

# Screening for estrogenic and antiestrogenic activities of plants growing in Egypt and Thailand

Ali M. El-Halawany<sup>1,2</sup>, Riham Salah El Dine<sup>1,2</sup>, Mi Hwa Chung<sup>1</sup>, Tsutomu Nishihara<sup>3</sup>, Masao Hattori<sup>1</sup>

<sup>1</sup>Department of Metabolic Engineering, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, <sup>2</sup>Department of Pharmacognosy, Faculty of Pharmacy, Cairo University 11562, <sup>3</sup>Faculty of Pharmaceutical Sciences, Hyogo College of Medicine, 1-3-6 Minatojima, Chuo-Ku, Kobe 650-0045, Japan

Submitted: 07-11-2010

Revised: 21-12-2010

Published: 08-06-2011

## ABSTRACT

**Background:** There is a growing demand for the discovery of new phytoestrogens to be used as a safe and effective hormonal replacement therapy. **Materials and Methods:** The methanol extracts of 40 plants from the Egyptian and Thailand folk medicines were screened for their estrogen agonist and antagonist activities. The estrogenic and antiestrogenic effects of the tested extracts were carried out using the yeast two-hybrid assay system expressing ER $\alpha$  and ER $\beta$ . In addition, all the extracts were subjected to a naringinase treatment and retested for their estrogenic activity. **Results:** The methanol extracts of *Derris reticulata* and *Dracaena lourieri* showed the most potent estrogenic activity on both estrogen-receptor subtypes, while, the methanol extracts of *Butea monosperma*, *Erythrina fusca*, and *Dalbergia candenatensis* revealed significant estrogenic activity on ER $\beta$  only. *Nigella sativa*, *Sophora japonica*, *Artabotrys harmandii*, and *Clitorea hanceana* showed estrogenic effect only after naringinase treatment. The most potent antiestrogenic effect was revealed by *Aframomum melegueta*, *Dalbergia candenatensis*, *Dracena loureiri*, and *Mansonia gagei*.

**Key words:** Estrogenic activity, leguminosae, yeast two-hybrid assay

### Access this article online

**Website:**

www.phcogres.com

**DOI:**

10.4103/0974-8490.81958

**Quick Response Code:**

## INTRODUCTION

Estrogens are key regulators of the cellular processes involved in the development and maintenance of the reproductive function. Estrogens have neuroprotective effects and reduce premenopausal mood fluctuations in women. In the eye, they lower intraocular pressure. Estrogens are arterial vasodilators and may have cardiovascular actions. In the liver, they stimulate the uptake of serum lipoproteins as well as the production of coagulation factors. They also prevent and reverse osteoporosis and increase cell viability in various tissues. They may protect against colon cancer, since colon cancer appears to be less likely to develop in postmenopausal women who are receiving estrogen-replacement therapy. Topically they increase collagen production and reduce the depth of the skin wrinkles.<sup>[1]</sup>

There are two subtypes of estrogen receptors and several

isoforms of each subtype. The first subtype is estrogen-receptor alpha (ER $\alpha$ ) which was first cloned in 1986.<sup>[2]</sup> The second subtype is estrogen-receptor beta (ER $\beta$ ) which was discovered recently.<sup>[3]</sup> These subtypes vary in structure; their encoding genes are located in different chromosomes. Although the DNA-binding domains of both estrogen receptors are very similar, the overall degree of similarity between the two receptors is low. This is particularly true for the ligand-binding domain of which only 55% of the amino acid sequence is shared.<sup>[4]</sup> As a result, some ligands bind to the two receptors with different affinities.

Phytoestrogens are plant-derived compounds that structurally or functionally mimic mammalian estrogens and, therefore, are considered to play an important role in the prevention of cancers, heart diseases, menopausal symptoms, and osteoporosis.<sup>[5]</sup> These naturally occurring, plant-derived estrogens, defined broadly as phytoestrogens, include the flavonoids (kaempferol and quercetin), the isoflavonoids (genistein, daidzein, formononetin, and equol), the lignans (enterolactone and enterodiol), the coumestanes (coumestrol), the mycotoxins (zearalenol), and stilbens (resveratrol). Several of them are ingested as precursors and then converted by the microflora of the mammalian gut.<sup>[6]</sup>

**Address for correspondence:**

Dr. Masao Hattori, Department of Metabolic Engineering, Institute of Natural Medicine, University of Toyama 2630 Sugitani, Toyama-930 0194, Japan. E-mail: saibo421@inm.u-toyama.ac.jp

The interest in plant-derived estrogens or phytoestrogens has recently been increased by the realization that hormone replacement therapy (HRT) is not as safe or effective as previously thought.<sup>[7]</sup> Two large-scale trials of HRT, the Women's Health Initiative in the USA and the Million Women Study in the UK, have shown that combined HRT increases the risk of breast cancer, heart disease, stroke, and venous thromboembolism. These well-publicized results have led to the conclusion that HRT will not ensure future health, although short-term use is beneficial for the relief of severe menopausal symptoms.<sup>[8]</sup> The aforementioned reports warrant for further investigation of new sources of phytoestrogens to satisfy the increasing demands for safe and effective HRT.

The goal of the present study is to investigate several plants from the Thailand and Egyptian folk medicines for estrogenic effect. In addition, several plant ingredients used as spices and common foods in the Egyptian market will be investigated for their possible effect as estrogenic agents, which could have a great value of developing an available food supplement safely used for controlling menopausal syndrome. Moreover, an enzymatic treatment of the plant extracts will be carried out to discover its possible action as precursor for estrogenic agents by the action of gut microflora.

## MATERIALS AND METHODS

Naringinase was purchased from Sigma Co. (St. Louis, Mo. USA) and *O*-nitrophenyl  $\beta$ -*D*-galactoside (ONPG) was purchased from Nacalai Tesque Co. (Kyoto, Japan). 17  $\beta$ -Estradiol was purchased from Calbiochem Co. (Darmstadt, Germany) and tamoxifen and 20T-zymolyase from Seikagaku Kogyo Co. (Tokyo, Japan).

