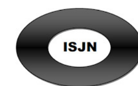
Direct RESEARCH JOURNALS

Vol.1 (2), pp. 8-13, November, 2016
International Standard Journal Number (ISJN)
e-ISJN: A4372-2603
Article Number: DRJA33276810
Copyright © 2016
Author(s) retain the copyright of this article
Direct Research Journal of Public Health and Environmental Technology (DRJPHET)
http://directresearchpublisher.org/journal/drjphet



Research Paper

Effects of the aqueous and methanolic leaf extracts of *Pteridium acquilinum* (*linnaeus*) on some female rats hormones

M. E. Khan [1*], E.T Williams[2], .A. Abel[2] and I. Toma[2]

[1]Department of Chemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria.
[2]Department of Chemistry, Adamawa State University Mubi, Adamawa State, Nigeria.
*Corresponding author E-mail: emamulu@yahoo.com

Received 9 July 2016; Accepted 8 October, 2016

This study screens the phytochemicals present in and evaluates the effect of aqueous and methanolic leaf extracts of *Pteridium acquilinum* (*dennstaedtiaceae*) [*Linnaeus]* on some reproductive hormones; follicle stimulating hormone (FSH) and luteinizing hormone (LH) in female Albino rats. Twenty female Wister albino rats weighing 120-140g (12 – 13 weeks old) were used. They were divided into four groups of five rats each. The rats in groups I and II were given 50 mg/kg body weight of aqueous and methanolic leaf extract respectively orally using orogastric tube. Group III served as negative control while group IV were treated with standard drug. Blood sample was collected from the rats weekly for four weeks (twenty eight days). The methods of Microwell (Sigma, USA) enzyme linked immunoassay (ELISA) were employed to estimate the serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH). The result shows that both the aqueous and methanolic extracts of the plant significantly ($p < 0.05$) increased the level of FSH throughout the period of the experiment. Similarly, the extracts also significantly ($p < 0.05$) increased the level of LH at week 2, but continue to decline at week 3 and 4 which show a similar pattern with the standard drug used. It can be concluded that, *P. acquilinum* leaf extracts have the potential to enhance fertility by increasing serum levels of FSH and LH which in turn increases the number of oocytes released at ovulation possibly through its antioxidant properties.

Key words: Hormones, Elisa, Albino Rats, and *Pteridium aquilinum*

INTRODUCTION

Fertility hormones regulate the reproductive cycle and are used to test for various associated conditions including infertility, menopause, early or delayed puberty as well as non-reproductive disorder. Female fertility is a biological process regulated by female hormones. The brain uses hormones to send signals to the body to trigger events. The body returns feedbacks to the brain to help regulate these events. This process repeats every cycle in women of child bearing age. The most common causes of female infertility are hormones commonly associated with ovulation, polycystic ovarian syndrome, pre-mature ovarian failure, damage to the fallopian tube or uterus and problem with cervix (Egba *et al*., 2014).

Endocrine disorder results from excessive production of hormones or insufficient production of one or more hormones or the lack of the tissues response to normal

circulating hormones (Michael, 2007). The female reproductive cycles function primarily by the interplay between the luteinizing hormone and follicle stimulating hormone. Also the female reproductive organs can be assayed by the serum level of these hormones. A large number of plant species have been screened for their anti-fertility efficacy. A review of reported that 577 plant species have been used traditionally in fertility regulation in females (Kumar *et al*., 2012). Reports have shown that women are increasingly using herbs amongst other things to combat the negative effect of industrial pollutants of fertility (Lans, 2007). *Pteridium aquilinum, (Linnaeus). [dennstaedtiaceae*], (bracken, brake or common bracken). Also known as "eagle fern," is a species of fern occurring in temperate and subtropical regions in both hemispheres. The extreme lightness of its spores has led to its global distribution, (Alonso-Amelot Avendano 2001). It is an herbaceous perennial plant, deciduous in winter. The large, roughly triangular fronds are produced singly, arising upwards from an underground rhizome, and grow to 1–3 m (3–10 ft) tall; the main stem, or stipe, is up to 1 cm (0.4 in) diameter at the base. An adaptable plant, it readily colonies disturbed areas. It can even be invasive in countries where it is native, such as England, where it has invaded heathers (Whitehead and Digby, 1997).

