academicJournals

Vol. 7(26), pp. 1802-1807, 15 July, 2013
DOI 10.5897/AJPP2013.2974
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Evaluating the anti-fertility activity of *Talinum paniculatum* (Jacq.) Gaertn in female wistar rats

Catthareeya Thanamool[1]*, Atcharaporn Thaeomor[1], Suthida Chanlun[2], Pittaya Papirom[2], Sajeera Kupittayanant[1]

[1]Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand.
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand.

Accepted 21 June, 2013

***Talinum paniculatum* (Jacq.) Gaertn (*T. paniculatum*) root and leaf methanolic extracts exhibited significant estrogenic activity in female Wistar rats. Both extracts (100 and 1,000 mg.kg^{-1} B.W) produced anti-immplantation activity and early abortifacient activity in a dose dependent manner (*P<0.001*). The phytochemical screening of the plant extracts showed presence of various phytosterols: campesterol, β-sitosterol, stigmasterol, stigmastan-3-ol, stigmast-22-en-3-ol and stigmastanol. The results suggested that *T. paniculatum* root and leaf extracts possess estrogenic activity and anti-fertility effect. This may be mainly due to the presence of phytosterols.**

Key words: *Talinum paniculatum* (Jacq.) Gaertn, estrogenic activity, anti-immplantation activity, early abortifacient activity, anti-fertility.

INTRODUCTION

Fertility control is a critical issue for women worldwide. About 1% of pregnant women lose their lives due to the unintended pregnant or obtain an abortion in order to avoid having unwanted child (Glasier et al., 2006). Generally, the serious adverse effects such as depression, gastrointestinal disturbance, massive painful uterine contraction, systemic illness, permanent infertility or death are frequently reported in women who utilized synthetic drugs or steroid contraceptions (O'Connell et al., 2007; Sanchez-Criado et al., 1997). Although herbal contraceptives could never reach the level of classical contraceptive pills, they are commonly cheaper and may be used with minimum undesirable side effects. Hence, there is a need for suitable medicinal plants with anti-immplantation and abortifacient activity that could be both safe and effective to use to control pregnancy.

Talinum paniculatum (Jacq.) Gaertn (*T. paniculatum*) or Som Java is recognized as having various medicinal properties (Jung et al., 2006). *T. paniculatum* is a wild deciduous perennial herb with well-developed root system. The medicinal-prepared *Talinum* spp. has long been used in folk medicine; particularly, in the treatment of type-2 diabetes, inflammatory skin problems, gastrointestinal disturbance, general weakness and reproductive disorders (Shimoda et al., 2001; Pak et al., 2005), for aphrodisiac effect and to increase vitality (Manuhara et al., 2012). The root has active constituents such as steroidal saponins and tannins. However, only tannins can be detected in the leaf (Yulia et al., 2005). Additionally, Filho et al. (2010) reported that campesterol, β-sitosterol, stigmasterol could be extracted from the leaf of *T. paniculatum*. In general, these compounds are known as phytosterols, and they possess both estrogenic and anti-estrogenic effects due to their estrogen-like structure. Despite these traditional medicinal properties, no scientific data are available regarding anti-fertility effect of the plant *T. paniculatum*. Therefore, this study was designed for evaluating the anti-fertility activity of

*Corresponding author. E-mail: D5110391@g.sut.ac.th; Tel: +66 4422 4644; Fax: +66 4422 4633.

Table 1. Treatment regiment for estrogenic activity evaluation.

