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ORIGINAL ARTICLE

Effect of *Vitex pinnata* L. Leaf Extract on Estrogenic Activity and Lipid Profile in Ovariectomized Rats

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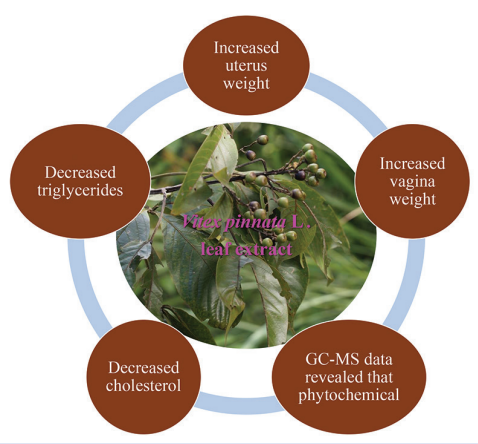
ABSTRACT

Background: The genus *Vitex* contains about 300 species distributed around the world. These genuses are used in the treatment of premenstrual syndrome. However, their estrogenic activity is not well understood. **Objectives:** To compare the estrogenic activity and lipid profile of ethanol extracts of leaves of *Vitex pinnata* L. with 17 β-estradiol in bilaterally ovariectomized (OVX) rats. **Methods:** Ethanol extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). Bilaterally OVX rats were divided into five groups,(n = 6) receiving different treatments, consisting of a vehicle (1% Tween), ethanol extract of *V. pinnata* at three different doses (100, 500, 1000 mg/kg) and standard drug, 17 β-estradiol at a dose of 1 mg/kg. All groups were administered orally, daily for 14 days. **Results:** GC-MS data revealed that the major chemical constituents of the extract were 3, 7, 11, 15-Tetramethylhexadecen-2-en-1-ol, Gamma-Stigmasterol, 9,12,15-octadecatrienoic acid and n-hexadecanoic acid. *V. pinnata* extracts at 1000 mg/kg slightly increased uterine and vaginal weight and endometrial thickness. Doses of extract at 500 and 1000 mg/kg induced a significant ($P < 0.05$) decrease of triglycerides and total cholesterol in serum of OVX rats. **Conclusion:** *V. pinnata* leaf extract exhibits estrogenic activity and reduces levels of serum triglycerides and cholesterol. The understanding of such activity of *V. pinnata* leaf extract has benefits for postmenopausal women.

Key words: 17 β-estradiol, estrogenic activity, lipid profile, ovariectomized rats, *Vitex pinnata*

SUMMARY

- GC-MS data revealed that the major chemical constituents of the extract were 3, 7, 11, 15-Tetramethylhexadecen-2-en-1-ol, Gamma-Stigmasterol, 9, 12, 15-octadecatrienoic acid and n-hexadecanoic acid
- *Vitex pinnata* extracts at 1000 mg/kg slightly increased uterine and vaginal weight and endometrial thickness
- *Vitex pinnata* extracts at 500 and 1000 mg/kg induced a significant ($P < 0.05$) decrease of total cholesterol and triglycerides in serum of ovariectomized rats.



Abbreviations used: GC-MS: Gas chromatography-mass spectrometry; OVX: Ovariectomized; *V. pinnata: Vitex pinnata*; (H and E): (Hematoxylin and eosin).

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INTRODUCTION

The genus *Vitex* L. is in the plant family Lamiaceae which comprises about 300 species in the tropics. It can be found in Malaysia, Indonesia, Philippines, Cambodia, and Thailand.[1] Chantaranothai recognized 18 species in Thailand.[2] Several species of the genus, for example, *Vitex negundo, Vitex doniana, Vitex polygama, Vitex trifolia, Vitex rotundifolia, Vitex altissima, Vitex peduncularis* and *Vitex agnus-castus* have long histories of use as phytoestrogens[3-7] in the alternative treatment of postmenopausal symptoms in many countries.[8] *Vitex pinnata* is known to contain carbohydrates, phenolic compounds, alkaloids, flavonoids, saponins, tannins, steroids, amino acids, and proteins.[9] The leaves of *V. pinnata* (syn *Vitex pubescens* Vahl.) have been applied on cuts and wounds[10] and have been eaten to treat hypertension and fever. The root is consumed for backache, body pain, and fatigue.[1] A previous phytochemical study reported the isolation of the ecdysteroids, pinnatasterone, 20-hydroxyecdysone, and turkesterone.[11] Another study reported a new iridoid glucoside, pinnatoside, and three known flavonoids, namely viscioside, apigennin, and luteolin from the bark of *V. pinnata*.[12] Moreover, Kamal *et al.*[13] have reported phytochemicals of *V. pinnata*, principally stigmasterol, β-sitosterol and flavonoids (5-hydroxy-3, 7,4–trimethoxyflavone, 5-hydroxy-7,4-dimethoxy-flavone and

