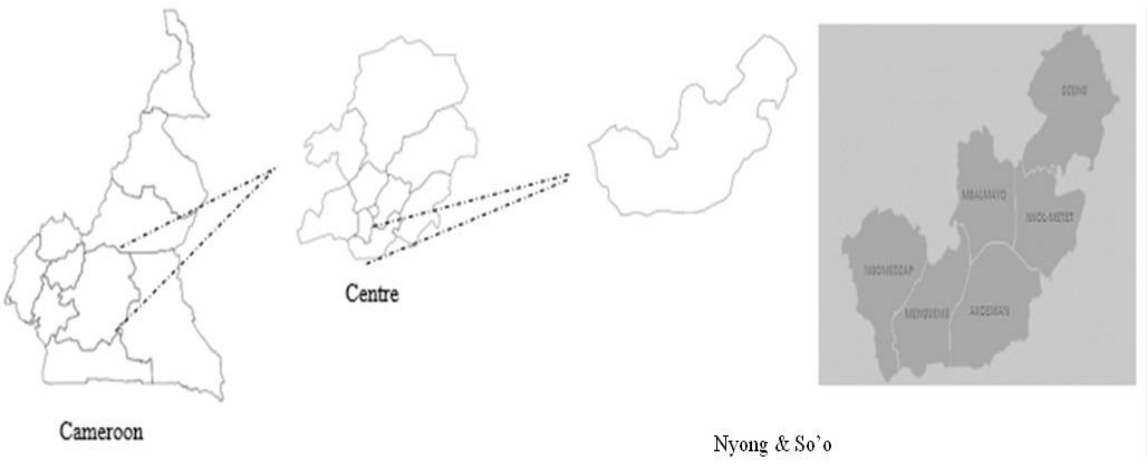

Fig. 1. Geographic position of the Department of Nyong & So'o within the Centre Region of Cameroon

2.5 Evaluation of Estrogenic Activities *of Mammea africana* in Ovariectomized Rats

This test was performed according to the protocol described by the OCDE. [10]. Forty-five rats were ovariectomized and then randomized after a hormonal decline (2 weeks). Animals were divided into nine groups: a negative control received distilled water at 10mL/kg, positive control was treated with E_2V (at 1 mg/kg); three groups were treated with *M. Africana* extract at doses of 40, 80, and 160mg/kg; and three groups were co-treated with this dose combined with E_2V (0.75mg/kg). A sham-operated group received distilled water at 10mL/kg. After 3 days of treatment *per os*, animals were sacrificed by decapitation under light anesthesia with diazepam and ketamine. The uterus, vagina, and mammary gland were dissected. The uterus was weighted and a section of the uterus was homogenized with a phosphate buffer (0.1M, pH 7.4). The homogenate was stored at -15°C for subsequent protein level assay. The other section of the uterus, vagina and mammary gland were fixed in formaldehyde 10% buffered for further histological analysis.

2.6 Vaginal Cytology Differentiation Analysis

Vaginal cytology was examined according to the protocol described by Oumarou et al. [11]. An eyedropper containing 10µL of saline at 0.9% was introduced into the vagina. The mix of saline and cells of the vagina was placed on ringed slides, fixed, and stained with the Papanicolaou method. Cellular differentiation was observed under a light microscope.

2.7 Assays for Seric Estradiol, LH, FSH, Cholesterol and Ovarian Cholesterol Levels

Seric total cholesterol and ovarian cholesterol were assessed using commercial diagnostic kits from Fortress, UK. Estradiol, Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH) seric levels were assessed by the ELISA method using Calbiotech kits.

2.8 Histomorphometric Analysis of Uterine, Vaginal, Ovarian and Mammary Gland Tissues

After fixation in 10% buffered formaldehyde (2 weeks), the organs (uterus, vagina, ovary, and mammary gland) were trimmed and dehydrated in alcohol (70%, 80%, 95%, and 100% (three baths)) for 1 hour each, and then stayed in two xylene baths for 2 hours per bath. The organs were then impregnated in a molten paraffin solution at 60°C for 5h. The paraffin blocks containing the tissues were used to make serial cuts of 7µm using a Reichert-Jung 2030 microtome and then stained with hematoxylin-eosin. Stained sections were visualized and images were captured by using a light microscope (Leitz Wetzlar Germany 513) connected with a digital camera Celestron 44,421 linked to a computer, where images were transferred. Histomorphometric assessments (luminal epithelium of the uterus and vaginal heights) were carried out using J image software (version 1.4.3.67).

For the evaluation of folliculogenesis, the tenth section of the right ovary was chosen. The

different stages of the ovarian cells were determined according to the method described by Kaplan and Türk [12], as described below:

- Primordial follicles: composed of oocytes surrounded by one layer of squamous follicular cells;
- Primary follicles: composed of oocytes surrounded by one layer of cuboidal follicular cells;
- Secondary follicles: those with more than one follicular cell layer;
- Tertiary follicles: those with granulosa cells with four distinct subtypes. The first is the *corona radiate*, which surrounds the *zona pellucida*; the second is the *membrana* which is interior to the basal *lamina*; the third is the *peri-antral* which is adjacent to the *antrum*; and the last is the *cumulus oophorous*.
- De Graaf follicles: characterized by a single large *antrum* of follicular fluid.
- Atresic follicles are made up of oocytes with complete apoptosis of all constituent cells.
- *Corpus luteum*: ruptured follicles with hypertrophied follicular cells and cavity filled with blood-filled cavity formed by ruptured follicles with hypertrophied follicular cells.