Group	Treatment and dosage	Route
1. OVX control	Vehicle control (1 mL/rat)	Orally
2. OVX	Root extract (100 mg.kg^{-1} BW)	Orally
3. OVX	Root extract (1,000 mg.kg^{-1} BW)	Orally
4. OVX	Leaf extract (100 mg.kg^{-1} BW)	Orally
5. OVX	Leaf extract (1,000 mg.kg^{-1} BW)	Orally

T. paniculatum root and leaf.extracts in female Wistar rats.

MATERIAL AND METHODS

Animals

Bilaterally ovariectomized (OVX) immature rats weighing between 120-150 g and of 6-week-age were used for estrogenic activity testing. Adult male and female Wistar rats weighing between 200-250 g and of 10-week-age were used for anti-fertility activity evaluation. The rats were individually housed in 24 x 15 x 15 cm cages under a 12:12-hr light-dark illumination cycles, at a constant temperature of 25 $\pm$ 0.5°C and 45-50% humidity. All rats were fed with the standard laboratory food containing 0.8% calcium (CP. Co. Ltd, Thailand). Water was provided a*d libitum*. All procedures involving animals were performed in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resource, National Research Council of Thailand. The experiments performed on rats were conducted under strict compliance according to the advice of the Institutional Animal Care and Use Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Plant materials

The plant, *T. paniculatum*, was collected from northeastern area of Thailand during November 2010. A voucher specimen (BKF174387) was deposited and identified by Botanist at the Royal Forest Department of Thailand, Bangkok, Thailand. *T. paniculatum* was cleaned, dried in a constant temperature (50°C), and powdered by grinding machine. The powder of root or leaf (10 g) was extracted separately using methanol (500 mL) in a Soxhlet apparatus for 12 h. The extracts were concentrated in a rotary evaporator, dried by freeze dryer and finally stored at -20°C until use. The yields of the root and leaf extracts were 6.67 and 9.62%, respectively.

Phytochemical screening

Phytochemical screenings of the crude extracts were performed using GC-MS (A Agilent Technologies 7890A gas chromatograph, coupled with an Agilent Technologies 5975C (EI) mass spectrometer). The separation was performed on an HP-5MS column; 30 m x 0.25 mm ID x 0.25 mm film thickness. The temperature of the column was programmed from 50 to 300°C at 10°C /min. The injector temperature and the detector temperature were 250°C. Helium was used as the carrier gas with a constant flow rate of 1.0 µL/min. All separated compounds were identified from the recorded mass spectra by comparing the mass spectra from the NIST and Wiley libraries.

Estrogenic activity evaluation

Female immature Wistar rats were anesthetized using thiopental sodium (25 mg.kg^{-1}BW) intraperitoneally, and OVX was carried out via paralumbar incision just caudal to the 13th rib. After 14 days of endogenous hormonal decline (Tanee et al., 2007). OVX rats were divided into 5 groups of 5 rats in each group. First group received the vehicle (Tween 80 in sesame oil, 10% v/v) and served as control. Group 2-5 were treated with different doses of *T. paniculatum* root and leaf extracts (100 and 1,000 mg.kg^{-1} BW, respectively). Treatment regimen for the experiment is shown in Table 1. The extracts were given for 5 consecutive days; vaginal opening and vaginal smear were observed daily between 9:00-10:00 am.

Vaginal smear was performed to examine cellular differentiation and to evaluate the presence of leukocytes, nucleated cells, or cornified cells. Vaginal smear samples were collected between 9:00-10:00 am daily by gently inserting the tip of dropper into the vagina, flushing normal saline (0.9% NaCl) in and out, and placing the fluid onto microscope slides and stained by methylene blue dripping (Urasopon et al., 2008). The appearance of cornified cells was used as an indicator of estrogenic activity (Cook et al., 1933; Parhizkar et al., 2011). The obtained vaginal cells were counted in 3 randomly chosen areas of the slide, and the percentage of cornified cells (%Co) was calculated using the following formula:

Percentage of cornified cell (%Co) = (Cornified cells x 100) / (Cornified cells + nucleated cells + leucocytes)

Fertility evaluation

The experimental protocols were designed by evaluating the anti-immplantation activity and abortifacient activity as described previously (Mukhram et al., 2012). Briefly, adult female rats of proestrous stage were selected and left overnight with fertile male (1 female : 1 male). After 24 h of intercoursal period, the rats were separated and the spermatic clumps were observed. The rat that showed thick clumps of spermatozoa in vaginal smears was designated as in 1st day pregnancy.

