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

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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 ERα and ERβ. 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 ERβ 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*.

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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.[1]

There are two subtypes of estrogen receptors and several isoforms of each subtype. The first subtype is estrogen-receptor alpha (ER*α*) which was first cloned in 1986.[2] The second subtype is estrogen-receptor beta (ER*β*) which was discovered recently.[3] 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.[4] 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.[5] These naturally occurring, plant-derived estrogens, defined broadly as phytoestrogens, include the flavonoids (kaempferol and quercitin), the isoflavonoids (genistein, daidzein, formonetin, 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.[6]

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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.[7] 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.[8] 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 *β-D*-galactoside (ONPG) was purchased from Nacalai Tesque Co. (Kyoto, Japan). 17 *β*-Estradiol was purchased from Calibiochem 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, cyhr2, 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 rERa (codons 252–600) was amplified from cDNA by PCR.[9]

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 × 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 37ºC for 4 h. The solution was then extracted with BuOH (10 ml × 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 *β*-galactosidase activity in the yeast two-hybrid screen expressing ER*a* and ER*β* was carried out.

Yeast two-hybrid assay

The yeast two-hybrid assay was carried out according to the method of Nishikawa.[9,10] Briefly, yeast cells expressing ER*a* and ER*β* 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 *β*-estradiol and the isolated compounds for 4 h at 30°C, and *β*-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× *g*, 5 min) and resuspended in 200 *μ*L of Z-buffer (0.1 M sodium phosphate, pH 7.0, 10 mM KCl, and 1 mM $MgSO_4$) containing 1 mg/mL of zymolyase at 37°C for 15 min. The reaction was started by the addition of 40 *μ*L of 4 mg/mL *O*-nitrophenol

β-D-galactopyranoside (ONPG) as a substrate. When yellow color developed (incubation time: *t*), 100 *μ*L of 1 M Na_2CO_3 was added to stop the reaction. The absorbance of the solution (150 *μ*L) was measured at 420 and 550 nm. The *β*-galactosidase activity was determined using the following formula:

$$U=1000\times(A_{420}-1.75A_{550})/(t\times0.05A_{600})$$

Antiestrogenic assay

To examine the antagonistic activity of the test compounds, the inhibition of *β*-galactosidase activity which had been induced by 10^{-7} M 17*β*-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 ± 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*a* and ER*β* at 100 and 10 *μ*g/mL concentrations, respectively. In addition, a naringinase treatment was carried out for all the methanol extracts.[11] Naringinase enzyme is a mixed enzyme of *β*-glucosidase and *a*-rhamnosidase activities. This treatment is used as a partial-mimic to the metabolism process (deglucosilation), which takes place in the gastrointestinal tract (GIT).[11] 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*β*) at a concentration of 100 *μ*g/mL.

Induction of *β*-galactosidase activity in the yeast two-hybrid assay

Yeast expressing estrogen receptor α (ERα)

17*β*-Estradiol was used as a positive control with maximum *β*-galactosidase activity (U) of 786.8±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 *μ*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 β (ERβ)

17*β*-Estradiol showed maximum *β*-galactosidase activity (U) of 1224.0±29.9 at 10^{-7}M. The methanol extracts of *Erythrina fusca* (T4) and *D. lourieri* (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 *μ*g/mL. *Nigella sativa* (E8), *S. japonica* (E11), *Bauhinia malabarica* (T7), *Clitoria hanceana* (T10), and *Diospyros ehretiodes* (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 β-Estradiol-induced *β*-galactosidase activity in the yeast two-hybrid assay (anti-estrogenic assay)

The activity of 17*β*-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 *β*-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 meleguita* (E1), *D. lourieri* (T17), *D. candeatensis* (T9), *Mansonia gagei* (T20), *Artabotrys harmandii* (T24), and *Cassia tora* (T11) showed the most potent antiestrogenic activity. While *B. horsfieldi* (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. meleguita* (E1) and *A. harmandii* (T23) showed an unexpected decrease in their activity by naringinase treatment.

Table 1: Induction of β-galactosidase in the yeast two-hybrid assay expressing ERα and ERβ by the methanol and naringinase-treated extracts of plants growing in Egypt

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

Each value represents the mean ± S.E of three independent experiments (n=3). Asterisks denote significant differences from the control at *$P < 0.05$, **$P < 0.01$. 17 β-Estradiol was used at 10^{-7}M.

