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.

Key words: Estrogenic activity, leguminoseae, yeast two-hybrid assay

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 (ERα) which was first cloned in 1986.[2] The second subtype is estrogen-receptor beta (ERβ) 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]
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 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 the yeast strain used in this study was Y190 (MATα, ura3-52, his3-D200, ade2-101, trp1-901, leu2-3, 112, gdhDgad80D,URA3:GAL-lacZ, cyhr2, LY32: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 rERα (codons 252–600) was amplified from cDNA by PCR.

**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 37°C 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 ERα and ERβ 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 ERα and ERβ 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 μL of Z-buffer (0.1 M sodium phosphate, pH 7.0, 10 mM KCl, and 1 mM MgSO4) containing 1 mg/mL of β-nicotinamide at 37°C for 15 min. The reaction was started by the addition of 40 μL of 4 mg/mL O-nitrophenol...
β-D-galactopyranoside (ONPG) as a substrate. When yellow color developed (incubation time: h), 100 µL of 1 M Na₂CO₃ was added to stop the reaction. The absorbance of the solution (150 µL) was measured at 420 and 550 nm. The β-galactosidase activity was determined using the following formula:

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

**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 17β-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, lignants, 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 ERα and ERβ at 100 and 10 µg/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 α-rhamnosidase activities. This treatment is used as a partial-mimic to the metabolism process (deg glucosilation), 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 (ERβ) at a concentration of 100 µg/mL.

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

**Yeast expressing estrogen receptor α (ERα)**

17β-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 µg/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)**

17β-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. 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 µg/mL. *Nigella sativa* (E8), *S. japonica* (E11), *Bambina malabaria* (T7), *Clitoria bancana* (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 17β-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 10⁻⁷ M. As shown in Table 3, the methanol- and the naringinase-treated extracts of *Aframomum meleguita* (E1), *D. loureiri* (T17), *D. candeatensis* (T9), *Mansonia gages* (T20), *Artabotrys harmandii* (T24), and *Cassia tora* (T11) showed the most potent antiestrogenic activity. While *B. borisfieldi* (T1), *D. reticulata* (T3), *Clerodendrum petasites* (T13), *E. fusca* (T4), *Poeniculum vulgar* (T13), *Clitoria ternatea* (T5), and *Lannea malabarica* (T7) 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                  |
|     |                                 |           | NT      | 46.8 ± 7.8                   | 46.8 ± 7.8                   |
| E2  | Cyperus esculentus (Cyperaceae)   | Tuber     | MeOH    | 41.7 ± 7.2                   | 41.7 ± 7.2                   |
|     |                                 |           | NT      | 43.8 ± 8.4                   | 43.8 ± 8.4                   |
| E3  | Sesamum indicum (Papilionaceae)   | Seeds     | MeOH    | 32.1 ± 3.4                   | 32.1 ± 3.4                   |
|     |                                 |           | NT      | 38.8 ± 9.0                   | 38.8 ± 9.0                   |
| E4  | Linum usitatissimum (Linaceae)    | Seeds     | MeOH    | 20.7 ± 6.5                   | 20.7 ± 6.5                   |
|     |                                 |           | NT      | 24.4 ± 9.5                   | 24.4 ± 9.5                   |
| E5  | Hordeum vulgare (Gramineae)       | Fruit    | MeOH    | 5.2 ± 2.6                    | 5.2 ± 2.6                    |
|     |                                 |           | NT      | 44.1 ± 6.5                   | 44.1 ± 6.5                   |
| E6  | Petroselinum crispum (Lamiaceae)  | Leaves   | MeOH    | 49.4 ± 4.5                   | 49.4 ± 4.5                   |
|     |                                 |           | NT      | 21.4 ± 7.7                   | 21.4 ± 7.7                   |
| E7  | Brassica oleracea (Cruciferae)    | Leaves   | MeOH    | 50.4 ± 7.1                   | 50.4 ± 7.1                   |
|     |                                 |           | NT      | 22.3 ± 7.8                   | 22.3 ± 7.8                   |
| E8  | Nigella sativa (Ranunculaceae)    | Seeds    | MeOH    | 30.8 ± 1.7                   | 30.8 ± 1.7                   |
|     |                                 |           | NT      | 21.9 ± 10.9                  | 21.9 ± 10.9                  |
| E9  | Vitis agnus-castus (Lamiaceae)    | Fruits   | MeOH    | 39.6 ± 4.4                   | 39.6 ± 4.4                   |
|     |                                 |           | NT      | 15.8 ± 4.3                   | 15.8 ± 4.3                   |
| E10 | Lens culinaris (Fabaceae)         | Seeds    | MeOH    | 19.6 ± 8.4                   | 19.6 ± 8.4                   |
|     |                                 |           | NT      | 22.3 ± 5.1                   | 22.3 ± 5.1                   |
| E11 | Sophora japonica (Fabaceae)       | Seeds    | MeOH    | 36.3 ± 4.8                   | 36.3 ± 4.8                   |
|     |                                 |           | NT      | 71.2 ± 3.6                   | 71.2 ± 3.6                   |
| E12 | Cicer arietinum (Fabaceae)         | Seeds    | MeOH    | 45.1 ± 3.0                   | 45.1 ± 3.0                   |
|     |                                 |           | NT      | 41.7 ± 6.4                   | 41.7 ± 6.4                   |
| E13 | Vigna unguiculata (Faba)          | Seeds    | MeOH    | 20.0 ± 7.1                   | 20.0 ± 7.1                   |
|     |                                 |           | NT      | 19.9 ± 9.0                   | 19.9 ± 9.0                   |
| E14 | Trigonella foenum grecum. (Faba)   | Seeds    | MeOH    | 19.4 ± 17.1                  | 19.4 ± 17.1                  |
|     |                                 |           | NT      | 24.0 ± 5.9                   | 24.0 ± 5.9                   |
| E15 | Phasoleus vulgaris (Faba)         | Seeds    | MeOH    | 21.8 ± 6.2                   | 21.8 ± 6.2                   |
|     |                                 |           | NT      | 21.7 ± 6.2                   | 21.7 ± 6.2                   |
| E16 | Vicia faba (Faba)                | Seeds    | MeOH    | 40.1 ± 3.2                   | 40.1 ± 3.2                   |
|     |                                 |           | NT      | 42.1 ± 9.7                   | 42.1 ± 9.7                   |
|     |                                 |           | DMSO    | 40.0 ± 7.0                   | 40.0 ± 7.0                   |
|     |                                 |           | 17β-Estradiol | 786.8 ± 32.5**              | 1224.0 ± 29.9**             |
|     |                                 |           |         |                              | 128.0 ± 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. β-Estradiol was used at 10 μg/mL.

