Estrogenic Activity of *Pueraria phaseoloides* Roxb. Benth Evaluated in Ovariectomized Rats


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This study investigated the potential estrogenic bioactivity of tropical kudzu (*Pueraria phaseoloides* Roxb. Benth) crude extracts. Three- to five-week old Sprague-Dawley rats were ovariectomized (OVX) and randomly treated 10 days post-OVX with plant extract at 3 doses (1050, 787.5, 525 mg/kg•d) with a positive control of 17β-estradiol (50µg/kg•d) in corn oil as vehicle for 15 days. Estrogenic activity was determined by analyzing body and uterine weight changes, vaginal cell cornification, uterine morphometry, and histopathology characteristic of estrogen-induced responses. The plant produced a dose-dependent increase in uterine weight and epithelial cell heights. The 2 higher doses of the plant induced differentiation of vaginal cornification in the OVX rats. Estrogenic potency of the plant crude extract used was significantly lower than 17β-estradiol. Data suggested that the plant crude extract exhibited weak estrogenic effects in immature ovariectomized rat model.

**Key Words:** *Pueraria phaseoloides*, estrogenic activity, isoflavones, phytoestrogens, vaginal cornification, uterotrophic bioassay

INTRODUCTION
Phytoestrogens, specifically isoflavones, are receiving great commercial interest at present. Isoflavones are found almost exclusively in legumes. Due to the structural similarity they possess with estrogenic steroids present in the human body, they mimic the effects of naturally occurring estrogenic compounds in the body. In fact, a variety of phytoestrogens have already been identified that bind stronger to the ERβ than ERα, which can induce estrogen-like actions (Davis et al. 1999; Nikov et al. 2000). Specifically, the biological effects associated with ingesting isoflavones indicate that dietary supplements rich in these compounds might be useful for alleviating menopausal health concerns. Epidemiological data have shown that a diet rich in isoflavones, such as those in soy, may reduce the risk of cardiovascular disease and breast and endometrial cancer (Adlercreutz 1995). It has also been suggested that the consumption of food containing phytoestrogens contributes to a lower occurrence of hormone-related cancers in Japanese and Chinese women compared with women in Western countries (Adlercreutz and Mazur 1997; De Kleijn et al. 2001; Xu et al. 2004). Phytoestrogen might also help prevent the onset of hormonal carcinogenesis by decreasing genotoxic estrogen metabolites (Dixon and Ferreira 2002).

Known phytoestrogen compounds have already been isolated from some *Pueraria* species (Morris 1999; Lee et al. 2001; Kim et al. 2003). *Pueraria mirifica*, an indigenous Thai herb, also contains phytoestrogenic substances including miroestrol,
puerarin, deoxymieroestrol, kwakhurin, and other phytoestrogens that belong to the isoflavone and coumestrol class (Bounds and Pope 1960; Ingham et al. 1988; Ingham et al. 1989; Chansakaow et al. 2000). It was observed that *P. mirifica* plant greatly influences menstrual cycles and may suppress ovulation by lowering serum levels of gonadotropins in cynomolgus monkeys (Trisomboon et al. 2005). The plant showed different effects to FSH and LH in ovariecтомized and gonadectomized rats (Malaivijitnond et al. 2004). Clinical trial of crude drug derived from the plant powder showed effectiveness of treatment of menopausal symptoms. It is believed that phytoestrogens contained in the plant, especially isoflavones, support female sexual characteristics including breast and skin appearances (Muangman and Cherdshewasart 2001). Estrogenic activity of the plant chemicals was related to metabolic activation (Lee et. al. 2002). Toxicity test of the plant powder and extract did not show any significant toxicity in both animal and human tests (Cherdshewasart 2003). High concentration of the plant extract showed antiproliferation to HeLa and MCF-7 cells (Cherdshewasart 2004a,b). Currently, phytoestrogenic compounds from *P. mirifica* are marketed as cosmetic and dietary supplement with indication for pre- and post-menopausal women.