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5-hydroxy-3,3,4,7-tetramethoxyflavone). The three compounds (β-Sitosterol, flavonoids and phenolic compounds) are known to have the estrogenic activity.[14,15] However, there is no data on the estrogenic properties and lipid profile of *V. pinnata*. Thus, we evaluated the estrogenic activity and lipid profile of *V. pinnata* leaf extract in ovariectomized (OVX) rats, an animal model of menopause.

Experimental

Plant materials

Leaves of *V. pinnata* were sampled and collected from the northeast of Thailand (February to March). Identification of a voucher specimen (no. W. Chatan 1748) was performed by Assistant Prof. Dr. Wannachai Chatan, Department of Biology, Faculty of Science, Mahasarakhan University, Thailand, and kept in the Natural Medicinal Mushroom Museum or MSUT, Mahasarakham University, Maha Sarakham Province, Thailand. The leaves were washed, air-dried, powdered and ethanolic extraction. The extract was filtered by using filter paper. Evaporation was done in a rotary evaporator. It was dried with a lyophilizer and then kept at −20°C until use.

Characterization of the extract

Composition of leaf extract of *V. pinnata* was analyzed for its chemical constituents by GC-MS (GC-MS) (GC-MS 7890A Agilent Technology). The identification of the extract composition was based on comparisons with mass spectra and retention indices of authentic reference compounds where possible.

Used animals

The thirty female Wistar rats (200–230 g) were chosen in the present study. The rats were maintained by using the guidelines of the Committee on the Care and Use of Laboratory Animal Resources, National Research Council.[16] The experimental protocol was approved by the Institutional Animal Care and Use Committee, Khon Kaen University, (approval no. 76/2017). The rats were kept in polypropylene cages (under the standard conditions 12 h light and 12 h dark cycles; 25°C ± 2°C) and had free access to water *ad libitum* and a commercial pellet diet.

Design of experiments

Estrogenic activity was evaluated in bilaterally OVX rats.[17] The parameters assessed were histology of vaginal and uterine wet weight. The OVX rats were separated into five groups (n = 6). All the rats in each group received the treatment for 14 days. Group 1 (OVX [OVX]; control group) received 1 ml of 1% (v/v) Tween 80, Group 2 (standard group) received 17 β-estradiol at a dose 1 mg/kg. Group 3, Group 4, and Group 5 (test group) received plant extract (1 ml) at doses of 100, 500, and 1000 mg/kg B. W., respectively.

Body weight and relative organ weight

After 14 days of treatment, the rats were sacrificed under CO_2 anesthesia. The uterus and vagina were removed and weighed. Relative organ weight (%ROW) of the vaginal and uterine weights were calculated as in Eq. 1.[18]

$$\% \text{ ROW} = ([\text{OR}/\text{BW}])\ 100 \quad (1)$$

Where OR = the absolute organ weight of the rat and BW = the body weight of the rat.

Histological examination

The six excised uteri and vagina from each group were fixed in formalin and processed for histological preparations. Slides stained with hematoxylin and eosin were examined under microscope for the changes in cellular organization of the uterus and vagina.[19]

Lipid profile assessment

Serum levels of total cholesterol, triglyceride, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol were measured by using the automated enzymatic method of SYNCHRONLX20Pro, at Khon Kaen University Community Outreach Center.

Statistical analysis

Statistical analysis was carried out using one-way (ANOVA) followed by Duncan's multiple comparison tests. The results are presented as mean ± standard error mean from six rats in each group. $P < 0.05$ ($P < 0.05$) were considered significant.

