Ethnobotanical survey of medicinal plants used against infertility in the Nyong & So'o Division (Cameroon) and pro-fertilizing activities of *Mammea africana* (Clusiaceae) aqueous extract rats

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 to evaluate the effect of *Mammea africana*, the most used medicinal plant extract on reproductive parameters in female rats.

Methods: Firstly, a transversal and analytic investigation was carried out on a sample of 22 traditional healers with semi-structured interviews. Secondly, two pharmacological assays were carried out to evaluate *M. africana* aqueous extract activities in 8-10 weeks female Wistar rats. For the first assay, non-ovariectomized (Novx) received *per os* respectively distilled water (DW) at 10mL/kg, *M. africana* extract at 40, 80, and 160 mg/kg during 14 days. In the end, cervical smears were realized then animals were sacrificed, and extract activities were evaluated on reproductive parameters. For the second assay, estrogenic activities of *M. africana* extract were evaluated on estrogenic-dependent tissues *per os* in ovariectomized (Ovx) rats. They received respectively distilled water (DW), estradiol valerate (E_2V) at 1 mg/kg, the extract at aforesaid doses, and 3 groups 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 Nyong & So'o Division to manage reproductive failure. Among them, *Mammea africana* the most used 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]. It affects both sexes, men and women. In a couple, pure man factors account for 20-35% of infertility while 40-50% of factors are solely due to women, 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 with personal, social, and economic consequences. In Africa, pregnancy disabilities have a particular concern because of the extent of the problem and the social stigma attached to it. 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 et So'o division is reported as a 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 effect [2]. Among these plants, *Mammea africana* is most famous in the Nyong & So'o Division in 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 improved 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 in Cameroon and evaluate the activities of the most 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 of Nyong & So'o Division, Centre Region (Cameroon) (3°31'0" N et 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* (Figure 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. 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.

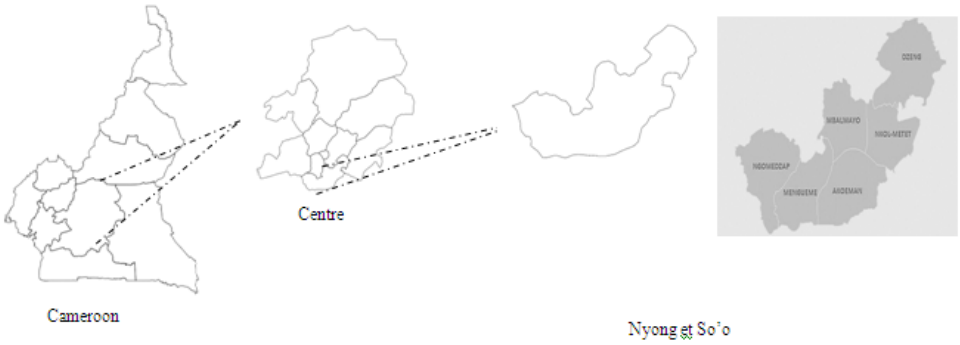


Figure 1: Geographic position of the Department of Nyong et So'o within the Centre Region of Cameroon

2.2. Plant material

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 a powder form using an electric grinder 500 g of barks of *M. africana* were macerated in 10 L of water for 24 h and then, boiled for 30 min. The decoction was filtered using Whatman N°3 paper and the filtrate obtained was lyophilized to obtain finally 35.41 g of a crude extract with a yield of 7.08 %.

2.2.3. Determination of study doses

The recommendations of the traditional healer allowed obtaining from 600mL of macerate 0.90g of crude extract 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 by [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 material

Eight to ten weeks-old female Wistar rats, weighted between 120-130g 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 6 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 3 estrous cycles was carried out before starting the assay. Rats were divided into 4 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 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 12h non-water fasting. Arterio-venous blood was collected after decapitation. Blood samples were centrifuged at 3500rpm (15min 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.