Tropical kudzu, *Pueraria phaseoloides* (Roxb.) Benth, is the counterpart leguminous weed species that naturally grows in the Philippines. This forage legume is used traditionally as a cattle feed and ground cover for many agricultural crops. Its usage can be traced back to century-old Chinese herbal medicine where kudzu root has been indicated to reduce addiction to alcohol (Keung and Vallee 1998; Shebek and Rindone 2000; Lukas et al. 2005) similar to that of Chinese kudzu, *Pueraria lobata* (Lin et al. 1996). To date, this plant is a noxious weed in the Philippines having overgrown roadside landscapes, uncultivated, and abandoned lots. Moreover, in the Philippines, which currently encourages herbal medicine research through the Traditional and Alternative Medicine Act of 1997 (Republic Act No. 8423), no plant indicated to alleviate post-menopausal conditions is presently included in the National Integrated Research Program for Medicinal Plants (NIRPROM) priority list for pharmacologic and clinical studies. NIRPROM is a Philippine Council for Health Research and Development - Department of Science and Technology (CHRD-DOST) governed body tasked to spearhead herbal medicine research in the Philippines.

In the light of studies above and the evidence of their direct affinity with their now-acknowledged plant relatives positive for phytoestrogens, this study hypothesized that *P. phaseoloides* also contains some still-unsigned phytoestrogenic substances. Moreover, no research so far has been conducted to assess its potential estrogenic properties that may be tapped as a locally available alternative compound for HRT. Thus, the present study aimed to evaluate the estrogenic activity in *P. phaseoloides* with immature, ovariecтомized rat using a four-fold parametric criterion: uterotrophic assay, vaginal cornification, morphometric and histopathological analysis.

**MATERIALS AND METHODS**

**Animals**

Three- to five-wk old female Sprague-Dawley rats weighing 28-47 g were obtained from the Bureau of Food and Drugs Rat Breeding Laboratory (Alabang, Muntinlupa, Philippines). The animals were transported and housed at the Laboratory Animal Room of Limnological Station, University of the Philippines Los Baños (UPLB) (College, Laguna, Philippines). Experimental condition was maintained at temperature of 23 ± 2°C, and relative humidity of 55 ± 5% on an approximate conventional 12-h light/dark cycle. Rat cages were randomly arranged to limit variation based on temperature and light.

The animals were bilaterally ovariecтомized following standard rodent ovariecтомy procedure. A period of 10 d was allotted for wound healing and acclimatization.

**Pueraria phaseoloides** *P. phaseoloides* was collected at Mt. Makiling, Los Baños, Laguna, Philippines over the period of September to December, 2004. The voucher specimen of the plant was properly identified by plant systematists from UPLB Museum of Natural History. The stem and leaves were separated and placed in brown paper bags for oven drying at 60°C for 2-3 h. Dried stem and leaves were combined and then ground using a Thomas-Wiley mill until a fine substance was achieved. The pulverized material was then soaked in 95% ethanol for 3 d, which was then occasionally swirled and agitated. The resulting mixture was filtered and the supernatant that was collected concentrated under vacuum reduced pressure through the rotatory evaporator. The resultant thick syrupy mass was kept under 4°C, and then used to compute for different doses based on a constant volume per body weight basis.
**Food and Treatment Groups**
To minimize the amounts of phytoestrogens, the conventional rat pelletized food was replaced with a mixture of pulverized corn, mungbean, and sunflower seeds. Vitamin and mineral premix was also given to ensure adequate nutrient supplementation. Access to food was unrestricted and water was administered through an automatic watering system.

Three treatment groups were assigned to receive 3 different treatment preparation of the plant extract: high dose (1050 mg/kg•d), middle dose (787.5 mg/kg•d), and low dose (525 mg/kg•d) based on LD₅₀ of the plant (5250 mg/kg•bw) and initial body weight. 17ß-estradiol (17ß-E2) was given on a 50 µg/kg•d body weight basis for positive control, which was within the treatment range used in the study by Padilla-Blanks et al. (2001) resulting to significant increase in body weight and about 3-fold increase in uterine wet weight. Corn oil was used as the vehicle for crude kudzu extract and 17ß-E2. Only corn oil was used for the negative control (Yamasaki et al. 2001a). All groups received treatment via gastric gavage at 0.5 ml per 50-g body weight, daily for 15 d to cover at least 3 reproductive cycles in the animal.

Using a triple beam balance, rat body weight was measured initially before treatment and then every three days for the 15-d duration of the treatment.

**Uterotrophic Assay: Uterine wet weight determination**
Rats were sacrificed by cervical dislocation on day 15 of the study. But prior to this, the body weight and vaginal smear of each rat were obtained. The horns and body of the uterus were excised and their wet weights determined. Absolute uterine weights were calculated as dividing the uterine wet weight by the body weight and multiplying by 100 (Padilla-Blanks et al. 2001).