The subject of this research is to determine the phytochemical content of the medicinal extract of bracken plant harvested from Michika LGA Adamwa, Nigeria and to ascertain the its fertility boost on some female albino rat `s reproduction.

MATERIALS AND METHODS

Sample collection and extraction process

Matured green leaves of *P. acquilinum* were collected in and around Michika Local Government Area, Adamawa State on 21 July 2015. Plant species was authenticated by Mr. T. M. Adamu in the state Ministry of Forestry, Mubi Adamawa State and a specimen of the plant was kept in the laboratory. 400 g of dried powdered leaves of *P. acquilinum* accurately weighed and percolated with 10L of distilled methanol for 72hrs. After which there was decantation, filtration, and concentration using rotary evaporator (R110) at 35^0C to obtain methanol soluble fraction, (F_m01). [30.5g], then, the process repeated on another crude sample for water and fraction F_w01 [35.6] obtained. These were kept in the refrigerator for further separation and analysis.

The experimental animals

The animals used in this study were female Wistar albino rats weighing 120-140 g (12 – 13 weeks old) obtained from Vetinary Research Institute Vom (Jos). Plateau State, Nigeria, and housed in a standard cage with free access to standard dry pellet diet, cabbage, apple, banana, bread and water ad libitum.

Experimental design

Before the beginning of the experiment, all the rats were separated into four groups of three each and kept in different cages for two weeks for environmental acclimatization. Serum was collected from each of the groups for the preliminary quantitative hormones (FSH and LH) test and results noted. Groups 1 aqueous extract and Group 2 methanolic extract were treated with 50 mg/kg/day methanolic extract of *P. acquilinum* while group 3 which served as control was given normal feed and group 4, standard drug, 20 mg/kg/day of a prescribed hormone boosting drug as the positive control, all of them, for 28 days. After treatment, serum was collected from each group and tested for the quantity of hormones again.

Specimen collection:

On the 28th day, the rats from each group were anaesthetized in chloroform. Under anaesthesia, their blood was collected and poured into EDTA bottles at very low temperature. It was centrifuged and the serum collected and preserved in plane bottles, for analysis.

Serum Sample

Blood samples were collected through cardiac puncture in centrifugal tubes at interval of seven days each and the serum separated by centrifugation. The Serum was then aspirated into blood sample bottles for use in the experiment.

Phytochemical screening

The phytochemical screening of the extracts were carried out as described by Nweze *et al*., (2004) and Senthilkumar and Reetha (2009). The samples were screened for its carbohydrates, saponins, alkaloids, flavonoids, steroids, anthocyanins, phenols and tannins, glycosides and terpenoids contents.

Luteinizing hormone concentration determination

The desired number of coated wells of microtiter was secured in the holder. 50 µL of specimens were dispensed into appropriate wells. 100 µL of enzymes

conjugate reagent was dispensed into each well, and then mixed gently for 30 seconds. The mixture was incubated at room temperature for 45 min. The incubation mixture was removed by flicking the contents into sink, the microtiter wells were rinsed and flicked 5 times with distilled water, and the wells were stricked sharply onto absorbent paper to remove residual droplets. 100 µL of 3, 3, 5, 5-Tetramethylbenzidine or TMB reagent was dispensed into each well and gently mixed for 10 seconds. It was incubated at room temperature in the dark for 20 min. The reaction was stopped by adding 100 µL of stop solution to each well and gently mixed for 30 seconds to ensure that all blue colour changed to yellow completely. The optical density was read at 450 nm with a microplate reader within 15 min.

Table 1. Results of qualitative phytochemical screening of the aqueous and methanolic leaf extracts of *P. aquilinum*

Phytochemicals	Aqueous Extract	Methanolic Extract
Tannins	+	+
Alkaloids	+	+
Phenol	-	-
Terpenoids	+	+
Steroid	+	+
Anthocyanins	+	+
Flavonoid	+	+
Glycoside	+	+
Carbohydrate	+	+

+ means present, - means below detectable limit.