Pregnant rats were randomly separated into 5 groups of 5 rats each. First group received the vehicle (Tween 80 in sesame oil, 10% v/v) and served as control. Group 2 through 5 were treated with different doses of *T. paniculatum* root and leaf extracts (100 and 1,000 mg.kg^{-1} BW, respectively). Treatment regimen for the anti-fertility experiment is shown in Table 2. All groups were orally administered the vehicle and plant extracts during 1st -7th day of pregnancy. On the 8th day, the bilateral laparotomy was carried out under surgical stage of anesthesia (pentobarbital sodium 15 mg.kg^{-1}BW) in sterile conditions. The numbers of implantation sites and corpora lutea in ovaries were observed in order to evaluate the anti-immplantation activity. The lateral abdomens were sutured and rats were left in cages for recovery. The vehicle and plant extracts were further treated for 7 days (9th -14th day of pregnancy). On the 15th day, pregnant rats were scarified to evaluate the early abortifacient

Table 2. Treatment regiment for the anti-fertility experiment.

Group	Treatment and dosage	Route
1. Pregnant control	Vehicle control (1 mL/rat)	Orally
2. Pregnant	Root extract (100 mg.kg^{-1} BW)	Orally
3. Pregnant	Root extract (1,000 mg.kg^{-1} BW)	Orally
4. Pregnant	Leaf extract (100 mg.kg^{-1} BW)	Orally
5. Pregnant	Leaf extract (1,000 mg.kg^{-1} BW)	Orally

activity.

The percentages of antiimplantation and early abortifacient activities were calculated. The summation of antiimplantation and early abortifacient activity gives percentage of anti-fertility activity of the tested materials. The calculation formulas are shown below:

%Anti-immplantation activity = 100- (No.of implantations / No.of coporalutea) x100

% Abortifacient activity = (No.of resorptions / No.of copora lytea) x 100

%Anti-fertility activity = % Antiimpantation activity + % Abortifacient activity

Statistical analysis

Statistical analysis of the differences between the group were analyzed by one-way analysis of variance (ANOVA) followed by the Turkey's multiple comparison tests. *P<0.001* was considered as statistically significant. All data are expressed to the mean value ± SD.

RESULTS

GC/MS Analysis

The GC/MS analysis of the root extract showed the presence of 5 phytosterols which were β-sitosterol (17.37%), stigmasterol (4.23%), stigmastan-3-ol (4.10%), stigmast-22-en-3-ol (1.84%) and campesterol (1.56%), respectively. 12 known compounds were fatty acids (0.50%-11.32%) and 2 unknown compounds were detected. The leaf extract showed the presence of 4 phytosterols which were β-sitosterol (10.60%), stigmastanol (2.76%), stigmasterol (0.85%) and campesterol (0.80%). 11 known compounds; phytols (69.32%), α-tocopherol (0.99%), fatty acids (0.43-3.41%) and 2 unknown compounds were identified.

Estrogenic activity

The effects of *T. paniculatum* root and leaf extract on vaginal epithelial cell differentiation in OVX rats are shown in Figure 1. Oral administration of the plant extracts caused significant increase in the percentage of cornfield cells compared with control group (*P<0.001*). When the extracts were given, the opened vaginas were observed (data not shown).

Antiimplantation and early abortifacient activity

Dose dependent response of antiimplantation and early abortifacient activity to *T. paniculatum* extracts on pregnant rats is illustrated in Table 3. With an increase in the dose of both root and leaf extracts (100 and 1,000 mg.kg^{-1} BW), the percentage of anti-implantation activity was significantly increased as confirmed by decreasing the number of implantation site on 8th day of pregnancy (*P < 0.001*). The extracts also produced a significant early abortifacient activity which is indicated from the implantation scars in the uterine horn on the 15th day of pregnancy (Figure 2).