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 meothanol and naringinase-treated extracts of plants growing in Thailand

| No. | Botanical name                          | Part used | Extract | ERα 10 µg/mL | ERβ 10 µg/mL |
|-----|-----------------------------------------|-----------|---------|--------------|--------------|
|     |                                         |           | MeOH    | 100 µg/mL    | 100 µg/mL    |
| T1  | Bauhinia horsfieldii Mac.Br. (Fabaceae) | Wood      | MeOH    | 42.6 ± 8.3   | 35.8 ± 2.4   |
|     |                                        |           | NT      | 38.9 ± 6.7   | 43.2 ± 3.5   |
| T2  | Butea monosperma Taub. (Fabaceae)       | Wood      | MeOH    | 38.8 ± 6.5   | 35.6 ± 5.6   |
|     |                                        |           | NT      | 42.6 ± 5.1   | 53.3 ± 7.1   |
| T3  | Derris reticulata Craib. (Fabaceae)     | Wood      | MeOH    | 51.8 ± 4.1   | 21.3 ± 3.1   |
|     |                                        |           | NT      | 65.5 ± 4.8   | 55.4 ± 2.2   |
| T4  | Erythrina fusca Lour. (Fabaceae)        | Wood      | MeOH    | 72.1 ± 5.9   | 220.2 ± 2.3  |
|     |                                        |           | NT      | 48.3 ± 5.3   | 282.2 ± 3.9  |
| T5  | Clitoria ternatea (Fabaceae)            | Aerial parts | MeOH  | 39.8 ± 4.7   | 19.4 ± 3.0   |
|     |                                        |           | NT      | 18.3 ± 8.6   | 33.2 ± 2.9   |
| T6  | Acacia concinna (Fabaceae)              | Aerial parts | MeOH  | 24.9 ± 7.4   | 47.7 ± 4.9   |
|     |                                        |           | NT      | 19.4 ± 5.2   | 54.4 ± 5.3   |
| T7  | Bauhinia malabarica Roxb. (Fabaceae)    | Leaf      | MeOH    | 26.3 ± 7.2   | 31.5 ± 5.1   |
|     |                                        |           | NT      | 15.4 ± 5.6   | 35.1 ± 1.1   |
| T8  | Crotalaria verrucosa (Fabaceae)         | Aerial parts | MeOH  | 24.1 ± 7.1   | 24.2 ± 4.9   |
|     |                                        |           | NT      | 20.6 ± 1.5   | 53.7 ± 1.2   |
| T9  | Dalbergia candenatensis (Fabaceae)      | wood      | MeOH    | 32.5 ± 10.3  | 50.4 ± 6.2   |
|     |                                        |           | NT      | 37.2 ± 4.8   | 85.3 ± 3.4   |
| T10 | Clerodendrum petasites S.Moore (Verbenaceae) | Wood   | MeOH    | 43.9 ± 7.8   | 29.5 ± 1.8   |
|     |                                        |           | NT      | 28.3 ± 9.4   | 83.2 ± 5.9   |
| T11 | Cassia tora Fish. (Fabaceae)            | Seeds     | MeOH    | 36.7 ± 1.5   | 51.2 ± 5.4   |
|     |                                        |           | NT      | 50.7 ± 4.1   | 45.6 ± 4.3   |
| T12 | Caesalpinia sappan L. (Fabaceae)        | Wood      | MeOH    | 39.4 ± 4.7   | 67.6 ± 1.8   |
|     |                                        |           | NT      | 39.8 ± 3.4   | 45.5 ± 1.6   |