Determination of follicle stimulating hormone concentration

The desired number of coated wells of microtiter was secured by the holder. 50 uL of specimens were dispensed into each well. 100 µL of enzyme conjugate reagents was dispensed into each well and mixed thoroughly for 30 seconds to have a complete mixing in the step. It was incubated at room temperature for 45 min. The incubation mixture was removed by flicking the plate contents into a waste container. The microtiter wells were rinsed and flicked 5 times with distilled water, and were sharply striked onto absorb paper to remove all residual water droplets. 100 µL of 3, 3, 5, 5-Tetramethylbenzidine or TMB reagent was dispensed into each well and gently mixed for 10 seconds. It was again incubated at room temperature in the dark for 20 min. The reaction was stopped by adding 100 µL of stop solution into each well and mixed gently for 30 seconds to ensure that all blue colour changed to yellow completely. The absorbance was read at 450 nm with a microplate reader within 15 min.

Statistical analysis

The results are presented as mean ± SD / SEM. Data from hormonal test were analyzed statistically using ANOVA followed by a post test (Turkey-kramer Multiple Comparison Test). Differences between means were considered significant at 1% and 5% level of significance, $p < 0.01$ and 0.05.

RESULTS

Table 1 shows the qualitative phytochemical screening results. Most of the phytochemicals analyzed for were present; steroids, alkaloids, saponins, tannins, anthocyanins, carbohydrates, flavonoids, glycoside and terpenoids. Phenols where below detectable levels.The results of the effects of *P. aquilinum* extract on follicle stimulating hormones (FSH) levels are presented in (Table 2). It was observed that aqueous extract of the leaf within one week one (day 7) did not caused a significant ($p > 0.05$) increase in the level of FSH (0.7196 ± 0.2693) as compared to the normal control group (0.3662 ±0.1251) and the group treated with standard drug (0.5384 ±0.1653). In the same week, the methanolic leaf extract significantly ($p > 0.05$) increased the FSH level (1.3246 ± 0.5053) as compared to the two control groups. At week 2 (day 14) the aqueous leaf extract (ALE) caused a significant increase in the level FSH, higher than at week 1 which is contrary to what was observed in the control groups. In the methanolic leaf extract (MLE) group the level of the hormone dropped though not to a significant level , from 1.3246 ± 0.5053 at week 1 to 0.9232 ±0.0556 at week 2, this is similar to what was observed in the normal control and the standard control groups. It was observed that at week 3 (day 21). while the ALE decreased the level of the hormone significantly ($p>0.05$) from 0.7196 ± 0.2693 at week 1 to 0.5806 ±0.1830, in the MLE group, on the other hand, there was a significant ($p>0.05$) increase in the level of the hormone (FSH) from 1.3246 ± 0.5053 (week1) to 2.4764 ± 0.2380 (week 3). This is similar to what was observed in the group treated with standard drug (increased from 0.5384 ±0.1653 to 0.7392 ± 0.0210). The effects of the ALE and MLE on the levels of FSH at week 4 (day 28) showed a similar pattern. All the extracts caused a significant increase in the level of the hormone at week 4 as compared to what was observed at week 1; from 0.7196 ± 0.2693 to 1.6148 ±0.1802 and 1.3246 ± 0.5053 to 3.6758 ± 0.2066 for ALE and MLE respectively; this is contrary to what was seen in the group treated with the standard drug which was a decrease from 0.5384 ± 0.1653 to 0.3528 ± 0.0276.

Table 3 below shows the effects of ALE and MLE of *P. aquilinum* on Luteinizing hormones (LH) level. The results

Table 2. Effect of the plant extract *P. aquilinum* on F S H in milli international unit per milliliter (Miu/mL).

Group	Week 1	Week 2	Week 3	Week 4
(50mg/kgbwt)	0.7196 ± 0.2693[a]	0.9014 ± 0.0620[b]	0.5806 ± 0.1830[b]	1.6148 ± 0.1802[b]
MLE (50mg/kgbwt)	1.3246 ± 0.5053[b]	0.9232 ± 0.0556[b]	2.4764 ± 0.2380[c]	3.6758 ± 0.2066[c]
Control normal (1ml of normal saline)	0.3662 ± 0.1251[a]	0.2586 ± 0.1564[a]	0.2956 ± 0.1006[a]	0.4086 ± 0.0634[a]
Standard control (clomiphene at 20mg/kgbwt)	0.5384 ± 0.1653[a]	0.3700 ± 0.0233[a]	0.7392 ± 0.0210[b]	0.3528 ± 0.0276[a]

All values are present as mean ± SEM. Values with different superscript are significantly different at ($P > 0.05$).
ALE: aqueous leaf extract.
MLE: methanolic leaf extract.