Anti-fertility activity

Among the different dosages of the plant extracts, the significant dose dependent effect of anti-fertility activity was observed (*P<0.001*). The data showed that dose-related responses of anti-fertility activity produced by the leaf extracts are more effective than the root extracts. Compared with control group, the percentage of anti-fertility activity of the root extracts at the dose of 100 and 1,000 mg.kg^{-1} BW were found to be 47.11 ± 11.95 and 71.65 ± 9.98%, respectively; whereas the percentage of anti-fertility activity of the leaf extracts at the dose of 100 and 1,000 mg.kg^{-1} BW were found to be 81.21 ± 15.78 and 97.29 ± 13.95% (Table 3).

DISCUSSION

The estrogenic substances are well known for their adverse effect on the maintenance of pregnancy by affecting the equilibrium of reproductive hormones to regulate the hypothalamus-pituitary-gonadal axis (Havranex et al., 1973; Iguchi and Sato, 2000). Any disturbance in the level of these hormones may cause infertility by affecting ovulation, implantation and obstructing the uterine milieu (Hughes et al., 1991; McGarvey et al., 2001; Abu and Uchenda, 2011). The large consumption of estrogenic substances as well as phytoesterogens can enhance the luteolytic activity (Shibeshi et al., 2006). They also increase the sensitivity

Table 3 Anti-fertility activity of *T. paniculatum* root and leaf extracts in female Wistar rats.

Treatment	NIS	NER	Anti-implantation Activity (%)	Abortifacient Activity (%)	Anti-fertility Activity (%)
Vehicle control	12.20 ± 3.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Root extract 100 mg.kg^{-1}BW	10.40 ± 1.52	3.60 ± 1.34*	18.97 ±4.94	28.15 ± 9.57*	47.11 ± 11.95*
Root extract 1,000 mg.kg^{-1}BW	8.40 ± 0.55	5.20 ± 1.30*	27.28 ± 5.17	44.38 ± 7.28*	71.65 ± 9.98*
Leaf extract 100 mg.kg^{-1}BW	6.60 ± 0.55*	4.60 ± 1.52*	41.44 ± 8.47*	39.77 ± 9.20*	81.21 ± 15.78*
Leaf extract 1,000 mg.kg^{-1}BW	6.00 ± 1.87*	5.20 ± 0.84*	45.42 ± 12.66*	51.57 ± 5.09*	97.29 ± 13.95*

All values are expressed as mean ± SD of 5 rats in each group. NIS= number of implantation sites, NER= number of embryonic resorptions. * indicates statistically difference at *P<0.001* in comparison to vehicle control group.

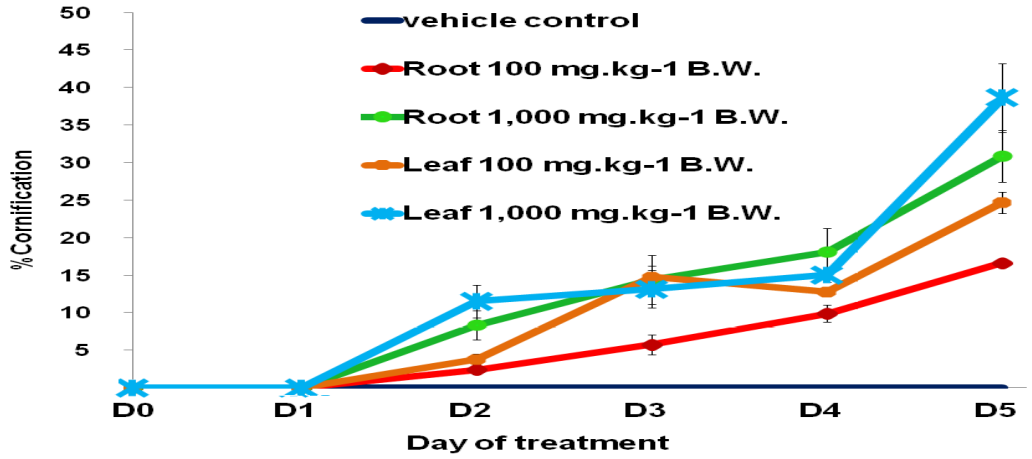

Figure 1. The chronic differentiation of vaginal epithelial cells of OVX rats treated by various doses of *T. paniculatum* root and leaf extracts. Data expressed as mean ± SD of 5 rats in each group. Since day 2, all dosages of the plant extracts caused significant increase in the percentage of cornfield cells compared with control group (*P < 0.001*). The measurement on the X axis of the graph represent day of the experiment. D0, 1 day before the experimental start; D1, first day of the experiment; D2-5 = day 2-5 of the experiment.