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 9 groups: a negative control received distilled water at 10mL/kg, positive control was treated with E_2V (at 1mg/kg), and three groups were treated with *M. africana* extract at the doses of 40, 80, and 160mg/kg, and three groups co-treated with this dose combined to E_2V (0.75mg/kg). A sham-operated group received distilled water at 10 mL/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 Fortress, UK. Estradiol, Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH) seric levels were assessed by 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%, 100% (three baths)) for 1 hour each, and then stayed in two xylene baths for 2h 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 44421 linked to a computer where images were transferred. Histomorphometric assessments (luminal epithelium of uterus and vagina 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 *corona radiata*: which surround the *zona pellucida*; the second is *membrana*: interior to the basal *lamina*; the third is *peri-antral*: adjacent to the *antrum* and the last is *cumulus oophorous*.
- De Graaf follicles: with one single large *antrum* of follicular fluid.
- Atresic follicles: composed of oocytes with radical apoptosis of all constituent cells.
- *Corpus luteum*: ruptured follicles with hypertrophied follicular cells and cavity filled with blood.

2.9. Statistical analysis

Comparison of proportions and chi-square test with a significance threshold of less than 0.05 were used for 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. Significance was set at $p < 0.05$.

3. Results

3.1. Ethnobotanical investigation

3.1.1 Sociodemographic characterization of traditional healers

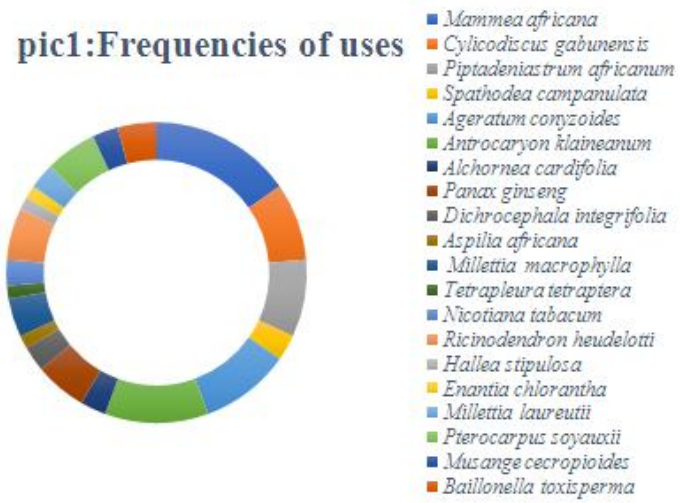
As shown in Table 1, 22 traditional healers were investigated. They were all married, mainly male, and aged between 70-80. The majority of them graduated their primary school and are male.

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

3.1.2 Frequencies of uses of different species

Figure 1 shows how much different species are used by traditional healers. *Mammea africana* was the most used plant with 50% then comes *Antrocaryon klaineanum* with 36.00% and *Ageratum conyzoides* with 31.80%.



As shown in Table 2, an ethnobotanical investigation in 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 affection linked to infertility. The most represented families were the *Asteraceae exéco* with the *Mimosaceae* with 3 species. 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 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* »

	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

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 the increase of 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 increased significantly estradiol ($p < 0.05$), FSH, and LH ($p < 0.01$) seric levels. The increase induced by the extract on endogenous estradiol level was in a dose-dependent manner. Furthermore, the extract reduced significantly both seric and ovarian cholesterol levels compared to the control (Figure 2).