**Vaginal Cellular Differentiation**
Vaginal smears were taken and scored daily to monitor cellular differentiation. A smear was also performed on all rats before dosing to establish a baseline, and to confirm that all ovariectomized animal smears showed no cornification. Vaginal smears were taken daily using a Pasteur pipette containing 8.5g/L of saline, placed on slides and observed under a light microscope using a 10X eyepiece and 10X objective. The same technician, unblinded, read smears immediately and cells were identified as leucocytes (PMNs), nucleated, or cornified epithelial cells. A raw score on a scale of 1 through 5 was assigned for cell populations ranging from entirely leucocytes (indicating a diestrus stage) to entirely cornified (indicating an estrous stage). The guide from Maligalig (2001) was used to evaluate staging of smears.

**Preparation of Tissues for Histopathology**
Collected uteri were immediately fixed in 4% formaldehyde in 0.1M phosphate buffered saline for 10 h. Collected tissue samples were processed through a series of alcohols and xylene, infiltrated with Paraplast paraffin under vacuum, and embedded in paraffin. Five-micron sections of cross and longitudinal sections of the mid-uterine body and tip of uterine horns were cut on a rotatory microtome and mounted on slides. Uterine sections were stained primarily to measure changes in the luminal and glandular epithelium by morphometric analyses (Grunert et al. 1987; Tinwell et al. 2000). Tissue sections were then sent to a veterinary pathologist for histopathological evaluation.

**Morphometric Analyses: Luminal and Glandular Epithelial Development of Uterus**
Quantitative measurements were done to morphometrically assess the variations in epithelial cell heights. Histologic responses were quantified using a Nikon Microscope interfaced with an image analysis system (Computer Imaging Laboratory, Institute of Biological Sciences, UPLB) using the program S-Capture and ImageJ 1.33 (National Institute of Health, USA). Following Markey et al. (2001) and Padilla-Blanks (2001) protocols, the following parameters were performed.

Epithelial cell height was measured in areas of epithelium in which the nucleus and basement membrane of single cuboidal/columnar epithelial cells are seen. To determine luminal cell height, 20 measurements from each animal for three animals (60 measurements per group) were randomly obtained. Measurements were done in longitudinally embedded tissues, always from the middle of the uterus and away from the oviduct or cervical segments. In determining cell height of glandular epithelium, 10 measurements from each animal for 3 animals (30 measurements per group) were randomly obtained. The mean for each animal was calculated and these animal means were used to determine the mean ± SD for each treatment group.

**Statistical Analysis**
Values were expressed as the mean ± SD. Data were assessed using Student’s t test and analysis of variance (ANOVA). Results were considered significant at p<0.05.
RESULTS

Body weight and uterine weight changes
No evidence of overt toxicity and abnormal clinical signs were observed in any of the groups during the 15-d treatment period. Although rat mortalities were noted due to impacted and non-healing wounds at the post-OVX incision area prior to treatment (n=2) and on the sixth treatment day (n=1). Results of the uterotrophic bioassay in rats are shown in Table 1. Treatment with various doses of *P. phaseoloides* as well as 17β-E2 caused 27-43% increase in body weight (BW) compared to initial recordings in all treatments (p<0.001). The increase in BW compared with untreated group (corn oil) was significant with 17β-E2 (50 µg/kg•d) and at the 2 higher doses of kudzu crude extract (KCE), i.e., 1050 and 787.5 mg/kg•day (p<0.005) but not with the low-dose KCE. However, the increase in BW with 3 doses of KCE was significantly lower than 17β-E2 treatment.

Similar dose response increase in the uterine weight was observed with increasing doses of plant extract. Data of uterine weights were expressed as uterine wet weight and absolute uterine weight (Table 1). *P. phaseoloides* extract administered to OVX rats at 525, 787.5, and 1050 mg/kg dose-dependently increased both wet (p<0.05, 0.005, 0.0005) and absolute uterine weights (p<0.05, 0.001, 0.0001), producing 7.6-, 6.9- and 4.8-fold increases in absolute uterine weight, respectively. However, the response was significantly less than that observed in 17β-E2 -treated rats (9.2 – fold), indicating lower potency (p<0.01).