### Yeast strain

The yeast strain used in this study is a kind gift from Professor Dr. Tsutomu Nishihara, Faculty of Pharmaceutical Sciences, Hyogo College of Medicine; 1-3-6 Minatojima, Chuo-Ku, Kobe, Japan.

The yeast strain used in this study was Y190 (*MATa*, *ura3-52*, *his3-D200*, *ade2-101*, *trp1-901*, *leu2-3*, *112*, *gal4Dgal80D*, *URA3::GAL-lacZ*, *cybr2*, *LYS2::GAL-HIS3*), obtained from Clontech (Palo Alto, CA). Yeast cells were transformed with the pGBT9-receptors and pGAD424-coactivators using a lithium acetate method and selected by growth on SD medium (lacking tryptophan and leucine). The yeast expression plasmids, pGBT9 and pGAD424, were purchased from Clontech (Palo Alto, CA). The LBD of rER $\alpha$  (codons 252–600) was amplified from cDNA by PCR.<sup>[9]</sup>

### Plant material

The plants (E1-5, 8, 10, 12-16) were purchased at Harraz herbal drug store, Cairo, Egypt. While the plant E11 was collected from the Medicinal Plant Station of Faculty of Pharmacy, Cairo University and was identified by its botanical staff, the other plants were collected from their natural habitats in Egypt and authentication of the plant was established by Assistant Prof. Dr. Sherif El-Khanagry, Agriculture Museum, El-Dokki, Cairo, Egypt. The plants T1–T27 were purchased from the herbal drug store “Cho Krom Pur,” Bangkok, Thailand, and identified by Dr. Katsuko Komatsu (Institute of Natural Medicine, University of Toyama). A voucher specimen was kept in the herbarium of the Institute of Natural Medicine, University of Toyama, Japan.

### Preparation of the methanol and aqueous extracts

The powdered plant (10 g each) was refluxed separately with MeOH and water (100 ml  $\times$  3) for 1.5 h. The extracts were concentrated and freeze-dried. To test their estrogenic activities, the extracts were dissolved in DMSO (10 mg/ml) as stock solutions.

### Preparation of the naringinase-treated extracts

The MeOH extract of the selected plants (40 mg, each) was incubated with naringinase enzyme (20 mg) in 2 ml of 0.2 M acetate buffer (pH=4.7) at 37°C for 4 h. The solution was then extracted with BuOH (10 ml  $\times$  3) and the combined BuOH extract was evaporated under vacuum to get the naringinase-treated extracts. The naringinase-treated extracts were dissolved in DMSO (10 mg/ml) as a stock solution for testing their estrogenic and antiestrogenic activities.

### Estrogenic and antiestrogenic assay methods

To examine the estrogenic activity of the extracts, the induction of  $\beta$ -galactosidase activity in the yeast two-hybrid screen expressing ER $\alpha$  and ER $\beta$  was carried out.

### Yeast two-hybrid assay

The yeast two-hybrid assay was carried out according to the method of Nishikawa.<sup>[9,10]</sup> Briefly, yeast cells expressing ER $\alpha$  and ER $\beta$  were separately grown overnight at 30°C with shaking in a synthetic defined medium (SD) lacking tryptophan and leucine. Yeast cells were treated with 17  $\beta$ -estradiol and the isolated compounds for 4 h at 30°C, and  $\beta$ -galactosidase activity was determined as follows: the growth of the yeast cells was monitored by measuring the turbidity at 600 nm. The treated yeast cells were collected by centrifugation (8000 $\times$  *g*, 5 min) and resuspended in 200  $\mu$ L of Z-buffer (0.1 M sodium phosphate, pH 7.0, 10 mM KCl, and 1 mM MgSO<sub>4</sub>) containing 1 mg/mL of zymolyase at 37°C for 15 min. The reaction was started by the addition of 40  $\mu$ L of 4 mg/mL *O*-nitrophenol

$\beta$ -D-galactopyranoside (ONPG) as a substrate. When yellow color developed (incubation time:  $t$ ), 100  $\mu$ L of 1 M  $\text{Na}_2\text{CO}_3$  was added to stop the reaction. The absorbance of the solution (150  $\mu$ L) was measured at 420 and 550 nm. The  $\beta$ -galactosidase activity was determined using the following formula:

$$U = 1000 \times (A_{420} - 1.75A_{550}) / (t \times 0.05A_{600})$$

### Antiestrogenic assay

To examine the antagonistic activity of the test compounds, the inhibition of  $\beta$ -galactosidase activity which had been induced by  $10^{-7}$  M  $17\beta$ -estradiol was measured at various concentrations of plant extracts. The tested concentrations must not be toxic to the yeast. In the yeast two-hybrid assay, the extracts were assessed as toxic when the difference between the absorption of the extract and DMSO (negative control) at 600 nm was 10% or more.

### Statistical analysis

Each set of experiments were repeated at least three times. Values are expressed as mean  $\pm$  S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis. The means were compared to DMSO in estrogenic assay and to estradiol (considered as 100% activity) in antiestrogenic assay.

## RESULTS

In the course of our search for new phytoestrogens of interest, 40 Egyptian and Thailand plants were investigated. The majority of the selected Egyptian plants (E1–E7, E10, and E12–16) are common foods and spices in the market. Some of the selected foods and spices are belonging to family Fabaceae [Table 1], which is known by its high phytoestrogen contents. The rest of the investigated Egyptian plants were selected based on their use as folk remedies for women diseases. On the other hand, the plants from Thailand were selected depending on their reported contents of phenolic compounds. Due to the growing interest for investigating the estrogenic activity of phenolic compounds other than the well-studied isoflavonoids, lignans, and coumestans, the presence of high concentration and diversity of phenolics in these plants comprised the bases for their selection.