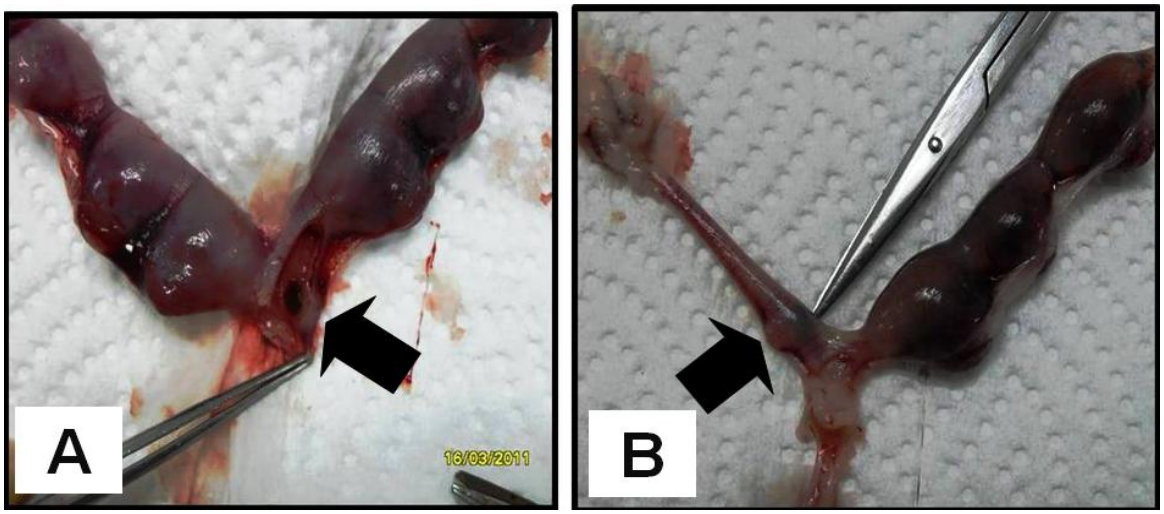

Figure 2. The 15th day of pregnant uteri show embryonic resorption scars (arrow) after the oral administration of *T. paniculatum* root extract (A, 1,000 mg.kg^{-1} BW) and *T. paniculatum* leaf extract (B, 1,000 mg.kg^{-1} BW) for 15 consecutive days.

of the response of the uterus to prostaglandins, which leads to the failure of implantation and increase the abortion rate (Woclawek-Potocka et al., 2005).

In this study, the evaluation of the anti-fertility of *T. paniculatum* root and leaf extracts was conducted during the times before and after implantation process. It was found that, *T. paniculatum* extracts enhanced the antiimplantation activity in a dose dependent manner in pregnant rats. The extracts also affected the conceptous after implantation period as illustrated by the increase in abortifacient activity. Among the tested groups, the group that was treated with leaf extract at the dose of 1,000 $mg.kg^{-1}$ BW exhibited the most potent anti-fertility activity, which is confirmed by the decreasing implantation sites and increasing abortifacient activity. In addition, the leaf extract contained high amount of phytols which could be metabolized to phytanic acid after oral ingestion by hepatic enzyme (Mize et al., 1966). This compound has been reported to obliquely activate estrogen responsive genes via the activating nuclear receptors peroxisome proliferator-activated receptors (PPARs) and heterodimerizes with retinoid X receptor (RXR) (Nuñez et al., 1997; Elmaza and Nau, 2004; Heim et al., 2002). Hence, the potent anti-fertility effects of the leaf extract may be due to the mimicking of estrogenic-like action; which synergistic occurs in response to their phytosterols and signals integrated emanating from phytol signaling pathways (Björnström and Sjöberg, 2005; Goldstein et al., 2003).