2.9 Statistical Analysis

A Comparison of proportions and a chi-square test with a significance threshold of less than 0.05 were used for the ethnobotanical investigation. For the pharmacological test, data were expressed as mean ± Standard Error on Mean (SEM). Analysis of variances (ANOVA) followed by the Dunnett test for multiple comparisons and the student t-test, were used between different groups using GraphPad Prism software version 8.0.1.244. The significance level was set at p0.05.

3. RESULTS

3.1 Ethnobotanical Investigation

3.1.1 Sociodemographic characterization of traditional healers

As shown in Table 1, 22 traditional healers were investigated. They were all married, mainly male, and aged between 70-80. The majority of them graduated their primary school and are male.

3.1.2 Frequencies of uses of different species

Fig. 2 shows how many different species are used by traditional healers. *Mammea africana* was the most used plant with 50% followed by *Antrocaryon klaineanum* with 36.00% and *Ageratum conyzoides* with 31.80%.

As shown in Table 2, an ethnobotanical investigation in the Nyong & So'o division revealed the use of 20 species of plants that belong to 13 families. These plants are commonly used against diseases or affections linked to infertility. The most represented families were the *Asteraceae exéco* and the *Mimosaceae*, with 3 species each. Diseases or affections linked with infertility registered are among others chlamydia, vaginitis, fallopian tubes blocked, difficulty childbirth, dysmenorrhea, and cleaning of male or female reproductive apparatus. Barks are the main parts of plants that are commonly used. Medicinal plants are usually prepared by decoction or maceration in water, and the maximum duration of the treatment is 4 weeks.

Table 1. Characterization of traditional healers

Parameters	Variables	Frequency	(%)
Gender	Male	17	77.27
	Female	5	22.73
Age	[45-50]	1	4.54
	[50-60]	5	22.73
	[60-70]	4	18.18
	[70-80]	12	54.55
Family situation	Married	22	100
	Single	-	-
Academic level	None	-	-
	Primary	17	77.27
	Secondary	4	18.18
	Academic	1	4.55

Table 2. Ethnobotanical investigation of medicinal plants used for reproductive failure in the Nyong & So'o Division

Municipalities	Vernacular names (Ewondo)	Scientific name	Family	Disease or affection treated (linked with infertility)	Parts of the plant	Method of preparation and solvent	Duration of the treatment	The number at the HNC[1]
Mbalmayo	*Abolzok*	*Mammea africana* Sabine	Clusiaceae	Chlamydia, vaginitis, Fallopian tubes blocked, difficult childbirth, dysmenorrhea	Barks	Maceration and decoction in water	2 weeks	43678/HNC
	Adoum	*Cylicodiscus gabunensis* Harms	Mimosaceae	difficult childbirth, Cleaning of male reproductive apparatus	Barks	Decoction in water	4 weeks	40031/HNC
	Atui	*Piptadeniastrum africanum* (Hook.f.) Brenham	Mimosaceae	Cleaning of male reproductive apparatus	Barks	Decoction in water	4 weeks	12115/SR[2]F-Cam
	Ewewôn	*Spathodea campanulata* P. Beauv.	Bignoniaceae	difficult childbirth	Barks	Decoction in water	4 weeks	45706/HNC
	Opkwari'	*Ageratum conyzoides*	Asteraceae	Chlamydia, vaginitis	Leaves	Maceration and decoction in water with honey	3 weeks	6575/SFK-Cam
	Angongui	*Antrocaryon klaineanum* Pierre	Anacardiaceae	Salpingitis, vaginitis	Barks	Decoction in water	4 weeks	66180/HNC
	Ewouwoùs	*Alchornea cardifolia* (Schum & Thonn.) Mull.Arg.	Euphorbiaceae	Cleaning of reproductive apparatus of both male and female	Leaves	Trituration	3 days	40512/HNC
	Ngouien	*Panax ginseng* C.A.Meyer	Araliaceae	Sexual failure	Roots	Trituration	3 days	/
	Ekekoa	*Dichrocephala integrifolia* (L.f.) Kuntze	Asteraceae	Chlamydia, vaginitis	Leaves	Maceration in water	3 weeks	61603/HNC

[1] ***HNC*** *: « Herbier National du Cameroun »*
[2] ***SRF-CAM:*** *« Section de Recherche Forestière du Cameroun »*