The estrogenic activity was investigated using the yeast two-hybrid assay expressing ER $\alpha$  and ER $\beta$  at 100 and 10  $\mu$ g/mL concentrations, respectively. In addition, a naringinase treatment was carried out for all the methanol extracts.<sup>[11]</sup> Naringinase enzyme is a mixed enzyme of  $\beta$ -glucosidase and  $\alpha$ -rhamnosidase activities. This treatment is used as a partial-mimic to the metabolism process (deglucosilation),

which takes place in the gastrointestinal tract (GIT).<sup>[11]</sup> The estrogenic activity of the naringinase-treated extracts was investigated using the formerly mentioned bioassay systems. Moreover, the antiestrogenic activity of the methanol extracts, before and after naringinase treatment, was investigated using yeast two-hybrid assay (ER $\beta$ ) at a concentration of 100  $\mu$ g/mL.

### Induction of $\beta$ -galactosidase activity in the yeast two-hybrid assay

#### Yeast expressing estrogen receptor $\alpha$ (ER $\alpha$ )

$17\beta$ -Estradiol was used as a positive control with maximum  $\beta$ -galactosidase activity (U) of  $786.8 \pm 32.5$  at  $10^{-7}$ M. The methanol- and the naringinase-treated extracts of *Derris reticulata* (T3) and *Dracaena loureiri* (T17) showed a significant estrogenic activity at 100  $\mu$ g/mL [Table 2], while the methanol extracts of *Sophora japonica* (E11) and *Alpinia siamense* (T15) showed a significant estrogenic activity only after naringinase treatment [Tables 1 and 2].

#### Yeast expressing estrogen receptor $\beta$ (ER $\beta$ )

$17\beta$ -Estradiol showed maximum  $\beta$ -galactosidase activity (U) of  $1224.0 \pm 29.9$  at  $10^{-7}$ M. The methanol extracts of *Erythrina fusca* (T4) and *D. loureiri* (T17) showed a concentration-dependent increase in their estrogenic activity [Table 2]. On the other hand, *Butea monosperma* (T2), *D. reticulata* (T3), and *Dalbergia candeatensis* (T9) and *A. siamense* (T15) showed a significant estrogenic activity at 100  $\mu$ g/mL. *Nigella sativa* (E8), *S. japonica* (E11), *Bauhinia malabarica* (T7), *Clitoria hanceana* (T10), and *Diospyros ebreioides* (T18) showed a significant activity only after naringinase treatment. The naringinase treatment of all the previously mentioned active extracts showed a significant increase in their estrogenic activities except *D. reticulata* (T3) and *D. candeatensis* (T9).

### Inhibition of $17\beta$ -Estradiol-induced $\beta$ -galactosidase activity in the yeast two-hybrid assay (anti-estrogenic assay)

The activity of  $17\beta$ -estradiol at  $10^{-7}$ M was considered as 100% and all the other extracts were calculated as a percentage inhibition of estradiol activity. Tamoxifen (positive control) inhibited the estradiol-induced  $\beta$ -galactosidase activity by 78% at a concentration of  $10^{-5}$ M. As shown in Table 3, the methanol- and the naringinase-treated extracts of *Aframomum melegueta* (E1), *D. loureiri* (T17), *D. candeatensis* (T9), *Mansonia gagei* (T20), *Artabotrys harmandii* (T24), and *Cassia tora* (T11) showed the most potent antiestrogenic activity. While *B. horsfieldii* (T1), *D. reticulata* (T3), *Clerodendrum petasites* (T13), *E. fusca* (T4), *Foeniculum vulgare* (T13), *Clitoria ternatea* (T5), and *Lannea grandis* (T24) showed intermediate activity. In addition, *A. melegueta* (E1) and *A. harmandii* (T23) showed an unexpected decrease in their activity by naringinase treatment.

**Table 1: Induction of  $\beta$ -galactosidase in the yeast two-hybrid assay expressing ER $\alpha$  and ER $\beta$  by the methanol and naringinase-treated extracts of plants growing in Egypt**