Table 3. Effect of the plant extracts *p. aquilinum* on Luteinizing Hormones in (Miu/ml).

Group	Week 1	Week 2	Week 3	Week 4
Aqueous Extract (50mg/kgbwt)	3.7204 ± 2.0983[a,b]	8.2830 ± 0.6775[d]	1.7860 ± 0.0963[a]	3.4014 ± 0.1459[b]
Methanolic Extract (50mg/kgbwt)	4.3116 ± 0.3796[a,b]	5.5666 ± 0.3266[b]	2.4358 ± 0.1758[b]	2.6572 ±0.1223[a]
Control (1m of normal saline)	6.0700 ± 2.0430[a]	0.4022 ± 0.0752[a]	1.4598 ± 0.1258[a]	2.7094 ± 0.1204[a]
Standard Control (20mg/kgbwt)	3.8684 ± 2.1542[a,b]	7.2380 ± 0.4439[c]	5.5154 ± 0.2769[c]	3.7790 ± 0.1547[c]

All values are present as mean ± SEM. Values with different superscript are significantly different at ($P > 0.05$).
ALE: aqueous leaf extract.
MLE: methanolic leaf extract.

showed that at week 1(day 7) the group treated with ALE, MLE and the standard drugs showed lower values (3.7204 ± 2.0983, 4.3116 ± 0.3796, 3.8684 ± 2.1542 respectively). When compared with the normal control group (6.0700 ± 2.0430). It was observed that at the end of week 2 day (14) the extract caused a significant ($p > 0.05$) increase in the levels of LH in groups 1 and 2 (8.2830 ± 0.6775 and 5.5666 ± 0.3266) which is similar to what was observed in the group that was given the standard drug (7.2380 ± 0.4439). In the same period, the level of the hormone decreased significantly ($p > 0.05$) in the untreated group. As seen in (Table 3), the extracts (ALE and MLE) and the standard drug decreased the level of the hormone (LH) to significant ($p > 0.05$) level at weeks 3 and 4 (day 21 and day 28). While the level of the hormone decreased in all the treated groups, it was observed to have increased in the control group within the same period as compared to the level of the hormone in week 1 (day 7).

DISCUSSION

The use of herbs in the management of ailment has been a regular practice in Africa with considerable therapeutic success. *P. aquilinum*is a plant highly praised for its nutritional and therapeutic benefits. Phytochemical screening has revealed many bioactive agents of plant extract that can affect the regulation of oestrous cycle, conception and reproduction (Orafidiya *et al.,* 2000; Olufisayo and Oluremi, 2008). Therefore the presence of these phytochemicals may account for the alterations in the levels of the circulating hormones observed in this study. Administration of the plant extract can influence hormonal response in host animals (Egba *et al*., 2014). The result showed that oral administration of the plants extract significantly ($p < 0.05$) increased the levels of follicles and the luteinizing hormones which also show similar activity or effect with the standard drug in the test groups compared to the negative control. This findings are in accordance with study of (Hafez and Hafez, 2000) who showed that alcoholic and aqueous extracts of celery leaves when administration at two dose (500 and 100 mg/kg) to the female mice lead to the significant ($p < 0.05$) increase in FSH and LH because these leaves contain chemical compounds that induce gonadotroping release in hormone (GnRH) from Hypothalamus which stimulate pituitary gland secretion.

The increase in the levels of FSH by the extract is obviously due to folliculogenesis and quickening of maturation of the follicle in the pre-ovulatory phase (Kumar *et al*., 2012). It is possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of stimulating hormone is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. The increase observed in the level of this hormone may adversely affect conception in female animals. Other researchers had earlier observed the inhibitory effect of other plant parts on the release of the gonadotropins (Benie *et al*., 1990; Banerje *et al*., 1999; AL-Qarawi *et al*., 2000 and Gonzales *et al*., 2003).