Municipalities	Vernacular names (Ewondo)	Scientific name	Family	Disease or affection treated (linked with infertility)	Parts of the plant	Method of preparation and solvent	Duration of the treatment	The number at the HNC[1]
	Eyalguedié	*Aspilia.africana* (Pers.) CD. Adams	Asteraceae	Chlamydia, vaginitis	Leaves	Decoction in water	3 weeks	51881/HNC
	Ekukué	*Millettia macrophylla*	Fabaceae	Hormonal disorder	Leaves	Trituration	4 weeks	49654HNC
	Apkwar	*Tetrapleura tetraptera* (Schum & Thonn.) Taub	Mimosaceae	difficult childbirth	Barks	Decoction in water	4 weeks	59241/HNC
Metet	*Ta'a*	*Nicotiana tabacum* Linn.	Solanaceae	Salpingitis	Leaves	Decoction in water	2 weeks	34737/HNC
Akoeman	*Essessang*	*Ricinodendron heudelotti* Baill.	Euphorbiaceae	Cyst, chlamydia, vaginitis	Seeds	Decoction in water	4 weeks	42573/HNC
	Elolom	*Hallea stipulosa* (DC.) Leroy	Rubiaceae	Dysmenorrhea	Barks	Decoction in water	4 weeks	66387/HNC
Ngomezap	*Mfol*	*Enantia chlorantha* Oliv.	Annonaceae	Dysmenorrhea, Cleaning of the reproductive apparatus of both male and female	Leaves	Decoction in water	4 weeks	25918/SRF-Cam
	Awangua	*Millettia laureutii* De Wild	Fabaceae	difficult childbirth	Barks	Decoction in water	4 weeks	43282/HNC
	Oyekui	*Pterocarpus soyauxii* Taub.	Fabaceae	Dysmenorrhea, menopausal symptoms	Leaves Heartwood	Maceration	4 weeks	56984HNC
Mengueme	*Asseng*	*Musange cecropioides* C. Sm. ex. R	Moraceae	Chlamydia, vaginitis	Leaves	Maceration and decoction in water	3 weeks	44062/HNC
	Adzap	*Baillonella toxisperma* Pierre	Sapotaceae	difficult childbirth	Barks	Decoction in water	4 weeks	54060/HNC

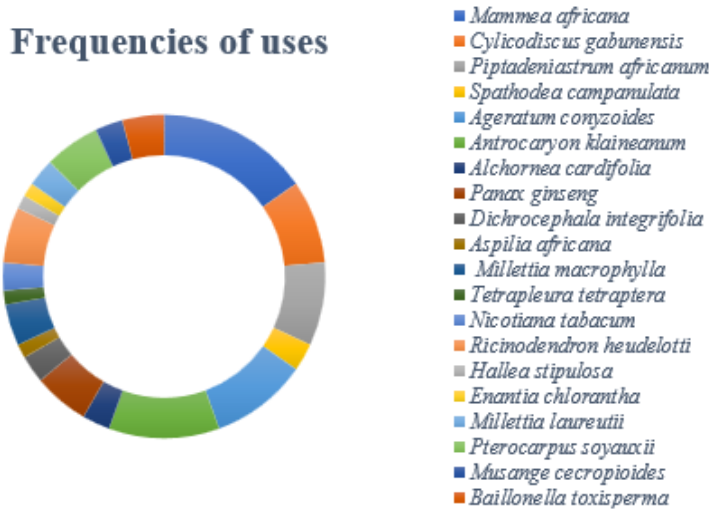

Fig. 2. Frequencies of different species used by traditional healers

3.2 Effects of *Mammea africana* Extract on Some Parameters of the Reproductive System

3.2.1 Effects of *Mammea africana* on seric estradiol, LH, FSH, cholesterol and ovarian cholesterol levels in non-Ovx rat

Globally, in non-Ovx rats, *Mammea africana* aqueous extract induced an increase in the investigated hormones. Indeed, the plant extract at 80mg/kg induced a significant increase in all hormones investigated compared to the control. Indeed, this dose of the extract significantly increased estradiol ($p < 0.05$), FSH, and LH ($p < 0.01$) seric levels. The increase in endogenous estradiol levels induced by the extract was dose-dependent. Furthermore, the extract significantly reduced both seric and ovarian cholesterol levels compared to the control (Fig. 3).

3.2.2 Effects of *Mammea africana* on folliculogenesis and estrous cycle in non-Ovx rats

Mammea africana aqueous extract promoted the maturation of ovarian follicles by inducing a dose-related and significant increase in the number of *corpus luteum* in the ovaries compared to the control. Only the extract at the dose of 160 mg/kg resulted in a significant increase in the number of secondary follicles. The different doses of the extract resulted in a non-significant decrease in the number of atretic and primordial follicles as compared to the control. By observing the ovarian histological section (Fig. 4), there was a normal structure of the ovary in all groups but with *corpus luteum* and follicles at different stages of development in animals treated with the plant extract (Table 3).