Vaginal Cellular Differentiation
Vaginal cells exhibited a distinct pattern of maturation in response to 17β-E2, beginning as a population of leukocytes, advancing to nucleated cells, and terminating as fully cornified cells. Figure 1 shows the changes in vaginal cytology during the 15-d exposure period to doses of kudzu extract and 17β-E2. As expected, exposure to 17β-E2 resulted in a persistent estrous endocrine status, which was reflected by the appearance of cornified epithelial cells in vaginal smears from OVX rats within 3 days following the initial dose and showed total cornification of cells in 5 days. Some cornified cells were also observed following exposure to high dose of KCE (1050 mg/kg•d) after 8 days of treatment, but failed to reach to

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**Table 1.** Body weight and uterine weight of immature ovariectomized SD rats treated with tropical kudzu crude extract (KCE), 17β-estradiol or vehicle for 15 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Uterine Wet Weight (g)</th>
<th>Absolute Uterine Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1050 mg/kg KCE</td>
<td>5</td>
<td>37.94 ± 8.28</td>
<td>64.58 ± 7.54</td>
<td>0.17 ± 0.02</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>787.5 mg/kg KCE</td>
<td>6</td>
<td>35.73 ± 4.00</td>
<td>55.40 ± 7.19</td>
<td>0.11 ± 0.05</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>525 mg/kg KCE</td>
<td>6</td>
<td>35.98 ± 4.55</td>
<td>49.17 ± 5.15</td>
<td>0.08 ± 0.05</td>
<td>0.16 ± 0.10</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>5</td>
<td>43.88 ± 3.16</td>
<td>77.44 ± 2.60</td>
<td>0.24 ± 0.03</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>5</td>
<td>39.50 ± 2.70</td>
<td>51.82 ± 4.64</td>
<td>0.02 ± 0.00</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

*a*All values represented as means ± SD.  
*b*Statistically different from the initial body weight [p < 0.001].  
*c*Change in BW statistically significant compared with negative control (corn oil) [p=0.01].  
*d*Statistically significant compared with negative control (corn oil) at p<0.05 [ * p<0.01; ** p<0.001; *** p<0.0001].  
*e*Statistically significant compared with positive control (17β-E2) at p<0.05.

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**Figure 1.** Vaginal cellular differentiation induced in ovariectomized immature rats in the 5 treatments. (Scale used: 1-completely undifferentiated leucocyte, 2-leucocytes and some nucleocytes, 3-predominantly nucleocytes, 4-nucleocytes and some cornified cells, 5-totally cornified cells). Significant cellular differentiation of cells compared with the negative control was observed in 1050 and 787.5 mg/kg•d doses of KCE at particular days within the 15-d treatment period (* p<0.05). None of the KCE-treated groups was able to achieve total cornification of cells as observed significantly after 3 days with 17β-E2-treated rats.
the extended estrous stage. Middle dose KCE (787.5 mg/kg•d) induced a significant cellular differentiation compared with the negative control on the 12th day of exposure, but was not effective to induce cornification of cells until the last day of treatment period. The lowest dose of KCE did not stimulate differentiation relative to controls.

Uterine histologic observation: Luminal and glandular epithelial cell height
The uterus exhibited an increase in epithelial cell height in response to 17ß-E2 and KCE (Fig. 2 to 4). Figure 2 shows the results of luminal epithelial cell height measurements. The increase in cell height was highest in rats treated with 17ß-E2, which induced a significant increase of 205% relative to the control group (p<0.005). A dose-dependent increase in epithelial height was observed in rats treated with increasing dose of *P. phaseoloides* crude extract inducing 11, 23, and 116% increase at 525, 787.5, and 1050 mg/kg•d KCE, respectively. However, the increase in cell height was significant relative to the negative control only at the highest concentration of KCE (p<0.01), and not with the middle (p= 0.07) and low doses (p= 0.56) of the plant extract.

Similarly, significant difference in glandular epithelial cell heights was observed as a response of the uterus with varying treatment doses of KCE (Fig. 3). A relative increases in glandular cell height of 52, 62, and 169% were exhibited by the 3 doses of KCE and found to be statistically significant compared to the negative control (p<0.05, p<0.01). 17ß-E2 was able to increase the glandular epithelial cell height by as much as 252% of the negative control (p<0.001). Any increase in the epithelial cell height induced by the plant extract, either luminal or glandular, was found to be statistically lower than that of 17ß-E2 treatment.