RESULTS

Gas chromatography-mass spectrometry chromatogram of ethanol leaf extract from *Vitex pinnata*

The GC-MS analysis has shown the presence of 20 compounds: benzoic acid, methyl 4-ethoxybenzoate, 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol,2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis), hexadecanoic acid, methyl ester, n-hexadecanoic acid, hexadecanoic acid, ethyl ester, 9,12,15-Octadecatrienoicacid,3,7,11,15-Tetramethylhexadecen-2-en-1-ol, 9,12,15-Octadecatrienoic acid, cis-Vaccenic acid, Stearic acid, 2-Palmitoylglycerol, all-trans-Squalene, 1-Hexacosanol, alpha-tocopherol, Ergost-5-en-3beta-ol, Beta-Stigmasterol, gamma-sitosterol, and maragenin I. The details of the identified phytoconstituents and their therapeutic activities are presented in Tables 1 and 2.

The body weight and relative organ weight

The means of the initial body weights of OVX rats were not different among the groups after the administration of *V. pinnata* for fourteen days. However, at the end of the experiment, the results showed that the bilateral OVX enhanced the increase of the final body weight. When compared to OVX, the standard drug 17 β-estradiol (1 mg/kg B. W.) produced statistically significant ($P < 0.05$) decreases in body weight [Table 3].

V. pinnata extracts at 1000 mg/kg slightly increased uterine and vaginal weight and endometrial thickness [Table 3]. The effect was not dose dependent. The standard drug, 17 β-estradiol produced statistically significant ($P < 0.05$), 1.40 ± 0.4 increase in uterine and vagina weight [Table 3].

Histology

Figure 1 shows images of the representative of the transverse section of uteri taken from one animal per treatment group. The OVX rats present a typical atrophic condition of the uterine endometrium [Figure 1a]. The histology showed the typical atrophic features with thin endometrial layer of the uterus. This layer contained poor vascularity and atrophied uterine glands, which were covered by low cuboidal epithelial cells. Oral administration of 17 β-estradiol affected the structure and size of all uteri as illustrated by an expansion in endometrial thickness, an enlargement uterine gland and more numerous vascularity. The bulky epithelial layer was well enlarged, as showed by the columnar cell type in Figure 1b. While, *V. pinnata* extract at 100 and 500 mg/kg B. W. did not perform animportant change to the endometrial

Table 1: Identified phytoconstitutents from the ethanol leaf extract of *V. pinnata*

Retention time	Name of the compounds	Molecular formula	Molecular weight	Area
4.20	Sodium benzoate	$C_7H_5NaO_2$	144.10	2.31
7.50	Benzoic acid, 4-hydroxy-, methyl ester	$C_8H_8O_3$	152.15	0.53
10.77	4-((1E)-3-Hydroxy-1-propenyl)-2-me thoxyphenol	$C_{10}H_{12}O_3$	180.20	1.71
10.95	2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis)	$C_{11}H_{16}O$	196.243	0.38
13.30	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$	270.451	0.33
13.70	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.424	3.31
14.16	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O$	284.477	1.73
15.46	9,12,15-Octadecatrienoic acid methyl ester (Z, Z, Z)-	$C_{18}H_{30}O$	278.430	0.26
15.80	3,7,11,15-Tetramethylhexadecen-2-en-1- ol	$C_{20}H_{40}O$	296.531	16.39
15.93	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O$	278.430	3.87
15.98	cis-Vaccenic acid	$C_{18}H_{34}O$	282.468	1.18
16.34	Stearic acid	$C_{18}H_{36}O$	284.477	2.14
16.85	Stearic acid	$C_{18}H_{36}O$	284.477	1.46
20.89	2-Palmitoylglycerol	$C_{19}H_{38}O$	330.503	0.37
25.63	All-trans-Squalene	$C_{30}H_{50}$	410.718	0.71
29.45	1-Hexacosanol	$C_{26}H_{54}O$	382.706	0.46
29.65	Alpha-Tocopherol	$C_{29}H_{50}O_2$	430.717	0.44
31.29	Ergost-5-en-3beta-ol	$C_{28}H_{48}O$	400.69	0.99
31.95	Beta-Stigmasterol	$C_{29}H_{48}O$	412.702	2.80
33.23	Gamma-Sitosterol	$C_{29}H_{50}O$	414.706	7.58
34.78	Maragenin I	$C_{29}H_{46}O_2$	426.685	0.74
35.913	Alpha-Tocopherol	$C_{29}H_{50}O_2$	430.717	0.46