Table 3. Effects of *Mammea africana* aqueous extract on ovarian follicles in non-Ovx rat

	Novx + H_2O	Novx + MA 40	Novx +MA 80	Novx + MA 160
Activities on ovarian follicles				
Pdf	2.25 ± 0.58	2.00 ± 0.31	2.33 ± 0.48	1.50 ± 0.22
Pf	3.00 ± 0.63	2.00 ± 0.31	4.00 ± 0.33	3.00 ± 0.32
Sf	1.33 ± 0.17	1.33 ± 0.18	1.66 ± 0.20	2.33 ± 0.18[2]
Terf	1.20 ± 0.20	1.33 ± 0.19	1.33 ± 0.18	1.40 ± 0.24
Dgf	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.33 ± 0.18
Af	4.25 ± 0.96	3.50 ± 0.67	2.50 ± 0.54	2.00 ± 0.63
Tf	12.95 ± 0.75	11.23 ± 0.34	12.50 ± 0.58	11.09 ± 0.31
Cl	4.25 ± 0.19	6.00 ± 0.31[1]	6.75 ± 0.58[2]	6.80 ± 0.37[2]

Data are mean ± SEM (n = 5); [1]$p < 0.05$, [2]$p < 0.002$: significant difference compared to control. Nor + H_2O = control treated with distilled water (10mL/kg); Novx + MA 40 = non-Ovx rats treated with M. africana aqueous extract at 40mg/kg, Novx + MA 80 = non-Ovx rats treated with M. africana aqueous extract at 80mg/kg, Novx + MA 160 = non-Ovx rats treated with M. africana aqueous extract at 160mg/kg. Cl = corpus luteum, Terf = Tertiary follicle, Af = atretic follicle, Pdf = Primordial follicle, Pf = primary follicle, Sf = Secondary follicle, Dgf = De Graaf follicle, Tf = Total follicles

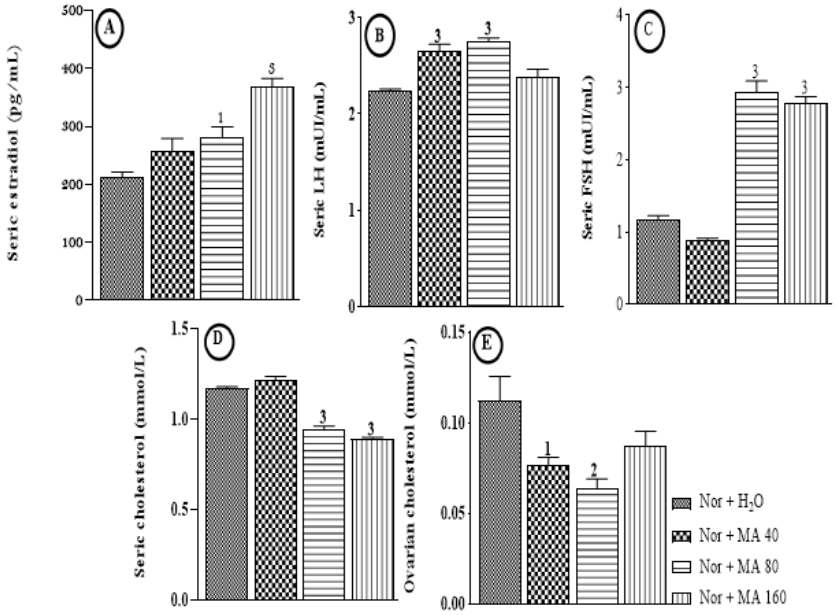

Fig. 3. Effects of aqueous extract of *Mammea africana* on estradiol (A), LH (B), FSH (C), seric (D), and ovarian (E) cholesterols levels in non-Ovx rats

Bars are mean ± ESM (n = 5); [1]p < 0.05; [2]p < 0.002, [3]p < 0.001: significant difference compared to control. Novx + H_2O = control treated with distilled water (10 mL/kg); Novx + MA 40 = non-Ovx rats treated with M. africana aqueous extract at 40mg/kg, Novx + MA 80 = non-Ovx rats treated with M. africana aqueous extract at 80mg/kg, Novx + MA 160 = non-Ovx rats treated with M. africana aqueous extract at 160mg/kg

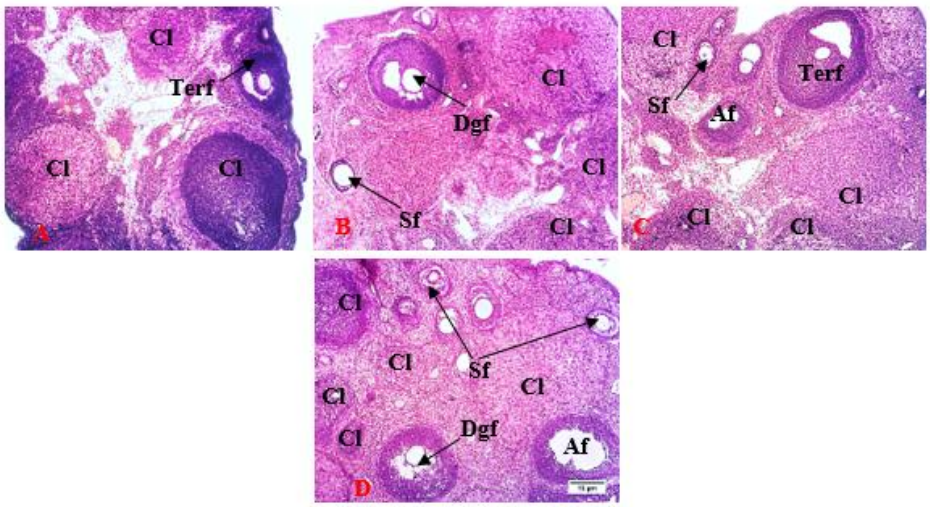

Fig. 4. Effects of *Mammea africana* aqueous extract on the duration of the estrous cycle and ovarian structure and follicles in non-Ovx rats (X25, HE)