No.	Botanical name	Part used	Extract	$\beta$ -Galactosidase activity (U)			
				ER $\alpha$		ER $\beta$	
				10 $\mu$ g/mL	100 $\mu$ g/mL	10 $\mu$ g/mL	100 $\mu$ g/mL
E1	<i>Aframomum melegueta</i> (Zingiberaceae)	Seeds	MeOH	34.2 $\pm$ 4.9	34.2 $\pm$ 4.9	93.4 $\pm$ 4.4	89.0 $\pm$ 7.1
			NT	46.8 $\pm$ 7.8	45.5 $\pm$ 6.2	88.3 $\pm$ 4.8	93.1 $\pm$ 12.4
E2	<i>Cyperus esculentus</i> (Cyperaceae)	Tuber	MeOH	41.7 $\pm$ 7.2	41.7 $\pm$ 7.2	90.9 $\pm$ 4.9	93.0 $\pm$ 4.3
			NT	43.8 $\pm$ 8.4	41.7 $\pm$ 9.2	92.9 $\pm$ 3.2	83.7 $\pm$ 10.3
E3	<i>Sesamum indicum</i> (Palidiaceae)	Seeds	MeOH	32.1 $\pm$ 3.4	32.0 $\pm$ 3.4	97.0 $\pm$ 5.3	93.9 $\pm$ 10.2
			NT	38.8 $\pm$ 9.0	46.2 $\pm$ 4.8	87.7 $\pm$ 6.5	101.0 $\pm$ 6.6
E4	<i>Linum ussitatissimum</i> (Linaceae)	Seeds	MeOH	20.7 $\pm$ 6.5	20.7 $\pm$ 6.5	124.2 $\pm$ 11.1	108.6 $\pm$ 17.0
			NT	24.4 $\pm$ 9.5	23.3 $\pm$ 6.7	117.8 $\pm$ 16.9	109.6 $\pm$ 11.1
E5	<i>Hordium vulgare</i> (Gramineae)	Fruit	MeOH	5.2 $\pm$ 2.69	5.1 $\pm$ 2.6	124.0 $\pm$ 4.3	128.7 $\pm$ 3.5
			NT	44.1 $\pm$ 6.5	42.9 $\pm$ 8.7	87.6 $\pm$ 12.4	110.3 $\pm$ 5.0
E6	<i>Petroselinum crispum</i> (Lamiaceae)	Leaves	MeOH	49.4 $\pm$ 4.5	49.3 $\pm$ 4.5	89.8 $\pm$ 13.8	77.5 $\pm$ 10.3
			NT	21.4 $\pm$ 7.7	15.5 $\pm$ 8.6	94.8 $\pm$ 11.2	99.7 $\pm$ 2.5
E7	<i>Brassica oleraceae</i> (Cruciferae)	Leaves	MeOH	50.4 $\pm$ 7.1	50.4 $\pm$ 7.2	88.7 $\pm$ 6.2	102.5 $\pm$ 20.2
			NT	22.3 $\pm$ 7.8	19.7 $\pm$ 9.6	91.7 $\pm$ 6.9	102.5 $\pm$ 5.0
E8	<i>Nigella sativa</i> (Ranunculaceae)	Seeds	MeOH	30.8 $\pm$ 1.7	54.4 $\pm$ 2.3	109.0 $\pm$ 12.6	92.8 $\pm$ 6.7
			NT	21.9 $\pm$ 10.9	51.6 $\pm$ 4.5	134.9 $\pm$ 4.9	289.3 $\pm$ 16.1*
E9	<i>Vitex agnus-castus</i> (Lamiaceae)	Fruits	MeOH	39.6 $\pm$ 4.4	39.5 $\pm$ 4.4	86.8 $\pm$ 10.1	87.4 $\pm$ 15.1
			NT	15.8 $\pm$ 4.3	22.2 $\pm$ 8.7	135.4 $\pm$ 5.4	125.6 $\pm$ 9.7
E10	<i>Lens culinaris</i> (Fabaceae)	Seeds	MeOH	19.6 $\pm$ 8.4	19.6 $\pm$ 8.4	91.3 $\pm$ 5.3	89.5 $\pm$ 5.4
			NT	22.3 $\pm$ 5.1	20.1 $\pm$ 8.2	128.4 $\pm$ 16.1	126.5 $\pm$ 13.1
E11	<i>Sophora japonica</i> (Fabaceae)	Seeds	MeOH	36.3 $\pm$ 4.8	36.0 $\pm$ 4.8	127.2 $\pm$ 8.6	117.3 $\pm$ 5.7
			NT	71.2 $\pm$ 3.6	271.7 $\pm$ 7.6**	725.7 $\pm$ 6.8**	2731.6 $\pm$ 36.1**
E12	<i>Cicer arietinum</i> (Fabaceae)	Seeds	MeOH	45.1 $\pm$ 3.0	45.1 $\pm$ 3.0	122.7 $\pm$ 9.8	117.4 $\pm$ 9.8
			NT	41.7 $\pm$ 6.4	36.2 $\pm$ 4.8	96.4 $\pm$ 8.2	104.0 $\pm$ 7.8
E13	<i>Vigna unguiculata</i> (Fabaceae)	Seeds	MeOH	20.0 $\pm$ 7.1	20.0 $\pm$ 7.1	98.5 $\pm$ 6.4	95.8 $\pm$ 7.7
			NT	19.9 $\pm$ 9.0	29.0 $\pm$ 0.4	131.7 $\pm$ 17.6	81.7 $\pm$ 10.2
E14	<i>Trigonella foenum greocum</i> (Fabaceae)	Seeds	MeOH	19.4 $\pm$ 17.1	19.4 $\pm$ 17.7	109.6 $\pm$ 18.7	106.4 $\pm$ 14.3
			NT	24.0 $\pm$ 5.9	21.1 $\pm$ 7.6	123.0 $\pm$ 7.3	93.2 $\pm$ 8.0
E15	<i>Phaseolus vulgaris</i> (Fabaceae)	Seeds	MeOH	21.8 $\pm$ 6.2	21.7 $\pm$ 6.2	113.8 $\pm$ 12.8	113.7 $\pm$ 7.7
			NT	21.7 $\pm$ 6.2	25.6 $\pm$ 10.6	112.0 $\pm$ 4.8	123.2 $\pm$ 10.3
E16	<i>Vicia faba</i> (Fabaceae)	Seeds	MeOH	40.1 $\pm$ 3.2	40.1 $\pm$ 3.2	130.6 $\pm$ 16.6	130.8 $\pm$ 7.7
			NT	42.1 $\pm$ 9.7	33.7 $\pm$ 7.7	108.0 $\pm$ 4.8	122.5 $\pm$ 13.1
	17 $\beta$ -Estradiol			786.8 $\pm$ 32.5**		1224.0 $\pm$ 29.9**	
	DMSO			40.0 $\pm$ 7.0		120.8 $\pm$ 7.0	

Each value represents the mean  $\pm$  S.E of three independent experiments (n=3). Asterisks denote significant differences from the control at \*P < 0.05, \*\*P < 0.01. 17  $\beta$ -Estradiol was used at 10<sup>-7</sup>M.

## CONCLUSION

It is found that, more than 300 plants were reported for their estrogenic activity and phytoestrogen contents.<sup>[12,13]</sup> In particular, isoflavones of the soybean, family Fabaceae, have attracted attention in the last years due to its high estrogenic activity. However, it can be assumed that other plants belonging to the same family could possess a significant estrogenic activity due to their reported high contents of flavonoids. Therefore, as a part of our search for new phytoestrogens, the extracts of many plants belonging to family Fabaceae were evaluated for their estrogenic effect. In addition, plants belonging to other families were evaluated for their possible estrogenic and/or antiestrogenic activities depending on their high phenolic contents.