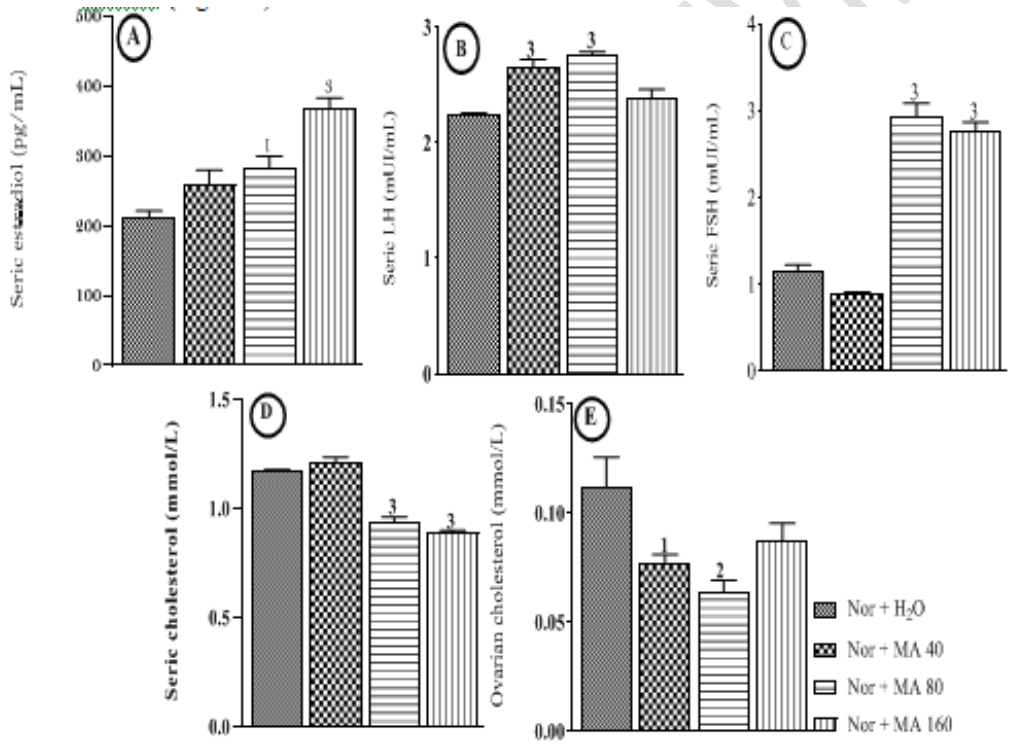


Figure 2: Effects of aqueous extract of *Mammea africana* on estradiol (A), LH (B), FSH (C), seric (D), and ovarian (E) cholesterols levels in non-Ovx rats.

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.

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 ovaries compared to the control. Only the extract at the dose of 160mg/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 (Figure 3), 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).

	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]

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

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.

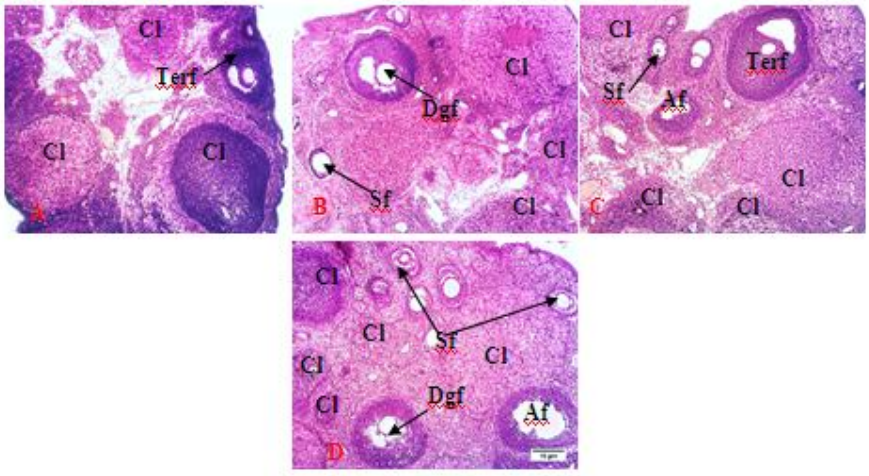


Figure 3: Effects of Mammea africana aqueous extract on the duration of the estrous cycle and ovarian structure and follicles in non-Ovx rats (X25, HE).

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

Figure 4 shows the effects of 14 days of treatment with *Mammea africana* aqueous extract on vaginal cytology and histology of uterus and vagina. The extract induced at the dose of 160mg/kg a significant increase ($p < 0.05$) in vagina epithelial height compared to control (Figure 4I). This dose also induced the stratification of vaginal epithelium characterized by intermediate cells observed on vaginal smears of non-Ovx treated rats with the extract at 160mg/kg. *Mammea africana* had not affected uterine epithelial height in non-Ovx rats compared to the control (Figure 4II).