Histopathological findings showed varying degrees of endometrial epithelial thickening, which is consistent to the dose used, i.e. the higher the dose the greater the degree of thickening and proliferation of cells in the stroma (Fig. 4). Uterine tissues demonstrated the characteristic hyperplasia and hypertrophy maximally seen in rats treated with 17ß-E2 becoming lesser in degree in the dose of tropical kudzu extract used. Thin layer of endometrium with very few blood vessels in the stratum vasculare was observed in the uterine tissues of corn oil-treated rats. Signs of actively functioning uterus like thick endometrium with numerous glands, distinct vascularization of the stratum vasculare were noted on 17ß-E2 treatment resembling findings with higher dose of the plant crude extract. Low- and middle-dose treatments had normal microscopic appearance.
DISCUSSION

The estrous cycle involves several physiological and morphological changes in the uterus, ovaries, and vagina. In rats, the cycle is completed and recurs at 4- to 5-d intervals (Maligalig 2001). The present experiment evaluated the effect of *P. phaseoloides* through gastric gavage in some reproductive parameters of ovariectomized rats. Gastric administration of the test substance in rats at the organism level simulates the most possible means by which the crude extract can be consumed by humans. Besides, this *in vivo* experiment replicated the myriad of pharmacokinetic and pharmacodynamic interactions that likely influence the estrogenic activity of the crude extract (Sutter 1995), which may not be demonstrated by *in vitro* assays. It should also be noted that a reliance on *in vitro* assays for predicting *in vivo* disrupter effects may generate false-negative and false positive results (Chearskul et al. 2004). Therefore, *in vivo* assay must be conducted to test the *in-vitro*-generated hypotheses (Vos 2003). *In vivo* studies indicated that 15-d uterotrophic assay in prepubertal rats or mice is an efficient method for determination of an estrogenic activity (Laws et al. 2000).

The effect of estrogen on immature rat uterus has established the importance of this organ for the bioassay of estrogen-like substances (Sonnenechin and Soto 1998; Yamasaki et al. 2001b). In the uterotrophic assay, laboratory animals were exposed to a chemical substance believed to have an effect on the uterus. Afterwards, the uterus was collected and its weight assessed. This procedure was an indirect measure of the proliferative effects of estrogen reflected by the increase in uterine weight (Sonnenechin and Soto 1998). As expected, exogenous administration of 17ß-E2 caused a significant increase in uterine weight.

Uterotrophic assay revealed that gastric administration of *P. phaseoloides* had a significant effect in increasing the absolute uterine weight. The effect of *P. phaseoloides* in the uterus of ovariectomized rats strongly indicates that some phytoestrogens of the plant led to an increase in uterine weight. This finding was consistent to the report of Tinwell et al. (2000) and Padilla-Blanks et al. (2001) that immature female rats and mice fed with phytoestrogen coumestrol-containing diet can cause a marked increase in uterine weight. However, the uterine
weight increase was significantly lower than that of 17β-E2-treated group, indicating that the plant exhibited only weakly estrogenic at high doses. Other studies with genistein and daidzen, the 2 major isoflavones found in leguminous plants, have also demonstrated weak estrogenic effects as reflected by changes in uterine weight (Santell et al. 1997; Picherit et al. 2000). Estrogen is known to elicit uterine growth response in a non-genomic mechanism (Grunert et al. 1987; Sonnenchein and Soto 1998), which involves inducing changes such as increase in vascular permeability, water imbibition, and cellular infiltration (Rockwell et al., 2002). This sequence of events subsequently leads to an increase in uterine weight. In ovariectomized rats, circulating level of estrogen is very low because of the removal of ovary, which is the primary source of the steroid hormone. Furthermore, immature rats were found to be most sensitive to increase in circulating estrogen in the blood as its reproductive system totally depends on estrogen for growth and differentiation. The study was conducted in ovariectomized, immature rat to rule out the effects of ovarian estrogen on uterotrophic response patterns. Hence, the increase in uterine weight in uterotrophic assay can very well be attributed to the effects of P. phaseoloides. Corn oil cannot cause the increase in uterine weight since it was found to be the most appropriate vehicle for substances that measures reproductive organ weights in rats, i.e. it does not cause an increase in uterine weight (Yamasaki et al. 2001a).

Estrogen also promotes cornification of the vaginal epithelial cells. Safranski et al. (1993) found that vaginal estrus characterized by full cornification of vaginal epithelial cells requires a higher surge of estrogen level. This could explain the abrupt appearance of fully cornified cells at day 4 in smears of rats treated with 17β-E2. Rats treated with 1050 mg/kg of the crude extract showed an increase in this differentiation pattern. This finding suggests the presence of some components of P. phaseoloides, which can induce estrogen-related changes. However, this effect did not match the pattern seen in rats treated with exogenous 17β-E2 where high dose of the plant extract was unable to reach full cornification in the whole 15-d duration of the experiment. Gray and Ostby (1998) reported vaginal mucification and vaginal histologic alterations in ovariectomized rats after 10-wk exposure to weak estrogenic compounds. Thus, a longer exposure period with this weak estrogenic crude extract dose may be able to achieve full cornification of cells.