RT: Retention time

Table 2: Therapeutic activity of the phytocompounds in *Vitex pinnata* leaf extract

RT	Name of the compound	Therapeutic activity**
4.20	Sodium benzoate	Antifungal, preservative
10.77	4-((1E)-3-Hydroxy-1-propenyl)-2-me thoxyphenol	Anticancer, antitumor
10.95	2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis)	Anti-HIV-integrase, increase tyrosine hyroxylase activity
13.30	Hexadecanoic acid, methyl ester	Antibacterial, antifungal,[20] increase aromatic amino acid decarboxylase activity
13.70	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant
14.16	Hexadecanoic acid, ethyl ester	Antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor
15.46	9,12,15-Octadecatrienoic acid, methyl ester (Z, Z, Z)-	Antioxidant, hypocholesterolemic, nematicide, pesticide
15.80	3,7,11,15-Tetramethylhexadecen-2-en-1-ol	Antiinflammatory, hyopcholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic
15.98	cis-Vaccenic acid	Antimicrobial
16.34	Stearic acid	Anhibit production of uric acid
29.65	Alpha-tocopherol	Inhibit oroduction of uric acid
31.95	Beta-Stigmasterol	Antiageing, analgesic, antidiabetic, anttiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
		Antiamyloid-beta, hypocholesterolemic

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases.[20] RT: Retention time

proliferation [Figure 1c and d], at 1000 mg/kg B. W. It made a slight increase in endometrial proliferation, but pathology signs were not detected [Figure 1e].

Figure 2 shows images the representative of the transverse section of vagina taken from one rat per treatment group. The atrophic vaginal epithelium was detected in OVX rats. Normally this layer consisted of one or two shriveled cuboidal or flattened squamous cells with a diminutive mucous cells Figure 2a. The result determined that the groups treated with the 17 β-estradiol displayed a normal squamous multilayered epithelium [Figure 2b], while the layer number was similar to the number in the OVX ratsand cornification was not found in the 100 and 500 mg/kg B. W. treatments [Figure 2c and d]. For the group treated with 1000 mg/kg B. W. extract, the epithelium thickness was slightly expanded [Figure 2e].

Lipid profile

The mean of triglycerides and serum total cholesterol were significantly ($P < 0.05$) decreased in the17 β-estradiol and the *V. pinnata* (500 and 1000 mg/kg B. W.) groups when compared to the OVX control group [Table 4].

Table 3: Body and relative organ weight

Treatment group	Final body weight (g)	Uterus and vagina weight (g)	Uterus and vagina weight/body weight (%)
OVX control	267.00±1.21[b]	0.22±0.1[b]	0.08±0.0[b]
17β-estradiol	213.33±50.7[a]	1.40±0.4[a]	0.67±0.2[a]
V. pinnata 100	269.83±2.62[b]	0.22±0.2[b]	0.08±0.0[b]
V. pinnata 500	276.33±10.76[b]	0.25±0.0[b]	0.09±0.0[b]
V. pinnata 1000	269.00±5.27[b]	0.32±0.7[b]	0.12±0.3[b]

[a,b]Mean within a column with different letters are different ($P<0.05$). *V. pinnata*: *Vitex pinnata*; OVX: Ovariectomized