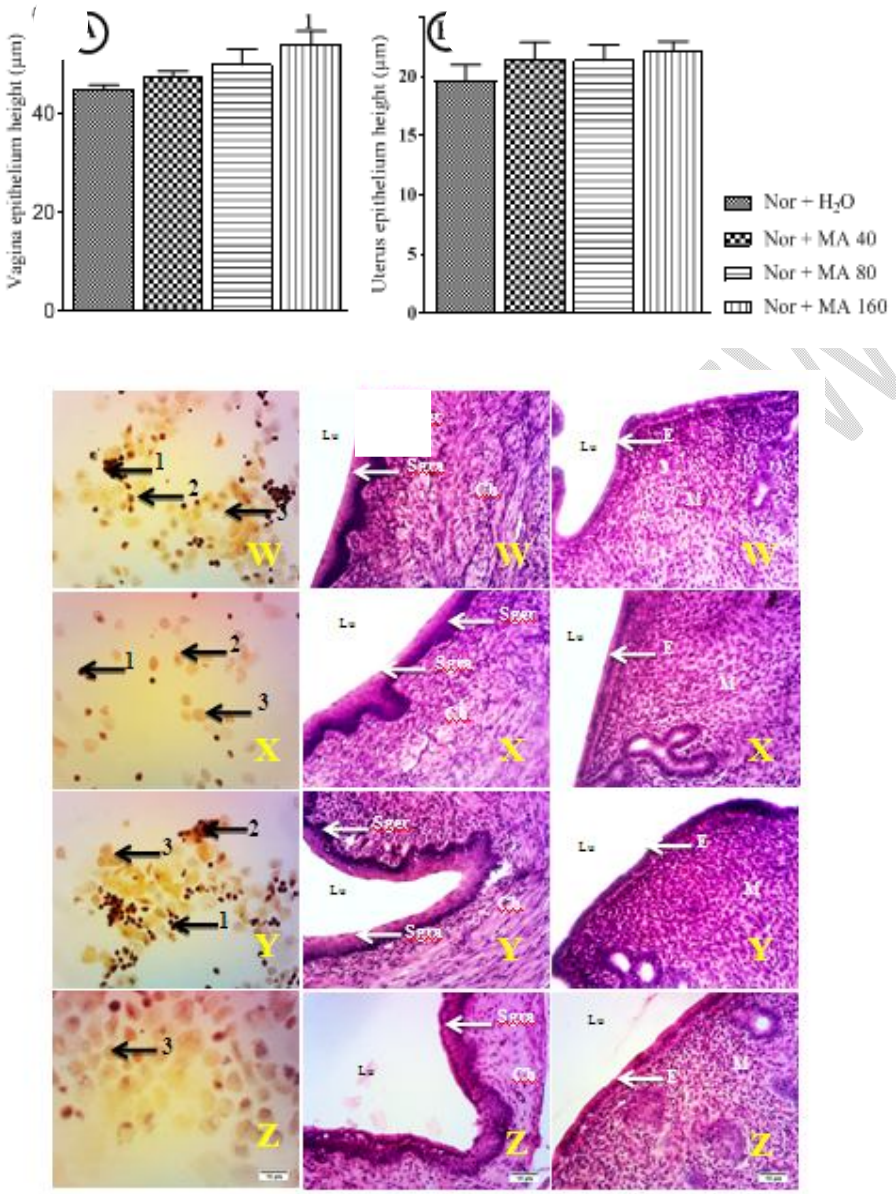


Figure 4: Effects of *Mammea africana* aqueous extract on vaginal (A) and uterine (B) epithelial height (I) and on cytology and histology of uterus and vagina in Novx animal (II). Bars are mean ± ESM (n = 5); [1]p < 0.05: significant difference compared to control. **Novx + H_2O** = control treated with 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 (Figure 5A) nor its epithelium height (Figure 5B), 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.