A = control treated with distilled water (10 mL/kg); B = non-Ovx rats treated with M. africana aqueous extract at 40mg/kg, C = non-Ovx rats treated with M. africana aqueous extract at 80mg/kg, D = non-Ovx rats treated with M. africana aqueous extract at 160mg/kg. Cl = corpus luteum, Terf = Tertiary follicle, Af = atretic follicle, Dgf = De Graaf follicle

3.2.3 Effects of *Mammea africana* on vagina and uterus

Fig. 4 shows the effects of 14 days of treatment with *Mammea africana* aqueous extract on vaginal cytology and the histology of the uterus and vagina. The extract induced, a dose of 160mg/kg a significant increase ($p < 0.05$) in vaginal epithelial height compared to control (Fig. 5-I). This dose also induced the stratification of vaginal epithelium, characterized by intermediate cells observed on vaginal smears of non-Ovx

treated rats given the extract at 160mg/kg. *Mammea africana* did not affect uterine epithelial height in non-Ovx rats compared to the control (Fig. 5-II).

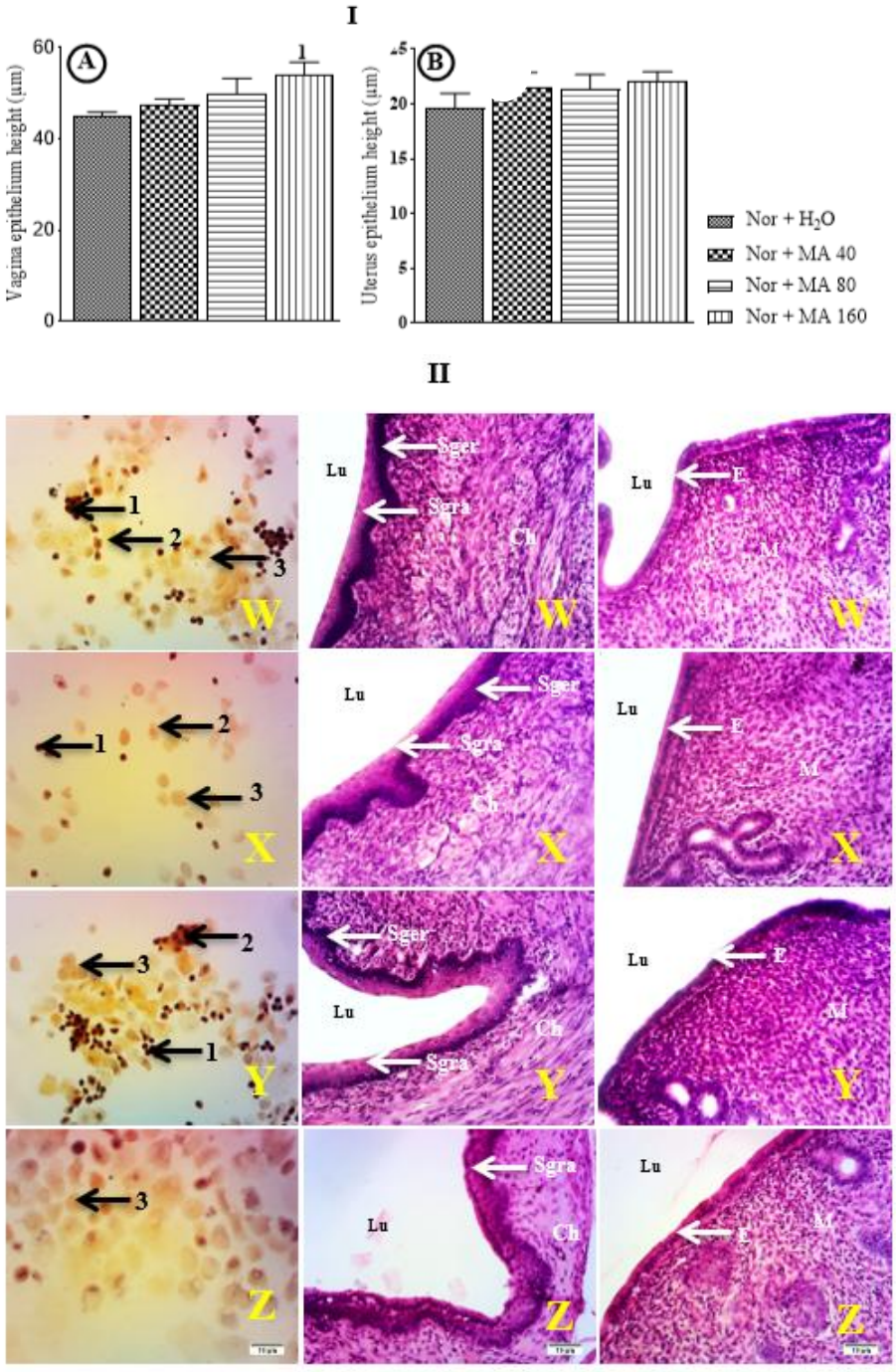

Fig. 5. Effects of *Mammea africana* aqueous extract on vaginal (A) and uterine (B) epithelial height (I) and on cytology and histology of uterus and vagina in Novx animal (II)