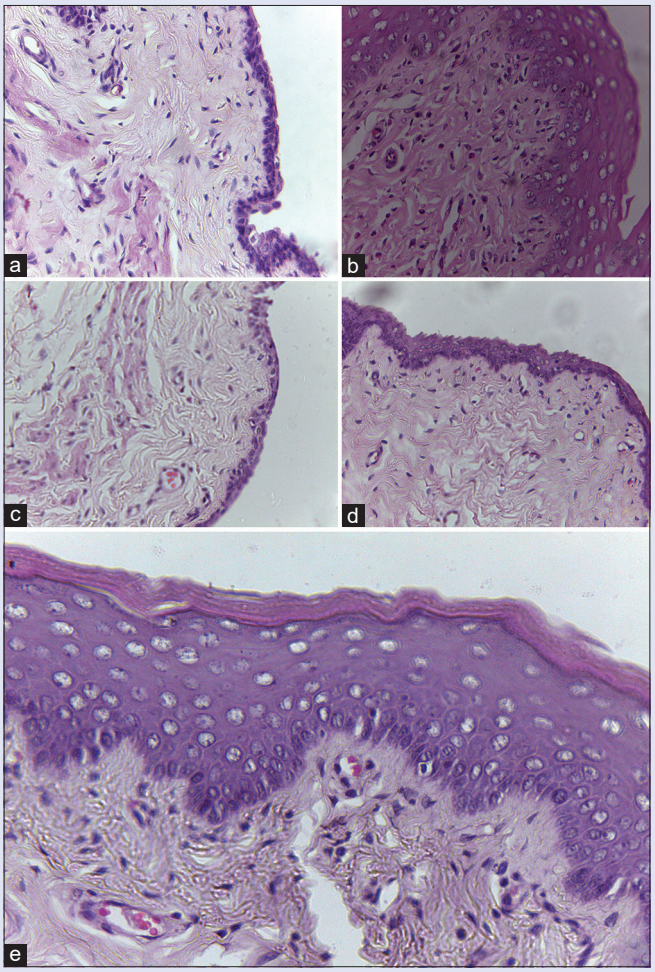

Figure 1: Photomicrographs of haematoxylin and eosin stained transverse section of uterus of *Vitex pinnata*; (a) OVX, (b) 17β-estradiol, (c) *Vitex pinnata* (100 mg/kg), (d) *Vitex pinnata* (500 mg/kg), (e) *Vitex pinnata* (1000 mg/kg) (H and E, ×40)

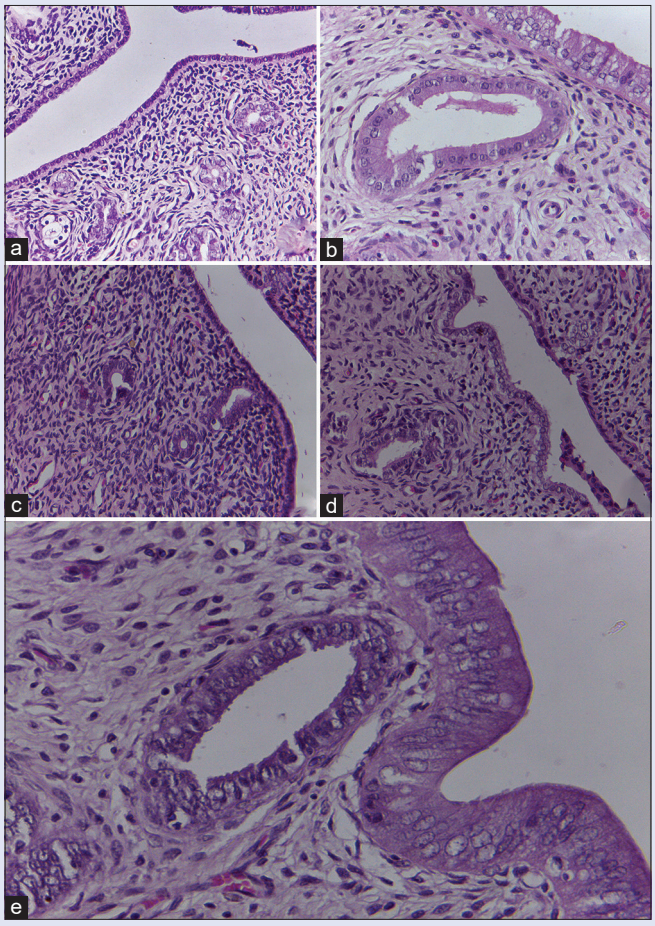

Figure 2: Photomicrograph of haematoxylin and eosin stained transverse section of vagina of *Vitex pinnata*; (a) OVX, (b) 17β-estradiol, (c) *Vitex pinnata* (100 mg/kg), (d) *Vitex pinnata* (500 mg/kg.), (e) *Vitex pinnata* (1000 mg/kg b.w.) (H and E, ×40)