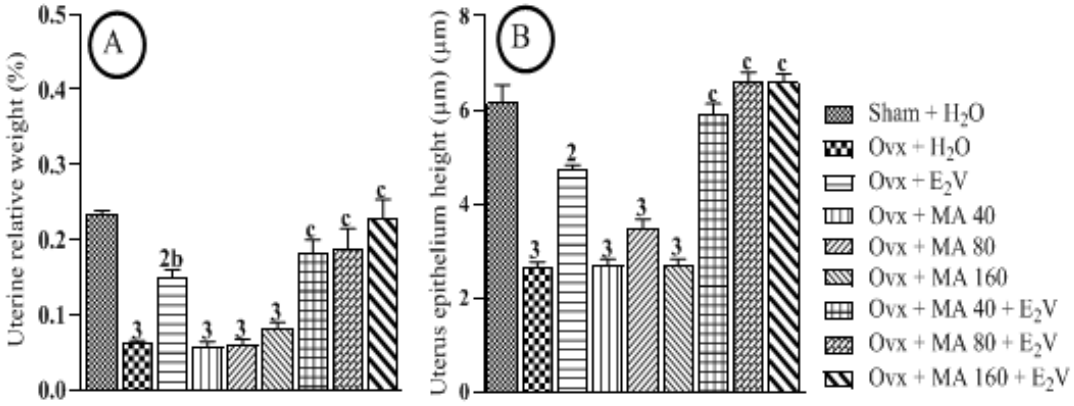


Figure 5: Effects of *M. africana* aqueous extract on the relative weight and epithelial height of the uterus.

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 0.75mg/kg.

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

The extract of *M. africana* at all the doses induced in 14 days-Ovx rat differentiation of intermediate cells in foliaceous cells compared to Ovx control. 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. *Mammea africana* aqueous extract induced differentiation of cellular layers of acini and increased production of eosinophilic secretions after three days of treatment in 14 days-Ovx animals compared to the Ovx control (Figure 6). 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.