CONCLUSION

It is found that, more than 300 plants were reported for their estrogenic activity and phytoestrogen contents.[12,13] 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 β-galactosidase in both yeast two-hybrid assay expressing ERα and ERβ. Most of these plants were reported to contain high amounts of flavonoids, specially flavanones and petrocarpans as in *B. monosperma* (T2),[14] prenylated flavonoids as in *D. reticulata* (T3),[15] and isoflavonoids as in *D. candenatensis* (T9).[16] 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. ehretoides* (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-β-

Table 2: Induction of β-galactosidase in the yeast two-hybrid assay expressing ERα and ERβ by the methanol and naringinase-treated extracts of plants growing in Thailand

No.	Botanical name	Part used	Extract	β-Galactosidase activity (U)			
				ERα		ERβ	
				10 μg/mL	100 μg/mL	10 μg/mL	100 μg/mL
T1	*Bauhinia horsfieldii* Mac.Br. (Fabaceae)	Wood	MeOH	42.6 ± 8.3	55.4 ± 13.5	35.8 ± 2.4	48.3 ± 1.2
			NT	38.9 ± 6.7	39.0 ± 5.8	43.2 ± 3.5	81.5 ± 5.2
T2	*Butea monosperma* Taub. (Fabaceae)	Wood	MeOH	38.8 ± 6.5	37.8 ± 5.8	35.6 ± 5.6	657.9 ± 8.5**
			NT	42.6 ± 5.1	46.50 ± 5.3	55.3 ± 7.1	1384.1 ± 73.5**
T3	*Derris reticulata* Craib. (Fabaceae)	Wood	MeOH	51.8 ± 4.1	92.1 ± 8.6**	21.3 ± 3.1	737.4 ± 68.8**
			NT	65.5 ± 4.8	118.9 ± 6.4**	55.4 ± 2.2	415.2 ± 44.3**
T4	*Erythrina fusca* Lour. (Fabaceae)	Wood	MeOH	72.1 ± 5.9	68.3 ± 5.3	220.2 ± 2.3**	1081.3 ± 17.2**
			NT	48.3 ± 5.3	72.1 ± 6.3	282.2 ± 3.5**	1731.7 ± 65.6**
T5	*Clitoria ternatea* (Fabaceae)	Aerial parts	MeOH	39.8 ± 4.7	33.4 ± 2.8	19.4 ± 3.0	62.5 ± 3.5
			NT	18.3 ± 8.6	19.2 ± 6.2	33.2 ± 2.9	93.1 ± 7.1
T6	*Acacia concinna* (Fabaceae)	Aerial parts	MeOH	24.9 ± 7.4	21.3 ± 8.0	47.7 ± 4.9	47.6 ± 5.5
			NT	19.4 ± 5.2	17.0 ± 7.8	54.4 ± 5.3	87.1 ± 3.7
T7	*Bauhinia malabarica* Roxb. (Fabaceae)	Leaf	MeOH	26.3 ± 7.2	24.9 ± 7.7	31.6 ± 8.5	48.9 ± 13.6
			NT	15.4 ± 6.6	18.1 ± 4.1	35.1 ± 5.1	141.3 ± 13.1**
T8	*Crotalaria verrucosa* (Fabaceae)	Aerial parts	MeOH	24.1 ± 7.1	23.7 ± 6.7	24.2 ± 4.9	32.8 ± 1.4
			NT	20.6 ± 1.5	24.3 ± 5.4	53.7 ± 1.2	52.1 ± 6.3
T9	*Dalbergia candenatensis* (Fabaceae)	wood	MeOH	32.5 ± 10.3	46.1 ± 7.6	50.4 ± 6.2	206.3 ± 18.2**
			NT	37.2 ± 4.8	45.3 ± 6.4	85.3 ± 3.4	284.2 ± 1.4**
T10	*Clitorea hanceana* Gopp. (Fabaceae)	Aerial parts	MeOH	43.9 ± 7.8	44.5 ± 8.6	29.5 ± 1.8	26.1.1 ± 16.4
			NT	28.3 ± 9.4	35.18 ± 6.8	83.2 ± 5.3*	779.0 ± 60.3**
T11	*Cassia tora* Fish. (Fabaceae)	Seeds	MeOH	36.7 ± 1.5	49.5±3.8	51.2 ± 5.4	61.8 ± 8.5
			NT	50.7 ± 4.1	46.3±13.4	45.6 ± 4.3	48.1 ± 4.3
T12	*Caeslpinia sappan* L. (Fabaceae)	Wood	MeOH	39.4 ± 4.7	45.9±9.4	67.6 ± 1.8	36.9 ± 7.8
			NT	39.8 ± 3.4	42.7±13.4	45.5 ± 1.6	11.3 ± 1.9
T13	*Clerodendrum petasites* S.Moore (Verbenaceae)	Wood	MeOH	26.4 ± 4.1	17.0 ± 7.9	28.8 ± 3.2	66.0 ± 7.1
			NT	21.0 ± 5.2	35.8 ± 8.4	46.4 ± 1.9	85.8 ± 16.2
T14	*Foeniculum vulgare* Mill. (Lamiaceae)	Fruits	MeOH	45.3 ± 10.5	45.3 ± 3.3	16.9 ± 1.3	50.7 ± 2.3