Bars are mean ± ESM (n = 5); [1] p < 0.05: significant difference compared to control. Novx + H_2O = control treated with distilled water (10 mL/kg); Novx + MA 40 = non-Ovx rats treated with M. africana aqueous extract at 40mg/kg, Novx + MA 80 = non-Ovx rats treated with M. africana aqueous extract at 80mg/kg, Novx + MA 160 = non-Ovx rats treated with M. africana aqueous extract at 160mg/kg. W= Novx + H_2O ; X= Novx + MA 40; Y = Novx + MA 80; Z= Novx + MA 160. 1 = polynuclear cells, 2 = parabasal cells; 3 = intermediate cells; Lu = lumen; Sger = stratum germativum; Sgra = stratum granulosum; Ch = chorion; E = Epithelium of the endometrium; S = Stroma of the endometrium

3.3 Estrogenic Activities of *Mammea africana* Extract in Ovariectomized Rat

3.3.1 Activities of *M. africana* extract on relative weight and luminal epithelium height of the uterus

The extract of *Mammea africana* at all the used doses had no effects on the relative weight of the uterus (Fig. 6A) nor its epithelium height (Fig. 6B), compared to the Ovx control. However, the extract combined with estradiol valerate at 0.75mg/kg maximized the effects of estradiol valerate alone. This maximization of the extract was significant ($p < 0.001$) and in a dose-dependent manner compared to Ovx control.

3.3.2 Activities of *M. africana* extract on histology and cytology

In comparison to the Ovx control, M. africana extract at all doses induced differentiation of intermediate cells in foliaceous cells in 14 days in Ovx rats. This observation is confirmed by the histology of the vagina. Indeed, the extract induced the cornification of the luminal epithelium of the vagina compared to the Ovx control. Nevertheless, *M. africana* aqueous extract has no effects on uterine luminal epithelium contrary to estradiol valerate at 1mg/kg. After three days of treatment, *Mammea africana aqueous* extract induced differentiation of cellular layers of acini and increased production of eosinophilic secretions in 14-day-old Ovx animals compared to the Ovx control (Fig. 7).Furthermore, *M. africana* extract maximized the vaginotrophic and mammotrophic effects of E_2V at 0.75mg/kg in Ovx compared to Ovx treated only with E_2V at 1mg/kg.

4. DISCUSSION

Infertility has a strong impact on families and communities. In women, infertility is most commonly caused by a range of abnormalities of the ovaries, uterus, fallopian tubes, and endocrine system, among others [13]. Furthermore, it is considered one of the unsolved problems of the human race [14]. Many people used medicinal plants to manage their primary health problems [15]. Indeed, medicinal plants are known as a prolific source of secondary

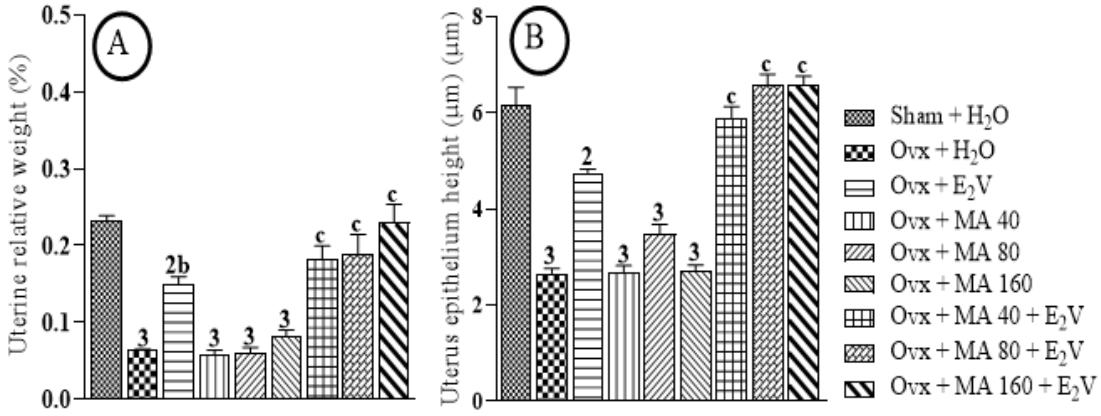

Fig. 6. Effects of *M. africana* aqueous extract on the relative weight and epithelial height of the uterus