According to the aforementioned data [Tables 1 and 2],

most of the tested leguminous plants showed a significant induction of  $\beta$ -galactosidase in both yeast two-hybrid assay expressing ER $\alpha$  and ER $\beta$ . Most of these plants were reported to contain high amounts of flavonoids, specially flavanones and petrocarpans as in *B. monosperma* (T2),<sup>[14]</sup> prenylated flavonoids as in *D. reticulata* (T3),<sup>[15]</sup> and isoflavonoids as in *D. candenatensis* (T9).<sup>[16]</sup> As the estrogenic activity of flavanones and isoflavonoids was reported by many authors, the activity of these plants might be due to these contents.

In case of the non-Fabaceae plants (E1–E9 and T13–T24), *N. sativa* (E8), *A. siamense* (T15), *D. lourieri* (T17), and *D. ebretoides* (T18) exhibited a significant estrogenic activity, while the other plants exhibited antiestrogenic activity [Table 3]. *N. sativa* is a commonly used plant in the Egyptian folk medicine. A similar activity was reported for *Nigella damascena* due to the presence of 3,4-dihydroxy- $\beta$ -

**Table 2: Induction of  $\beta$ -galactosidase in the yeast two-hybrid assay expressing ER $\alpha$  and ER $\beta$  by the methanol and naringinase-treated extracts of plants growing in Thailand**