In addition, the classical effects of estrogen and phytoestrogenic compounds, such as vaginal cornification and immature opened vagina, were used to detect and confirm the property of anti-fertility substances (Ahirwar et al., 2010). The *T. paniculatum* root and leaf extracts can have the same effects which strongly support anti-fertility activity of the plant. In this study, the GC/MS analysis of *T. paniculatum* crude extracts showed the presence of non-steroidal phytoestrogens such as campesterol, β-sitosterol, stigmasterol, stigmastan-3-ol, stigmast-22-en-3-ol and stigmastanol. These phytosterols have been claimed to possess estrogenic activity due to their affinity to estrogen receptors leading to infertility in animals (Dane and Patil, 2012; Suryawanshi, 2011). In conclusion, the anti-fertility activity of *T. paniculatum* root and leaf extracts might mainly be due to the estrogenic activity of the phytosterols.

ACKNOLEDGEMENT

This study was supported by the Office of the Higher Education Commission of Thailand.

REFERENCES

Abu AH, Uchendu C (2011). Effect of aqueous ethanolic extract of *Hymenocardia acida* stem bark on oestrous cycle of albino rats. J. Med. Plant Res. 5(8):1280-1283.

Ahirwar D, Ahirwar B, Kharya MD (2010). Evaluation of anti-fertility of *Trigonella foenum graecum* seeds. Der Pharnacia Sinica. 1(3):33-39.

Björnström L, Sjöberg M (2005). Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol. Endocrinol. 19:833-842.

Cook JW, Dodds EC, Hewett CJ (1933). A synthetic oestrus-exciting compound. Nature. 131:56-57.

Dane P, Patil S (2012). Evaluation of saponin from *Trigonella foenum Graecum* seed for its anti-fertility activity. Asian J. Pharm. Clin. Res. 5(3):154-157.

Elmaza MMA, Nau H (2004) Potentiation of the teratogenic effects induced by coadministration of retinoic acid or phytanic acid/phytol with synthetic retinoid receptor ligands. Arch. Toxicol. 78:660-668.

Filho SAV, Ramos MPO, Silva GDF, Duarte LP, Peres V, Miranda RRS, de Souza GHB, Belinelo HVJ (2010). Antinociceptive and edematogenic activity and chemical constituents of *Talinum paniculatum Willd*. J. Chem. Pharm. Res. 2:265-274.

Glasier A, Gülmezoglu MA, Schmid GP, Moreno CG, Van Look PF (2006). Sexual and reproductive health: a matter of life and death. Lancet.. 4:1595-1607.

Goldstein JT, Dobrzyn A, Clagett-Dame M, Pike JW, DeLuca HF (2003). Isolation and characterization of unsaturated fatty acids as natural ligands for the retinoid-X receptor. Arch Biochem Biophys. 420:185-193.

Havranex F, Stroufová A, Kozlová J, Herzmann J, Hejda J (1973). On the mechanism of the contraceptive action of oestrogens administered after ovulation. Ceska Gynekol. 38:617-619.

Heim M, Johnson J, Boess F, Bendik I, Weber P, Flūhmann B (2002). Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. The FASEB J. 16:718-720.

Hughes CLJ, Kaldas RS, Weisinger AS, McCants CE, Basham KB (1991). Acute and subacute effects of naturally occurring estrogens on luteinizing hormone secretion in the ovariectomized rat. Reprod. Toxicol. 5:127-132.

Iguchi I, Sato T (2000). Endocrine disruption and eevelopmental abnormalities of female reproduction. Am. Zool. 40:402-411.

Jung M, Park M, Lee HC, Kang TS, Kang ES, Kim SK (2006). Antidiabetic agents from medicinal plants. Curr. Med. Chem. 13:1203-1218.