Bars are mean ± SEM (n = 5); $^{2}p < 0.002$; $^{3}p < 0.001$: significant difference compared to Sham-operated control. $^{b}p < 0.002$; $^{c}p < 0.001$: significant difference compared to Ovx control. Sham + H_2O = sham-operated animals treated with distilled water; Ovx + H_2O = Ovariectomized animals treated with distilled water (10 mL/kg); Ovx + E_2V = Ovariectomized animals treated with estradiol valerate at 1 mg/kg Ovx + MA 40 = Ovariectomized animal treated with M. africana aqueous extract at 40mg/kg, Ovx + MA 80 = Ovariectomized animal treated with M. africana aqueous extract at 80mg/kg, Ovx + MA 160 = Ovariectomized animal treated with M. africana aqueous extract at 160mg/kg. Ovx + MA 40 + E_2V = Ovariectomized animal treated with M. africana aqueous extract at 40mg/kg and estradiol valerate at the dose of 0.75mg/kg, Ovx + MA 80 + E_2V = Ovariectomized animal treated with M. africana aqueous extract at 80mg/kg and estradiol valerate at the dose of 0.7mg/kg, Ovx + MA 160 + E_2V = Ovariectomized animal treated with M. africana aqueous extract at 160mg/kg and estradiol valerate at the dose of 0.75 mg/kg

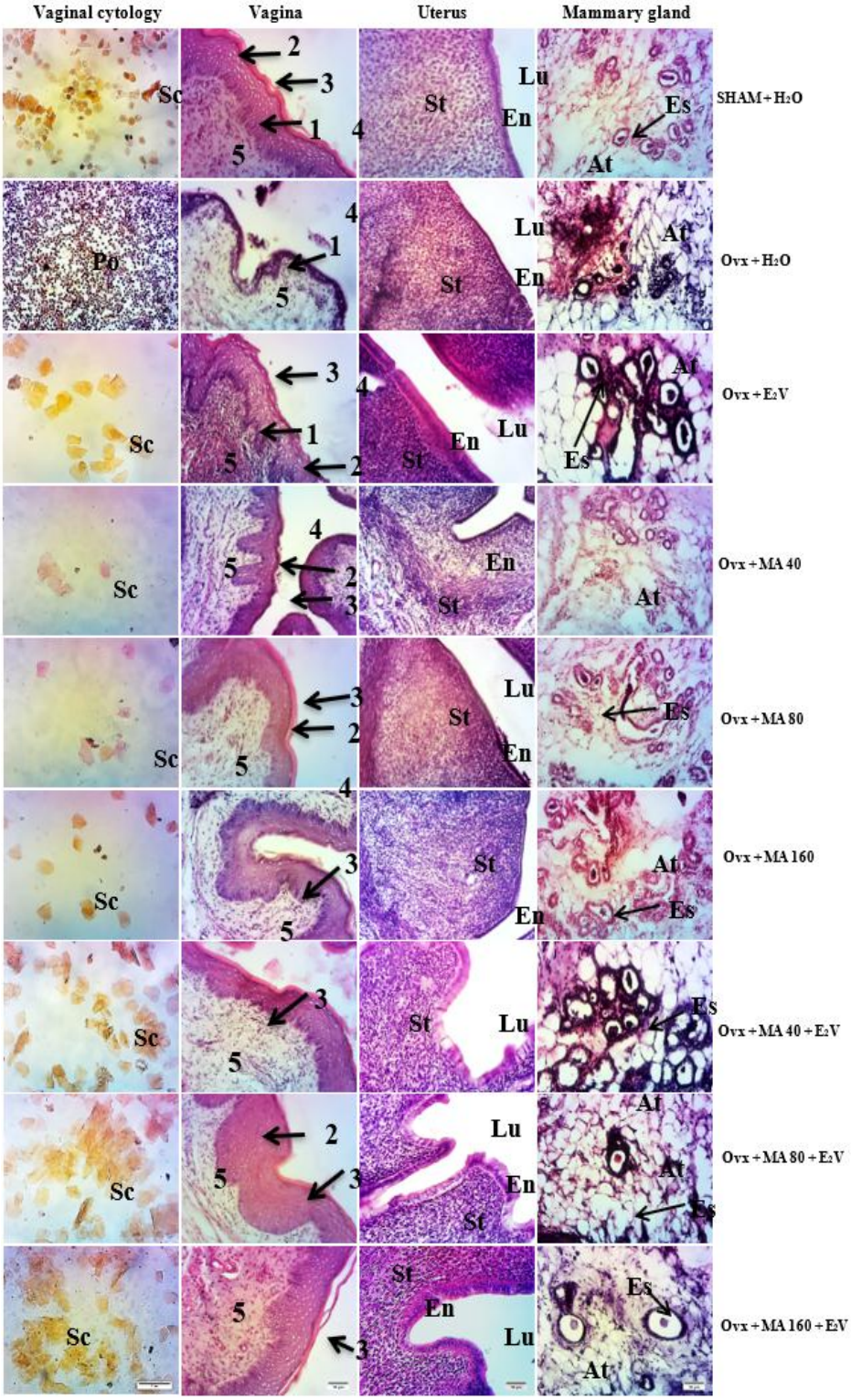

Fig. 7. Vaginal cytology (100X, Papanicolaou) and the microarchitecture of vagina, uterus, and mammary gland (HE, X100) after 3-day treatment with *Mammea africana* stem bark extract