No.	Botanical name	Part used	Extract	$\beta$ -Galactosidase activity (U)			
				ER $\alpha$		ER $\beta$	
				10 $\mu$ g/mL	100 $\mu$ g/mL	10 $\mu$ g/mL	100 $\mu$ g/mL
T1	<i>Bauhinia horsfieldii</i> Mac.Br. (Fabaceae)	Wood	MeOH	42.6 $\pm$ 8.3	55.4 $\pm$ 13.5	35.8 $\pm$ 2.4	48.3 $\pm$ 1.2
			NT	38.9 $\pm$ 6.7	39.0 $\pm$ 5.8	43.2 $\pm$ 3.5	81.5 $\pm$ 5.2
T2	<i>Butea monosperma</i> Taub. (Fabaceae)	Wood	MeOH	38.8 $\pm$ 6.5	37.8 $\pm$ 5.8	35.6 $\pm$ 5.6	657.9 $\pm$ 8.5**
			NT	42.6 $\pm$ 5.1	46.50 $\pm$ 5.3	55.3 $\pm$ 7.1	1384.1 $\pm$ 73.5**
T3	<i>Derris reticulata</i> Craib. (Fabaceae)	Wood	MeOH	51.8 $\pm$ 4.1	92.1 $\pm$ 8.6**	21.3 $\pm$ 3.1	737.4 $\pm$ 68.8**
			NT	65.5 $\pm$ 4.8	118.9 $\pm$ 6.4**	55.4 $\pm$ 2.2	415.2 $\pm$ 44.3**
T4	<i>Erythrina fusca</i> Lour. (Fabaceae)	Wood	MeOH	72.1 $\pm$ 5.9	68.3 $\pm$ 5.3	220.2 $\pm$ 2.3**	1081.3 $\pm$ 17.2**
			NT	48.3 $\pm$ 5.3	72.1 $\pm$ 6.3	282.2 $\pm$ 3.5**	1731.7 $\pm$ 65.6**
T5	<i>Clitoria ternatea</i> (Fabaceae)	Aerial parts	MeOH	39.8 $\pm$ 4.7	33.4 $\pm$ 2.8	19.4 $\pm$ 3.0	62.5 $\pm$ 3.5
			NT	18.3 $\pm$ 8.6	19.2 $\pm$ 6.2	33.2 $\pm$ 2.9	93.1 $\pm$ 7.1
T6	<i>Acacia concinna</i> (Fabaceae)	Aerial parts	MeOH	24.9 $\pm$ 7.4	21.3 $\pm$ 8.0	47.7 $\pm$ 4.9	47.6 $\pm$ 5.5
			NT	19.4 $\pm$ 5.2	17.0 $\pm$ 7.8	54.4 $\pm$ 5.3	87.1 $\pm$ 3.7
T7	<i>Bauhinia malabarica</i> Roxb. (Fabaceae)	Leaf	MeOH	26.3 $\pm$ 7.2	24.9 $\pm$ 7.7	31.6 $\pm$ 8.5	48.9 $\pm$ 13.6
			NT	15.4 $\pm$ 6.6	18.1 $\pm$ 4.1	35.1 $\pm$ 5.1	141.3 $\pm$ 13.1**
T8	<i>Crotalaria verrucosa</i> (Fabaceae)	Aerial parts	MeOH	24.1 $\pm$ 7.1	23.7 $\pm$ 6.7	24.2 $\pm$ 4.9	32.8 $\pm$ 1.4
			NT	20.6 $\pm$ 1.5	24.3 $\pm$ 5.4	53.7 $\pm$ 1.2	52.1 $\pm$ 6.3
T9	<i>Dalbergia candenatensis</i> (Fabaceae)	wood	MeOH	32.5 $\pm$ 10.3	46.1 $\pm$ 7.6	50.4 $\pm$ 6.2	206.3 $\pm$ 18.2**
			NT	37.2 $\pm$ 4.8	45.3 $\pm$ 6.4	85.3 $\pm$ 3.4	284.2 $\pm$ 1.4**
T10	<i>Clitoria hanceana</i> Gopp. (Fabaceae)	Aerial parts	MeOH	43.9 $\pm$ 7.8	44.5 $\pm$ 8.6	29.5 $\pm$ 1.8	26.1 $\pm$ 16.4
			NT	28.3 $\pm$ 9.4	35.18 $\pm$ 6.8	83.2 $\pm$ 5.3*	779.0 $\pm$ 60.3**
T11	<i>Cassia tora</i> Fish. (Fabaceae)	Seeds	MeOH	36.7 $\pm$ 1.5	49.5 $\pm$ 3.8	51.2 $\pm$ 5.4	61.8 $\pm$ 8.5
			NT	50.7 $\pm$ 4.1	46.3 $\pm$ 13.4	45.6 $\pm$ 4.3	48.1 $\pm$ 4.3
T12	<i>Caeslpinia sappan</i> L. (Fabaceae)	Wood	MeOH	39.4 $\pm$ 4.7	45.9 $\pm$ 9.4	67.6 $\pm$ 1.8	36.9 $\pm$ 7.8
			NT	39.8 $\pm$ 3.4	42.7 $\pm$ 13.4	45.5 $\pm$ 1.6	11.3 $\pm$ 1.9
T13	<i>Clerodendrum petasites</i> S.Moore (Verbenaceae)	Wood	MeOH	26.4 $\pm$ 4.1	17.0 $\pm$ 7.9	28.8 $\pm$ 3.2	66.0 $\pm$ 7.1
			NT	21.0 $\pm$ 5.2	35.8 $\pm$ 8.4	46.4 $\pm$ 1.9	85.8 $\pm$ 16.2
T14	<i>Foeniculum vulgare</i> Mill. (Lamiaceae)	Fruits	MeOH	45.3 $\pm$ 10.5	45.3 $\pm$ 3.3	16.9 $\pm$ 1.3	50.7 $\pm$ 2.3
			NT	21.3 $\pm$ 8.1	47.3 $\pm$ 4.5	43.9 $\pm$ 6.9	62.3 $\pm$ 21.5
T15	<i>Alpinia siamense</i> K.Schum. (Zingiberaceae)	Rhizome	MeOH	47.8 $\pm$ 9.4	41.4 $\pm$ 8.1	33.1 $\pm$ 1.3	87.5 $\pm$ 1.97*
			NT	47.6 $\pm$ 7.0	80.6 $\pm$ 5.6*	30.4 $\pm$ 1.9	137.3 $\pm$ 10.6**
T16	<i>Vitex trifolia</i> (Verbenaceae)	Leaf	MeOH	15.8 $\pm$ 1.5	14.8 $\pm$ 1.7	34.7 $\pm$ 3.2	54.7 $\pm$ 2.1
			NT	35.8 $\pm$ 8.4	35.8 $\pm$ 5.3	28.3 $\pm$ 5.6	33.8 $\pm$ 9.3
T17	<i>Dracaena loureiri</i> Gagnep. (Agavaceae)	Wood	MeOH	66.7 $\pm$ 5.4	88.1 $\pm$ 5.6*	533.8 $\pm$ 17.5**	1550.9 $\pm$ 209**
			NT	51.5 $\pm$ 3.3	88.7 $\pm$ 7.3*	655.4 $\pm$ 19.5**	1828.6 $\pm$ 37.4**
T18	<i>Diospyros ehretioides</i> Wall. (Ebenaceae)	Wood	MeOH	29.2 $\pm$ 9.2	48.2 $\pm$ 7.8	9.1 $\pm$ 1.8	7.6 $\pm$ 1.1
			NT	37.9 $\pm$ 3.7	58.6 $\pm$ 7.7	27.5 $\pm$ 3.5	107.9 $\pm$ 6.2*
T19	<i>Abutilon hirtum</i> (Malvaceae)	Aerial parts	MeOH	30.0 $\pm$ 1.8	35.8 $\pm$ 8.1	23.5 $\pm$ 6.5	30.4 $\pm$ 3.4
			NT	35.6 $\pm$ 1.3	42.4 $\pm$ 10.2	55.1 $\pm$ 7.3	53.7 $\pm$ 8.9
T20	<i>Mansonia gagei</i> Drumm. (Sterculiaceae)	Heart wood	MeOH	37.9 $\pm$ 6.6	32.3 $\pm$ 1.1	50.0 $\pm$ 1.5	34.9 $\pm$ 3.3
			NT	26.3 $\pm$ 1.7	15.7 $\pm$ 6.8	43.5 $\pm$ 2.6	44.2 $\pm$ 4.2
T21	<i>Vernonia elliptica</i> (Compositae)	Wood	MeOH	20.1 $\pm$ 1.6	19.4 $\pm$ 3.9	22.5 $\pm$ 2.4	12.8 $\pm$ 2.1
			NT	25.8 $\pm$ 4.2	16.3 $\pm$ 6.4	63.2 $\pm$ 8.0	53.5 $\pm$ 9.9
T22	<i>Mantingia calabura</i> (Tiliaceae)	Wood	MeOH	38.23 $\pm$ 1.7	22.6 $\pm$ 4.7	16.8 $\pm$ 1.3	4.8 $\pm$ 2.2
			NT	21.9 $\pm$ 3.2	16.7 $\pm$ 5.7	45.2 $\pm$ 2.2	51.9 $\pm$ 4.5
T23	<i>Artabotrys harmandii</i> Finet and Gagnep. (Annonaceae)	Wood	MeOH	27.6 $\pm$ 8.9	20.1 $\pm$ 6.1	46.7 $\pm$ 1.2	66.3 $\pm$ 2.4
			NT	41.4 $\pm$ 3.9	19.4 $\pm$ 3.9	66.7 $\pm$ 6.6	118.1 $\pm$ 9.5**
T24	<i>Lannea grandis</i> Engl. (Anacardiaceae)	Wood	MeOH	16.3 $\pm$ 6.3	21.9 $\pm$ 3.2	18.4 $\pm$ 3.2	43.2 $\pm$ 4.3
			NT	38.2 $\pm$ 6.5	22.6 $\pm$ 4.7	73.4 $\pm$ 3.5	92.2 $\pm$ 9.7
	Estradiol			3074.0 $\pm$ 133.8**		3367.0 $\pm$ 103.9**	
	DMSO			55.5 $\pm$ 1.5		86.9 $\pm$ 4.6	