DISCUSSION

Ovariectomy can cause estrogen deficiency, the reproductive cycle stops, and an increase in body weight and the changes in the plasma lipid levels (a risk factor for cardiovascular disease). When female rats do not have an estrogen, the vagina and uterus undergo atrophy of the endometrium. However, giving estrogenic substances helps to prevent atrophic changes of the organ and also stimulates mitosis in the epithelia of the endometrium in OVX females.[21] In the present study, treatment with 17 β-estradiol showed evidence of uterotrophic activity as indicated by uterine and vagina weight and histological changes of the uterus and vagina in OVX rats. Low and medium doses (100 and 500 mg/kg of extract had not effect on the uterus and vagina. Interestingly, the result showed that the ethanolic plant extract at high dose (1000 mg/kg) produced slightly increased the weight of the uterus and vagina and epithelium in OVX rats. GC-MS analysis has shown the presence of *V. pinnata* leaf extract found few phytosterols such as β-stigmasterol and gamma-sitosterol. Kuiper *et al.* 1998 found that phytochemicals such as flavonoids, steroids (phytosterols) and phenolic compounds are estrogenic substances.[15] Thus, the effects of the plant extract can be attributed to its weak estrogenic activity.

Phytochemical analysis of the leaves of *V. pinnata* revealed the presence of constituents that are known to exhibit medicinal as well as physiological activities. GC-MS has been the best technique used

Table 4: Mean lipid levels (mg/dl) of ovariectomized rats supplemented with various doses of *Vitex pinnata*

Treatment	Lipid profile (mg/dl)			
	Total cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol
OVX control	52.50±1.91[c]	93.17±17.90[a]	14.17±0.65[a]	17.67±3.81[b]
17β-estradiol	31.33±3.15[a]	57.83±7.96[b]	18.00±1.86[a]	1.77±2.36[a]
V. pinnata 100	48.33±5.58[c]	57.33±10.38[b]	19.00±2.96[a]	17.87±2.86[b]
V. pinnata 500	40.50±4.16[b]	52.67±4.88[b]	17.50±1.54[a]	10.57±5.88[b]
V. pinnata 1000	41.00±1.78[b]	51.33±6.49[b]	19.50±0.76[a]	13.07±3.71[b]

[a,b,c]Mean within a column with different letters are different ($P<0.05$). *V. pinnata*: *Vitex pinnata*; OVX: Ovariectomized; LDL: Low-density lipoprotein; HDL: High-density lipoprotein

for screening, identification and quantification of many bioactive compounds in plant extracts.[22] GC-MS data revealed that the ethanolic extract of *V. pinnata* contains n-Hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid and the unsaturated fatty acid stearic acid, which is known to have estrogenic activity. The estrogenic activity shown by the extract of *V. pinnata* can be attributed to the presence of unsaturated fatty acid.[23] Thus this type of GC-MS analysis is the first step toward understanding the nature of the active principles in theethanolic extract of *V. pinnata*.

This study showed that feeding *V. pinnata* extracts at doses of 500 and 1000 mg/kg B. W resulted in significant decreases in total cholesterol and triglyceride and this might have been a consequence of feeding phytoestrogen. Hwang *et al*. 2001[23] showed that phytoestrogens are potent low density lipoprotein antioxidants. Other reports have demonstrated that *V. agnus-castus* phytoestrogen also reduced LDL-cholesterol and triglyceride and produced HDL-cholesterol in OVX rats.[24] The results indicate that the leaf extract from *V. pinnata* possess hypolipidemic activityis more likely associated with the presence of unsaturated fatty acid which is known to have estrogenic activity. However, the mechanism of the lipid-lowering effects of phytoestrogen is not clear. Our results of gas chromatography-mass spectrometry verified that the phytoestrogen of *V. pinnata* L. Which is a technique used for screening/indentification/quantification of many chemical compounds in plant extracts.[25]

CONCLUSION

This study showed that a higher dose *V. pinnata* L. extract (1000 mg/kg) possesses the estrogenic activity and resulted in decreases of total cholesterol and triglycerides in OVX rats. GC-MS analysis data revealed that the ethanolic extract of *V. pinnata* contains phytosterols and unsaturated fatty acids. These phytosterols and unsaturated fatty acids are known to possess estrogenic activity. Furthermore, the results lend some support for the traditional use of this plant in the management of gynecologic disorders and lipid profiles in menopause women.

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Conflicts of interest

There are no conflicts of interest.

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