McGarvey C, Cates PS, Brooks N, Swanson IA, Milligan SR, Coen CW, O'Byrne KT (2001). Phytoestrogens and gonadotropin-releasing hormone pulse generator activity and pituitary luteinizing hormone release in the rat. J. Endocrinol. 124:1202-1208.

Mize CE, Avigan J, Baxter JH, Fales HM, Steinberg D (1966). Metabolism of phytol-U-^{14}C and phytanic acid-U-^{14}C in the rat. J. Lipid. Res. 7:692-697.

Manuhara YSW, Yachya A, Kristanti AN (2012). Effect of aeration and inoculum density on biomass and saponin content of *Talinum paniculatum* gaertn. hairy roots in balloon-type bubble bioreactor. J. Pharm. Biomed. Sci. 2(4):47-52.

Mukhram MA, Shivakumar H, Viswanatha GL, Rajesh S (2012). Anti-fertility effect of flower extracts of *Tabernaemontana divaricata* in rats. Chin. J. Nat. Med. 10(1):58-62.

Nuñez SB, Medin JA, Braissant O, Kemp L, Wahli W, Ozato K, Segars JH (1997). Retinoid X receptor and peroxisome proliferator-activated receptor activate an estrogen responsive gene independent of the estrogen receptor. Mol. Cell Endocrinol. 127:27-40.

O'Connell K, Davis AR, Kerns J (2007). Oral contraceptives: side effects and depression in adolescent girls. Contraception. 75:299-304.

Pak SC, Lim SC, Nah SY, Lee J, Hill JA, Bae CS (2005). Role of Korean red ginseng total saponins in rat infertility induced by polycystic ovaries. Fertil. Steril. 84(2):1139-1143.

Parhizkar S, Latiff AL, Rahman SA, Dollah, MA, Parichehr, H (2011). Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. AJPP. 5:137-142.

Sanchez-Criado CJE, Tebar M, Padron L (1997). The steroid antagonist RU486 given at pro-oestrus induces hypersecretion of follicle-stimulating hormone from oestrus afternoon to early metoestrus in the rat. Eur. J. Endocrinol. 137(3):281-284.

Shibeshi W, Makonnen E, Zerihun L, Debella A (2006). Effect of

Achyranthes aspera L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones. Afr. Health Sci. 6(2):108-112.
Shimoda H, Nishida N, Ninomiya K, Matsuda H, Yoshikawa M (2001). Javaberine A, new TNF-alpha and nitric oxide production ihibitor, from the roots of *Talinum paniculatum*. Heterocycles. 55(11):2043-2050.
Suryawanshi JAS (2011). Neem - natural contraceptive for male and female - an overview. Int J Biomol. Biomed. 1:1-6.
Tanee FS, Njamen, D, Magne Ndé, CB, Wanji, J, Zierau, O, Fomum, ZT, Vollmer, G (2007). Estrogenic effects of the ethyl-acetate extract of the stem bark of *Erythrina lysistemon* Hutch (Fabaceae). Phytomedicine. 14:222-226.
Urasopon N, Hamada Y, Asaoka k, Poungmali U, Malaivijitnond S (2008). Isoflavone content of rodent diets and its estrogenic effect on vaginal cornification in *Pueraria mirifica*-treated rats. Sci. Asia. 34:371-376.
Woclawek-Potocka I, Acosta TJ, Korzekwa A, Bah MM, Shibaya M, Okuda K, Skarzynski DJ (2005). Phytoestrogens modulate prostaglandin production in bovine endometrium: cell type specificity and intracellular mechanism. Exp. Biol. Med. 230:236-233.
Yulia LW, Razief N (2005). Study of phytochemistry of Java ginseng compare to Korean ginseng. In Priosoerganto BP, Suprayagi A, Tiuria R, Astuti DA (Eds) Development of animal health and production for improving the sustainability of livestock farming in the integrated agriculture system: Proceeding of the Mini Workshop Southeast Asia Germany Alumni Network (SEAG) at German Institute for tropical and subtropical agriculture, Indonesia, pp 45-49.