Each value represents the mean  $\pm$  S.E of three independent experiments (n=3). Asterisks denote significant differences from the control at \*P < 0.05, \*\*P < 0.01. Estradiol was used at 10<sup>-7</sup>M concentration.

phenylethyl alcohol and dihydroxy phenyl acetic acid.<sup>[17]</sup> *D. loureiri* (Agavaceae) showed a potent estrogenic activity on both ER subtypes. The estrogenic activity of this plant and its retrodihydrochalcones and homoisoflavones contents were reported before using another biological assay system.<sup>[18]</sup>

Among the plants exhibiting antiestrogenic activity, the methanol extracts of *Mansonia gagei*, *A. meleguita* (E1), *D. candenatensis*, and *C. tora* showed the most potent inhibition of estradiol-dependent induction of  $\beta$ -galactosidase in the yeast two-hybrid assay (ER $\beta$ ). The activity of *M. gagei* and *C. tora* was investigated by the authors and the activity was

**Table 3: Inhibition of 17  $\beta$ -estradiol-induced  $\beta$ -galactosidase in the yeast two-hybrid screen by different extracts of Egyptian and Thailand plants**

Plant	% inhibition of Estradiol Induced $\beta$ -Galactosidase Activity				
	MeOH	NT	Plant	MeOH	NT
E1	56.7 $\pm$ 3.4**	18.9 $\pm$ 2.5*	T7	14.2 $\pm$ 3.2*	10.8 $\pm$ 2.4
E2	2.9 $\pm$ 3.3	4.9 $\pm$ 3.1	T8	19.5 $\pm$ 3.2*	8.6 $\pm$ 1.6
E3	5.5 $\pm$ 5.9	10.9 $\pm$ 2.5	T9	42.2 $\pm$ 5.0**	39.9 $\pm$ 2.8**
E4	4.5 $\pm$ 3.3	7.9 $\pm$ 2.5	T10	4.6 $\pm$ 8.1	2.6 $\pm$ 2.5
E6	4.8 $\pm$ 5.1	0.7 $\pm$ 1.3	T11	35.7 $\pm$ 3.1**	33.7 $\pm$ 1.5**
E7	8.7 $\pm$ 2.1	2.3 $\pm$ 6.7	T12	10.2 $\pm$ 1.4	6.2 $\pm$ 1.5
E8	2.3 $\pm$ 0.5	5.5 $\pm$ 7.5	T13	28.5 $\pm$ 1.3**	33.5 $\pm$ 1.8**
E9	7.8 $\pm$ 2.2	7.7 $\pm$ 2.7	T14	13.8 $\pm$ 1.8*	30.5 $\pm$ 4.8**
E10	8.5 $\pm$ 4.4	4.8 $\pm$ 5.1	T15	—	—
E11	6.1 $\pm$ 1.28	4.7 $\pm$ 2.3	T16	11.3 $\pm$ 4.5	16.1 $\pm$ 4.3
E12	0.9 $\pm$ 2.5	3.4 $\pm$ 1.1	T17	47.2 $\pm$ 2.4**	35.2 $\pm$ 1.4**
E13	6.6 $\pm$ 2.1	9.1 $\pm$ 3.2	T18	8.1 $\pm$ 1.9	18.8 $\pm$ 4.3*
E14	8.3 $\pm$ 1.9	14.4 $\pm$ 2.1	T19	1.8 $\pm$ 1.4	0.5 $\pm$ 1.5
E15	4.0 $\pm$ 1.5	1.5 $\pm$ 2.5	T20	42.8 $\pm$ 2.7**	40.2 $\pm$ 2.0**
E16	4.5 $\pm$ 4.2	0.1 $\pm$ 1.6	T21	8.8 $\pm$ 1.5	5.1 $\pm$ 1.1
T1	27.4 $\pm$ 3.6**	12.6 $\pm$ 2.7*	T22	5.8 $\pm$ 11.9	26.9 $\pm$ 1.9**
T3	30.3 $\pm$ 5.4**	28.7 $\pm$ 2.2**	T23	38.5 $\pm$ 1.5**	25.4 $\pm$ 3.0**
T4	30.3 $\pm$ 5.8**	22.8 $\pm$ 1.7**	T24	24.0 $\pm$ 2.7**	18.4 $\pm$ 0.6*
T6	15.5 $\pm$ 3.8*	14.4 $\pm$ 2.4*			
17 $\beta$ -Estradiol	2333.41 $\pm$ 112.5		Tamoxifen	78.2 $\pm$ 2.4**	

Each value represents the mean  $\pm$  S.E of three independent experiments ( $n=3$ ). Asterisks denote significant differences from the control at \* $P < 0.05$ , \*\* $P < 0.01$ .  $\beta$ -Galactosidase activity ( $U$ ) of 17  $\beta$ -estradiol was considered (100%).  $\beta$ -Galactosidase activity ( $U$ ) of the tested compounds was calculated as a percentage inhibition of the 17  $\beta$ -estradiol activity. Tamoxifen was used at  $10^{-5}$ M concentration. T15 was toxic to the yeast in the tested concentration.

found to be due to their naphthoquinones, naphthopyrones, and acetyl naphthalene derivatives, respectively.<sup>[19,20]</sup> While, the naringinase-treated extract of *S. japonica* showed higher activity, as an agonist, than the methanol extract due to the hydrolysis of its content of flavonoid glycosides to the more active kaempferol and ginseng aglycones.<sup>[21]</sup> These findings encourage the author to continue the investigation of other plants showing significant estrogen agonist and/or antagonist effects in order to identify their phytoestrogen content.