Lutenizing hormone stimulates secretion of sex steroids from the gonads. In females, ovulation of matured follicles in the ovary is induced by a surge of luteinizing hormone secretion during the pre-ovulatory periods. Several researchers have demonstrated that luteinizing hormones release surges at the pre-estrous stage which are responsible for ovulation (Gallo,1981; Hashimato 1987). Any substance capable of inhibiting this release

could provoke disruption of ovulation by decreasing the number of mature follicles or induce an oestrous cycle disruption at rest (Gonzales *et al*., 2003). The increase in level of serum lutenizing hormone indicates the inhibitory effect of the extract on the release of lutenizing hormone which may trigger disruption of ovulation. This may result in impairment of the oestrous cycle; hamper conception and normal reproduction in the females. It is therefore possible that *P. acquilinum* contains anti-gonadotropic substance(s) which may affect the oestrous cycle and alter reproduction in females.

The result of this study indicates that aqueous and methanolic extracts of *P. acquilinum* significantly ($p > 0.05$) increased serum follicle and lutenizing hormones in female rats. Phytochemical constituents of *P. acquilinum* are alkaloids, saponins, tanins, phenols, anthraquinones, steroids, flavonoids, carbohydrates and cardiac glycosides (Prasad *et al*., 2012, and Okoli *et al.,* 2010). Yu *et al.* (2003) had reported that saponins lower serum androgens and 17β-estradiol, but elevate progesterone levels, suggesting that saponins modulate steriodogenesis in the ovary. Estradiol stimulates the growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of the cycle. It is well established that estradiol is directly responsible for growth and development of reproductive organs. In synergy with follicle stimulating hormone, estradiol stimulates granulose cell proliferation during follicular development (Alfa and Rasheed, 2008). Plants with estrogenic property can directly influence pituitary action by peripheral modulation of lutenizing hormone and follicle stimulating hormone, decreasing secretion of these hormones and blocking ovulation (Brinker, 1997) thus, the increase in the serum concentration of estradiol observed in the 50 mg\kg of the plants extract in groups 1 and 11 may be attributed to a decreased aromatase activity or substrate supplementation during estrogen synthesis (Hsia *et al*., 2007). Consequently such decreased in estradiol levels may hamper ovulation preparation of the reproductive tract for zygote implantation and the subsequent maintenance of pregnancy (Hsia *et al*., 2007). Kadohama *et al*., 1993, had reported that several plant alkaloids inhibit aromatase activity.

Thus it is possible that the aqueous and methanolic extracts of *P. acquilinum* contain biologically active phytochemicals which may be endocrine disrupting. Such substances in the plant extract may induce hormonal imbalances or disorders such as anti-fertility and contraception in hormone dependent organs like the ovary and mammary glands.

Conclusion

The findings in this study have important implications for female contraceptive development. Both the aqueous and methanolic extracts of the plants significantly ($p < 0.05$) increased the level of FSH throughout the period of the experiment. Similarly, the extracts also show significant ($p < 0.05$) increased levels of LH at week 2, but continue to decline at week 3 and 4 which showed a similar pattern with the standard drug used. It can be concluded that, *P. acquilinum* leaf extract has the potential to enhance fertility by increasing serum levels of FSH and LH which in turn increases the number of oocytes released at ovulation possibly through its antioxidant properties. Plant products as contraceptives will be more acceptable for economic reasons and for the fact that they are associated with fewer side effects than synthetic agents.

Authors` declaration

We declare that this study is an original research by our research team and we agree to publish it in the Journal.

REFERENCES

Alfa MM, Rasheed AM (2008). “In synergy with follicle stimulating hormone, estradiol stimulates granulose cell proliferation during follicular development” *Egypt J. Comp. Path and Clinic Path*, 21(40)3: 69-87.

Alonso-Amelot ME., Avendano M (2001). "Possible association between gastric cancer and bracken fern in Venezuela: An epidemiologic study."*International Journal of Cancer*. 91(2):252-259.

Al-Qarawi AA, Abdel-Rahman HA, El-Badry AA, Harraz F, Razig NA, Abdel-Magied EM (2000).The effect of *Cynomorium coccineum* and *Withania somnifera* on gonadotrophins and ovarian follicles of immature Wistar rats. *Phytother Res*; 14: 288-290.

Austin Daniel F. (2004). Florida ethnobotany. *CRC Press. p. 551.* ISBN 0-8493-2332-0.Retrieved 30 June 2010.10 (4). 793–803.

Banerje R, Pal AK, Kabir SN, Pakrashi A (1999). Antiovulatoryfaculty of the flowers of *Malvaviscus conzatii. Phytother Res*; 13: 169-171.