Vaginal cytology: Sc = superficial cell; Ic = Intermediate cell; Po = Polynuclear; Pc = Parabasal cell; Vagina: 1 = Stratum germinativum; 2 = Stratum granulosum, 3 = Stratum corneum, 4 = Lumen, 5 = Chorion, Uterus: Lu = uterine lumen; En = luminal epithelium; St = stroma; Mammary gland: At = adipose tissue; Es = Eosinophilic secretion

metabolites that can improve ovarian folliculogenesis and steroidogenesis [16]. This can be due to the estrogenic, progestogenic, anti-inflammatory, antibiotic, or antioxidant activities of plants. Furthermore, medicinal plants can also act on the hypothalamic-pituitary-gonad axis by inducting or inhibiting of ovulation or spermatogenesis [17]. In Cameroon, particularly in the Nyong & So'o Division, many traditional healers used the plant to manage infertility problems.

This study aimed to investigate plants commonly used against reproductive dysfunction in the Nyong & So'o division and evaluate the effects of the most commonly used plant (*Mammea africana*) on parameters of the reproductive system in female rats. This study documented 20 species of plants used for the management of reproductive failure by traditional healers. Indeed, many studies have reported that medicinal plants play an essential role in primary healthcare [18,19]. Among the plants reported in this study, *Mammea africana* was the most used plant, followed by *Antrocaryon klaineanum*, and *Ageratum conyzoides*. Ndjib et al. [20] reported that they are also used in Cameroon as an anti-haemorrhoidal treatment in the Centre and Littoral region. To assess and elucidate the pharmacological pathways of the most used one, *M. africana*, preliminary tests were carried out *in vivo* in female rats. *M. africana* aqueous extract induced the stratification and cornification of the vagina in Ovx rats. Furthermore, the same extract induced the differentiation of acini and the production of eosinophilic secretion in the same conditions. Thus, *M. africana* aqueous extract contains secondary metabolites that induce estrogenic activity. These activities are probably due to phytoestrogens like 7-dihydroxy-8-(12-methyl-butyl) – 4 –N –pentylcoumarins, 4-phenyl and 4-alkylcoumarins, which have been isolated from the stem bark of *M. africana* [21]. Indeed, it is well-documented that phytoestrogens can exhibit estrogenic activities [17]. The estrogenic effects of *M. africana* extract in the present study were selective. Indeed, there is no differentiation in the luminal epithelium of the endometrium of Ovx rats treated with the extract. Küpeli-Akkol et al. [22] showed that coumarin-based products can exhibit selective estrogen receptor modulation. Findings of the present study also showed that *M. africana* aqueous extract maximized the effects of estradiol valerate on the vagina, mammary gland as well as uterus in Ovx animals, suggesting the fixation of some *M. africana* compounds on estrogen receptors and confirming the extract-induced trophic effects. This observation is different from the result of Bulzomi et al. [23] which showed that phytoestrogens and flavonoids in particular can reduce the effect of endogenous estradiol.

The aqueous extract of *M. africana* increased the level of endogenous estradiol in Novx rats. This result reflected the impact of the extract on folliculogenesis. Estradiol is produced in the majority by the ovaries, especially by granulosa cells. In the present study, the extract increased the number of *corpus luteum* and reduced the number of atretic follicles. This suggests that *M. africana* secondary metabolites promote ovulation. Indeed, many compounds, as well as flavonoids, lignans, and coumestans derived from plants, can mimic the biological activities of endogenous estradiol. Phytoestrogens can bind to estrogen receptors or regulate steroidogenesis by modulating cytochrome P450 aromatase and/or 17β-hydroxysteroid dehydrogenase [24]. This point of view is confirmed by the increase in gonadotropin (LH and FSH) levels induced by the *M. africana* aqueous extract. The estrogenic effects of some compounds are often related to the stimulation of the hypothalamus-pituitary complex, increasing FSH and LH, which will thereafter induce ovarian steroidogenesis [25]. Furthermore, *M. africana* extract reduced ovarian as well as seric cholesterol. Cholesterol is a precursor of steroidogenesis; its reduction in the ovaries can reflect its use for the production of steroid hormones, as reflected by Ahangarpour et al. [26]. Previous studies showed that *Mammea africana* possessed hypocholesterolemic effects in diabetic rats [6]. Further studies are needed to assess *Mammea africana* activities in animal models of infertility.

5. CONCLUSION

Overall, many plants are commonly used for the management of reproductive failure in the Nyong & So'o Division (Cameroon). This study provides comprehensive information on therapeutic procedures employed by traditional healers in the treatment of diseases related to infertility. *Mammea africana,* the most used plant in this division, showed some good points for the management of infertility. Its aqueous extract stimulated ovarian follicle maturation, increased gonadotropin serine levels, and induced estrogenic activity. More research is needed to standardize the plant's use in the treatment of infertility.

CONSENT

It is not applicable.

ETHICAL APPROVAL

For the present study, prior authorization for the use of animals was obtained from the Cameroon National Ethics Committee (Reg. Nº. FWA-IRD 0001954).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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