			NT	21.3 ± 8.1	47.3 ± 4.5	43.9 ± 6.9	62.3 ± 21.5
T15	*Alpinia siamense* K.Schum. (Zingiberaceae)	Rhizome	MeOH	47.8 ± 9.4	41.4 ± 8.1	33.1 ± 1.3	87.5 ± 1.97*
			NT	47.6 ± 7.0	80.6 ± 5.6*	30.4 ± 1.9	137.3 ± 10.6**
T16	*Vitex trifolia* (Verbenaceae)	Leaf	MeOH	15.8 ± 1.5	14.8 ± 1.7	34.7 ± 3.2	54.7 ± 2.1
			NT	35.8 ± 8.4	35.8 ± 5.3	28.3 ± 5.6	33.8 ± 9.3
T17	*Dracaena loureiri* Gagnep. (Agavaceae)	Wood	MeOH	66.7 ± 5.4	88.1 ± 5.6*	533.8 ± 17.5**	1550.9 ± 209**
			NT	51.5 ± 3.3	88.7 ± 7.3*	655.4 ± 19.5**	1828.6 ± 37.4**
T18	*Diospyros ehretioides* Wall. (Ebenaceae)	Wood	MeOH	29.2 ± 9.2	48.2 ± 7.8	9.1 ± 1.8	7.6 ± 1.1
			NT	37.9 ± 3.7	58.6 ± 7.7	27.5 ± 3.5	107.9 ± 6.2*
T19	*Abutilon hirtum* (Malvaceae)	Aerial parts	MeOH	30.0 ± 1.8	35.8 ± 8.1	23.5 ± 6.5	30.4 ± 3.4
			NT	35.6 ± 1.3	42.4 ± 10.2	55.1 ± 7.3	53.7 ± 8.9
T20	*Mansonia gagei* Drumm. (Sterculiaceae)	Heart wood	MeOH	37.9 ± 6.6	32.3 ± 1.1	50.0 ± 1.5	34.9 ± 3.3
			NT	26.3 ± 1.7	15.7 ± 6.8	43.5 ± 2.6	44.2 ± 4.2
T21	*Vernonia elliptica* (Compositae)	Wood	MeOH	20.1 ± 1.6	19.4 ± 3.9	22.5 ± 2.4	12.8 ± 2.1
			NT	25.8 ± 4.2	16.3 ± 6.4	63.2 ± 8.0	53.5 ± 9.9
T22	*Mantingia calabura* (Tiliaceae)	Wood	MeOH	38.23 ± 1.7	22.6 ± 4.7	16.8 ± 1.3	4.8 ± 2.2
			NT	21.9 ± 3.2	16.7 ± 5.7	45.2 ± 2.2	51.9 ± 4.5
T23	*Artabotrys harmandii* Finet and Gagnep. (Annonaceae)	Wood	MeOH	27.6 ± 8.9	20.1 ± 6.1	46.7 ± 1.2	66.3 ± 2.4
			NT	41.4 ± 3.9	19.4 ± 3.9	66.7 ± 6.6	118.1 ± 9.5**
T24	*Lannea grandis* Engl. (Anacardiaceae)	Wood	MeOH	16.3 ± 6.3	21.9 ± 3.2	18.4 ± 3.2	43.2 ± 4.3
			NT	38.2 ± 6.5	22.6 ± 4.7	73.4 ± 3.5	92.2 ± 9.7
	Estradiol			3074.0 ± 133.8**		3367.0 ± 103.9**	
	DMSO			55.5 ± 1.5		86.9 ± 4.6	

Each value represents the mean ± 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^{-7}M concentration.

phenylethyl alcohol and dihydroxy phenyl acetic acid.[17] *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.[18]

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 *β*-galactosidase in the yeast two-hybrid assay (ER*β*). The activity of *M. gagei* and *C. tora* was investigated by the authors and the activity was

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

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

Each value represents the mean ± S.E of three independent experiments (n=3). Asterisks denote significant differences from the control at $*P < 0.05$, $**P < 0.01$. *β*-Galactosidase activity (*U*) of 17 *β*-estradiol was considered (100%). *β*-Galactosidase activity (*U*) of the tested compounds was calculated as a percentage inhibition of the 17 *β*-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 naphtoquinones, naphthopyrones, and acetyl naphthalene derivatives, respectively.[19,20] 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 flavnoid glycosides to the more active kaempferol and ginestein aglycones.[21] 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.

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