## REFERENCES

- Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. *N Engl J Med* 2002;346:340-52.
- Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, *et al.* Human oestrogen receptor cDNA: Sequence, expression and homology to v-erb-A. *Nature* 1986;320:134-9.
- Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A* 1996;93:5925-30.
- Witkowska HE, Carlquist M, Engstrom O, Carlsson B, Bonn T, Gustafsson JA, *et al.* Characterization of bacterially expressed rat estrogen receptor beta ligand binding domain by mass spectrometry: Structural comparison with estrogen receptor alpha. *Steroids* 1997;62:621-31.
- Setchell KD. Phytoestrogens: The biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* 1998;68:1333S-46.
- Benassayag C, Perrot-Appianat M, Ferre F. Phytoestrogens as modulators of steroid action in target cells. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002;777:233-48.
- Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. *Phytochemistry* 2004;65:995-1016.
- McPherson K. Where are we now with hormone replacement therapy? *BMJ* 2004;328:357-8.
- Nishikawa J, Saito K, Goto J, Dakeyama F, Matsuo M, Nishihara T. New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. *Toxicol Appl Pharmacol* 1999;154:76-83.
- Eguchi K, Ozawa M, Endoh YS, Nishikawa J, Nishihara T, Goto K, *et al.* Validity test for a Yeast Two-Hybrid assay to screen for estrogenic activity, and its application to insecticides and disinfectants for veterinary use. *Bull Environ Contam Toxicol* 2003;70:226-32.
- Ahn E, Nakamura N, Komatsu K, Nishihara T, Hattori M. Screening for medicinal plant extracts for estrogenic activity in combination with a glycosidase treatment. *J Trad Med* 2004;21:81-6.
- Fransworth N, Bingel A, Cordell G, Crane F, Fong H. Potential value of plants as sources of new antifertility agents II. *J Pharm Sci* 1975;64:717-54.
- Price KR, Fenwick GR. Naturally occurring oestrogens in foods—a review. *Food Addit Contam* 1985;2:73-106.
- Jassbi A, Singh P, Krishna V, Gupta P, Tahara S. Antioxidant study and Assignments of NMR spectral data for 3',4',7-trihydroxy flavanone 3',7--di-O-B-D-glucopyranoside (Butrin) and its hydrolysed products. *Chem Nat Comp* 2004;40:250-3.
- Chulabhorn M, Hsna P, Wirongrong K, Somask R. Two new pyranoflavanones from the stems of *Derris reticulata*. *Heterocycles* 2002;57:1287-92.
- Hamburger MO, Cordell GA, Tantivatana P, Ruangrungsi N. Traditional medicinal plants of Thailand, VIII. Isoflavonoids of *Dalbergia candanensis*. *J Nat Prod* 1987;50:696-9.
- Agradi E, Fico G, Cillo F, Francisci C, Tome F. Estrogenic activity of phenolic compounds from *Nigella damascena* evaluated using a recombinant yeast screen. *Planta Med* 2001;67:553-5.

18. Ichikawa K, Kitaoka M, Taki M, Takaishi S, Iijima Y, Boriboon M, *et al.* Retrodihydrochalcones and homoisoflavones isolated from Thai medicinal plant *Dracaena loureiri* and their estrogen agonist activity. *Planta Med* 1997;63:540-3.
19. El-Halawany AM, Chung MH, Ma CM, Komatsu K, Nishihara T, Hattori M. Anti-estrogenic activity of mansorins and mansonones from the heartwood of *Mansonia gagei* DRUMM. *Chem Pharm Bull (Tokyo)* 2007;55:1332-7.
20. El-Halawany AM, Chung MH, Nakamura N, Ma CM, Nishihara T, Hattori M. Estrogenic and anti-estrogenic activities of *Cassia tora* phenolic constituents. *Chem Pharm Bull (Tokyo)* 2007;55:1476-82.
21. El-Halawany AM, Chung MH, Abdallah HM, Nishihara T, Hattori M. Estrogenic activity of a naringinase-treated extract of *Sophora japonica* cultivated in Egypt. *Pharm Biol* 2010;48:177-81.

**Cite this article as:** El-Halawany AM, Salah El Dine R, Chung MH, Nishihara T, Hattori M. Screening for estrogenic and antiestrogenic activities of plants growing in Egypt and Thailand. *Phcog Res* 2011;3:107-13.  
**Source of Support:** Nil, **Conflict of Interest:** None declared.

PR now Indexed with PubMed

The screenshot shows the PubMed search interface. The search bar contains the query "Pharmacognosy Research [Jour] AND 2010[pdat]". The results section shows 12 results, with the first result selected. The selected result is titled "Is the term substitution relevant to Pharmacognosy and/ or vegetable crude drug industry?". The author is Selvam AB, from the Pharmacognosy Section, Botanical Survey of India, Howrah - 711 103, India. The abstract discusses the use of substitution in pharmacognosy and its potential for misinterpretation. The PMID is 21589761 and the PMCID is PMC3093034. There are links for "Free Full Text @ www.phcogres.com" and "FREE full text article in PubMed Central".

Announcement

**“Quick Response Code” link for full text articles**

The journal issue has a unique new feature for reaching to the journal’s website without typing a single letter. Each article on its first page has a “Quick Response Code”. Using any mobile or other hand-held device with camera and GPRS/other internet source, one can reach to the full text of that particular article on the journal’s website. Start a QR-code reading software (see list of free applications from <http://tinyurl.com/yzlh2tc>) and point the camera to the QR-code printed in the journal. It will automatically take you to the HTML full text of that article. One can also use a desktop or laptop with web camera for similar functionality. See <http://tinyurl.com/2bw7fn3> or <http://tinyurl.com/3ysr3me> for the free applications.