The uterus and ovaries are the most sensitive tissues to estrogenic regulation, and both tissues express ERα and ERβ, ERα being the predominant isoform in uterus while ERβ prevails in ovaries (Couse et al. 1997; Kuiper et al. 1997). Buchanan et al. (1998) found that vaginal epithelial proliferation was mediated indirectly through stromal ERα, and both epithelial and stromal ERα was required for estrogen-induced cornification and stratification. Phytoestrogens compete stronger with 17β-E2 for binding to ERβ than to ERα and trigger their estrogenic-like physiological responses by mainly acting through ERβ (Kuiper et al. 1998). This could probably explain the weak estrogenic activity exhibited by P. phaseoloides crude extract on the ovariectomized rat.

Several mechanisms of action have been proposed to underlie the purported beneficial effects of phytoestrogens on menopausal symptoms. This includes weak estrogen action on the release of pituitary hormones (Murkies et al. 1998). Phytoestrogens also stimulate the production of sex-hormone binding globulin (SHBG) by the liver (Pino et al. 2002). Higher SHBG levels result in more bound and thus less free estradiol, reducing the amount of estrogens available for binding with estrogen receptors. Because of their weak estrogenic potential (<0.1% that of estradiol), phytoestrogens did not elicit a strong estrogenic response and thus have anti-estrogenic property that inhibits the growth and proliferation of estrogen-dependent cancer cells (Makela et al. 1995; Chearskul et al. 2004). This action is contrary to the known fact that estrogen was typically involved, at least as a permissive agent, in initiation and progression of neoplasias of estrogen target organs (such as mammary gland, endometrium, vagina, and cervix) (Buchanan et al., 1998). Davis et al., (1999) reported that this anti-estrogenic property functions in in vivo tests by protecting estrogen receptor activation by the more potent steroidal agonists such as endogenous estradiol.

The implication of these preliminary findings may be clinically significant. From the above results and previous studies (Shi et al. 2003, Kintzios et al. 2004), it confirms that the isoflavone content in P. phaseoloides (Roxb.) Benth has the estrogenic activity, as related subspecies also exhibited the same effects. Local postmenopausal women, especially folks in the countryside where the plant naturally abounds, may benefit from taking P. phaseoloides extracts. However, the safe dose is yet to be firmly established clinically as more experiments are needed to settle other pharmacodynamic aspects of drug approval such as metabolism, toxicology, and isoflavone analysis of the plant samples. In addition, there is also a need to screen other wild varieties of the plant that contain the highest concentration of the active principle. The high-yielding variety is then selected or harvested from the plant invasion areas or set up a controlled-cultivation for
large-scale commercial production. Future formulation of commercially prepared drug forms (e.g. as tablet or syrup) may be underway as the active principle of the plant is identified. In the end, this study hopes to share its contribution in fulfilling the mandate of health sector in providing safe, effective, and affordable pharmaceutical products derived from commonly available plants.

CONCLUSION AND RECOMMENDATION

This study has shown that *P. phaseoloides* exhibited weak estrogenic effects *in vivo* in the rat uterus and vaginal cells. Also, the phytoestrogen(s) in *P. phaseoloides* has low potency for estrogenic action. Short-term moderate consumption of tropical kudzu is unlikely to cause physical problems due to its estrogenic effect. However, long term exposure to higher doses may cause pharmaceutical benefits or risks to humans.

The chemical nature of phytoestrogens in *P. phaseoloides* is still unknown and therefore warrants further investigation. In addition, there is also a need to screen other wild varieties of the plant that may be capitalized on for large-scale commercial production.

ACKNOWLEDGEMENTS

The authors are grateful to Our Lady of Fatima University Administration and First Year Medicine, Section A2, SY 2004-2005 for the financial assistance; Dr. MDG Maligalig, Dr. EB Rodriguez and Prof. N. Sabino for the use of laboratory facilities at University of the Philippines Los Baños; to Drs. PP Ocampo, Prof. JM Desamero and Prof. RM Neyra for stimulating discussions, and; to Beth Lontoc for providing excellent technical assistance in the whole project duration.

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