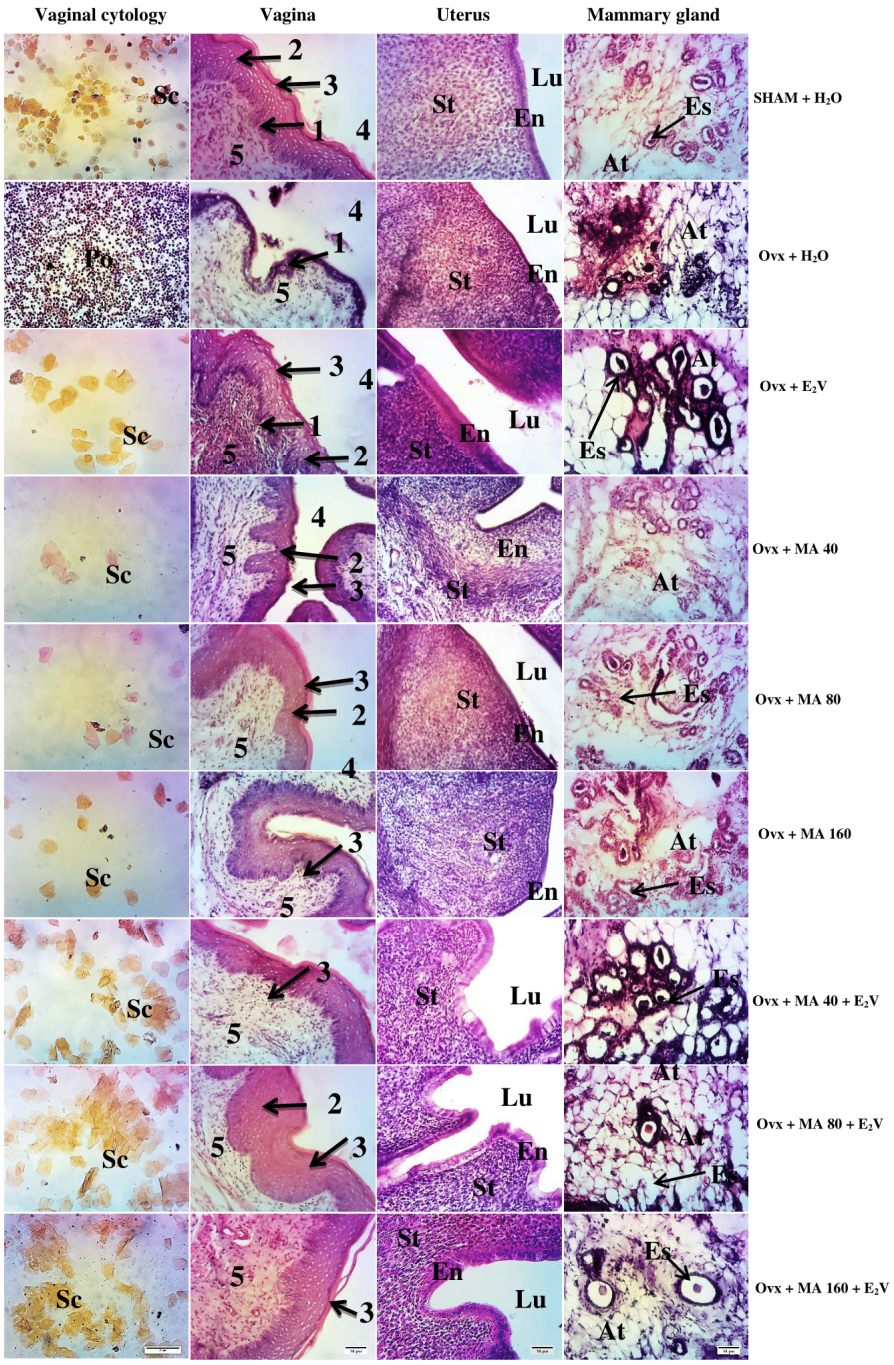


Figure 6: Vaginal cytology (100X, Papanicolaou) and the microarchitecture of vagina, uterus, and mammary gland (HE, X100) after 3-day treatment with *Mammea Africana* stem bark extract. **Vaginal cytology: Sc** = superficial cell; **Ic** = Intermediate cell; **Po** = Polynuclear; **Pc** = Parabasal cell; **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.

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 to be the one of 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 metabolites which 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 induction or inhibition 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 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 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 with *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 to 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 which induced estrogenic activities. These activities are probably due to phytoestrogens like 7-dihydroxy-8-(12-methyl-butyl) – 4 –N –pentylcoumarins and 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 coumarins-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* compound on estrogen receptors and confirming the extract-induced trophic effects. This observation is different from the result of Bulzomi *et a1*. [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 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 gonadotropins (LH and FSH) levels induced by *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 ovaries can reflect its use for the production of steroid hormones as reflected by Ahangarpour *et al.* [26]. Previous studies showed that *Mammea africana* possesses hypocholesterolemic effects in diabetic rats [6].

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 proceedings 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 induced maturation of ovarian follicles,

increase gonadotropins seric level, and induced estrogenic activities. Further studies are needed to standardize the uses of the plant to manage infertility.

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).

Data Availability

The data supporting the conclusions of the present study can be available upon request.

References

1. Jacobson M, Chin H, Mertens A, Spencer J, Fothergill A, Howards P. Research on infertility: definition makes a difference revisited. *Am J Epidemiol.* 2018;187(2):337-346.
2. Abebe M, Afework M, Abaynew Y. Primary and secondary infertility in Africa: a systematic review with meta-analysis. Fertil Res Pract. 2020;6(1):20.
3. James P, Taidy-Leigh L, Bah A, Kanu J, Kangbai J, Sevalie S. Prevalence and correlates of herbal medicine use among women seeking care for infertility in Freetown, Sierra Leone. Evid Based Complement Alternat Med. 2018; 2018:9493807.
4. Abondo-Ngono R, Tchindjang M, Essi MJ, Ngadjui TB, Beyeme V. Cartographie des acteurs de la médecine traditionnelle au Cameroun : cas de la région du Centre. Ethnopharmacologia. 2015; 53: 56-63
5. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210-229.
6. Tchamadeu M, Dzeufiet P, Nouga C, Azebaze A, Allard J, Girolami J, Tack I, Kamtchouing P, Dimo T. Hypoglycaemic effects of *Mammea africana* (Guttiferae) in diabetic rats. *Journal of ethnopharmacology*. 2010; 127(2), 368-372.
7. Okokon J, Udokpoh A. Antimalarial activity of *Mammea africana*. Afr J Tradit Complement Altern Med. 2006;3(4): 43-49.
8. Ouahouo B, Azebaze A, Meyer M, Bodo B, Fomum Z, Nkengfack A. Cytotoxic and antimicrobial coumarins from *Mammea africana*. Ann Trop Med Parasitol. 2004;98(7):733-739.