Benie T, El Izzi A, Tahiri C, Duval J, Thieulant ML (1990). *Combretodendron africanum* bark extracts as an antifertility agent. 1: estrogenic effects *in vivo* and LH release by cultured gonadotrope cells. *J Ethnopharm*; 29: 13-23.

Brinker F *Br J* (1997). Plants with estrogenic property can directly influence pituitary action by peripheral modulation. *Phytother*: 4: 123-145.

Egba SI, Udom IU, Okonkwo CO (2014). “the most common causes of female infertility are hormones” *Global Journal of Biochemistry and Biotechnology*. 9(1):24-29.

Gallo RV. Pulsatile LH (1981). Release during the ovulatory surgeon proestrous in the rat. *Biol. Reprod.*
24:100-104.

Gonzales GF, Rubio S, Chung A, Gasco M, Villegas L (2003). the effect of plant extract on the anterior pituitary. *Asia Journal Androl.,* 5: 349-52.

Hashimoto I, Isomoto N, Eto M, Kawaminami M,Sunazuka C, Ueki N (1987). Preovulatory secretion of progesterone, luteinizing hormone and prolactin in 4-day and 5-day cycling rats. *Biol. Reprod*; 36: 599-605.

Hafez ES, Hafez B (2000). Hormones, growth factor and reproduction; Reproduction in farm Animals. 7th edition. Lippincott Wiliams and Wilkins, Awoltekluwer, Co. Philadelphia. Pp31 – 55.

Hsia SM, Yeh CL, Kuo YH, Wang PS, Chiang W (2007). Effects of Adlay (*Coix lachryma*-jobi L. var. ma-yuen Stapf.) Hull extracts on the secretion of progesterone and estradiol *in vivo* and *in vitro*. *Exptal Biol & Med*; 232:1181-1194.

Kumar D, Kumar A, Prakash OJ (2012). "Folliculogenesis and quickening of maturation of the follicle in the pre-ovulatory phase". *Ethnopharmacol.*, 140: 1-32.

Kadohama N, Shintani K, Osawa Y (1993). Tobacco alkaloid derivatives as inhibitors of breast cancer aromatase. *Cancer Lett* 75: 175-182.

Lans C (2007). The negative effect of industrial pollutants of fertility *Ethnobiol.Ethnomed.*,3: 13.

Michael J (2007). Neurology and general medicine: Expert consult on line and print. Edinburgh, Churchill living stone. Pp. 45-76.

Nweze EL, Okafor JI, Njoku O (2004). Antimicrobial activities of methanol extract of *Trema guinensis* (Schumm and Thorn) and *Morinda Lucida* Benth used in Nigeria.*Biological Research Journal*, 2:39-46.

Olufisayo O, Oluremi EF (2008). The bioactive agents of plant extract that can affect the regulation of oestrous cycle, conception and reproduction. *Veterinary Research.* 2(34).42-46.

Orafidiya OO, Elujoba AA, Iwalewa FO, Okeke (2000). The bioactive agents of plant extract. I.N. *Pharm.Pharmacol. Lett* 10: 9-12.

Okoli CO, Ejike AC, Agwagah OC, Akah PA (2010). "Phytochemical constituents of *Pteridium acquilinum* are alkaloids, saponin,.." *Pharmacognosy Res.,* 2(1):36-40. *Not cited in the body of the paper*

Prasad MP, Shekhar S and Amit B, (2012). Phytochemical analysis and antioxidant potential of Piper species and its molecular characterization by RAPD markers. *International Journal of Fundamental and Applied Science*, 1(1):7–10.

Senthilkumar PK, Reetha D (2009). Screening of antimicrobial properties of certain Indian medicinal plants. *Journal of Phytology.* 1(3):193-198.

Whitehead SJ, Digby J (1997). "The morphology of bracken (*Pteridium aquilinum* (L.) Kuhn) in the North York Moors—a comparison of the mature stand and the interface with heather (*Calluna vulgaris* (L.) Hull) 1. The fronds". *Annals of Applied Biology*. 131 (1):103–16.doi:10.1111/j.1744-7348.1997.tb05399.x.

Yu WJ, Lee BJ, Nam.SY, Yang DC, and Yun.YW (2003). Saponins lower serum androgens and 17β-estradiol" *Biol. Pharm.Bull.*,26(11):1574-1580.