9. Nair A, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31.
10. OCDE. (2007). Essai n°440 : Bio-essai utérotrophique chez les rongeurs : Essai de dépistage à court terme des propriétés œstrogéniques, Lignes directrices de l'OCDE pour les essais de produits chimiques' ' *Éditions OCDE*.
11. Oumarou M, Zingue S, Bakam B, Ateba S, Foyet S, Mbakop F, Njamen D. *Lannea acida* A. Rich. (Anacardiaceae) ethanol extract exhibits estrogenic effects and prevents bone loss in an ovariectomized rat model of osteoporosis. Evid Based Complement Alternat Med. 2017; 2017:7829059.
12. Kaplan S, Türk A. Adalimumab increases follicle reserve and follicle development in rat ovary: The effect of adalimumab on ovarian reserve. Cureus. 2020;12(10):e11230.
13. Ombelet W. WHO fact sheet on infertility gives hope to millions of infertile couples worldwide. Facts Views Vis Obgyn. 2020;12(4):249-251.
14. Jaradat N, Zaid AN. Herbal remedies used for the treatment of infertility in males and females by traditional healers in the rural areas of the West Bank/Palestine. BMC Complement Altern Med. 2019;19(1):194.
15. World Health Organization. Traditional medicine. Fact sheet 134. 2003-5. Archived from the original on 2008-07-28. *World Health Organization*. 2008;1.4.
16. Khazaei M, Montaseri A, Khazaei M, Khanahmadi M. Study of *Foeniculum vulgare* Effect on folliculogenesis in female mice. Int J Fertil Steril. 2011;5(3):122-127.
17. Doyle B, Frasor J, Bellows L, Locklear T, Perez A, Gomez-Laurito J, Mahady G. Estrogenic effects of herbal medicines from Costa Rica used for the management of menopausal symptoms. Menopause. 2009;16(4):748-755.
18. Tsobou R, Mapongmetsem PM, Van Damme P. Medicinal Plants Used for Treating Reproductive Health Care Problems in Cameroon, Central Africa. Econ Bot. 2016; 70:145-159.
19. Agbor M, Naidoo S. Ethnomedicinal plants used by traditional healers to treat oral health problems in Cameroon. Evid Based Complement Alternat Med. 2015; 2015:649832.
20. Ndjib R, Zambou L, Bissemb O, Ngotta B, Amina M, Kenne P, Nyegue M, Dibong S. Performance, vulnerability, and importance of medicinal plants used in the treatment of vaginitis in four cities of Cameroon. *Int. J. Botany*, 2020 ;16 : 45-57.

21. Games D. Identification of 4-phenyl and 4-alkylcoumarins in *Mammea americana*, *Mammea africana*, and *Calophyllum ionophyllum* by gas chromatography-mass spectrometry. *Tetrahedron Lett*, 1972;13(31):3187-3190.
22. Küpeli-Akkol E, Genç Y, Karpuz B, Sobarzo-Sánchez E, Capasso R. Coumarins and Coumarin-Related Compounds in Pharmacotherapy of Cancer. Cancers (Basel). 2020;12(7):1959.
23. Bulzomi P, Bolli A, Galluzzo P, Leone S, Acconcia F, Marino M. Naringenin and 17beta-estradiol coadministration prevents hormone-induced human cancer cell growth. IUBMB Life. 2010 Jan;62(1):51-60.
24. Taxvig C, Elleby A, Sonne-Hansen K, Bonefeld-Jørgensen E, Vinggaard A, Lykkesfeldt A, Nellemann C. Effects of nutrition relevant mixtures of phytoestrogens on steroidogenesis, aromatase, estrogen, and androgen activity. Nutr Cancer. 2010;62(1):122-131.
25. Park Y, Choo W, Kim H, Chung K, Oh S. Inhibitory Aromatase effects of flavonoids from ginkgo biloba extracts on estrogen biosynthesis. Asian Pac J Cancer Prev. 2015;16(15):6317-6325.
26. Ahangarpour A, Lamoochi Z, Fathi Moghaddam H, Mansouri SM. Effects of *Portulaca oleracea* ethanolic extract on reproductive system of aging female mice. Int J Reprod Biomed. 2016 ;14(3):205-212.