| T13 | Clerodendrum petasites S.Moore (Verbenaceae) | Wood   | MeOH    | 26.4 ± 4.1   | 28.8 ± 3.2   |
|     |                                        |           | NT      | 21.0 ± 5.2   | 46.4 ± 1.9   |
| T14 | Foeniculum vulgare Mill. (Lamiaceae)    | Fruits   | MeOH    | 45.3 ± 10.5  | 16.9 ± 1.3   |
|     |                                        |           | NT      | 21.3 ± 8.1   | 43.9 ± 6.9   |
| T15 | Alpinia siamense K.Schum. (Zingiberaceae) | Rhizome | MeOH    | 47.8 ± 9.4   | 33.1 ± 1.3   |
|     |                                        |           | NT      | 47.6 ± 7.0   | 30.4 ± 1.9   |
| T16 | Vitex trifolia (Verbenaceae)            | Leaf      | MeOH    | 15.8 ± 1.5   | 34.7 ± 3.2   |
|     |                                        |           | NT      | 35.8 ± 8.4   | 32.8 ± 5.6   |
| T17 | Draacaena lourei Gagnep. (Agavaceae)    | Wood      | MeOH    | 66.7 ± 5.4   | 533.8 ± 7.5  |
|     |                                        |           | NT      | 51.5 ± 3.3   | 655.4 ± 19.5 |
| T18 | Diospyros ehretioides Wall. (Ebenaceae) | Wood      | MeOH    | 29.2 ± 9.2   | 9.1 ± 1.8    |
|     |                                        |           | NT      | 37.9 ± 3.7   | 27.5 ± 3.5   |
| T19 | Abouton hirtum (Malvaceae)              | Aerial parts | MeOH  | 30.0 ± 1.8   | 23.5 ± 6.5   |
|     |                                        |           | NT      | 35.6 ± 1.3   | 55.1 ± 7.3   |
| T20 | Mansonia gagei Drum. (Sterculiaceae)    | Heart wood | MeOH  | 37.9 ± 6.6   | 50.0 ± 1.5   |
|     |                                        |           | NT      | 26.3 ± 1.7   | 43.5 ± 2.6   |
| T21 | Vernonia elliptica (Compositae)         | Wood      | MeOH    | 20.1 ± 1.6   | 22.5 ± 4.2   |
|     |                                        |           | NT      | 25.8 ± 4.2   | 63.2 ± 8.0   |
| T22 | Mantingia calabura (Tiliaceae)          | Wood      | MeOH    | 36.23 ± 1.7  | 16.8 ± 1.3   |
|     |                                        |           | NT      | 21.9 ± 3.2   | 45.2 ± 2.2   |
| T23 | Artabotrys harmandi Finet and Gagnep.   | Wood      | MeOH    | 27.6 ± 8.9   | 46.7 ± 1.2   |
|     | (Annonaceae)                            |           | NT      | 41.4 ± 3.9   | 66.7 ± 6.6   |
| T24 | Laneea grandis Engl. (Anacardiaceae)    | Wood      | MeOH    | 16.3 ± 6.3   | 18.4 ± 3.2   |
|     |                                        |           | NT      | 38.2 ± 6.5   | 73.4 ± 3.5   |

|                | Estradiol    | DMSO       |                |
|----------------|--------------|------------|----------------|
|                | 3074.0 ± 133.8** | 55.5 ± 1.5 | 86.9 ± 4.6     |
|                | 3367.0 ± 103.9** |           |                |

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 10⁻⁷M concentration.

Among the plants exhibiting antiestrogenic activity, the methanol extracts of *Mansonia gagei*, *A. melegueta* (E1), *D. caderenatensis*, and *C. tora* showed the most potent inhibition of estradiol-dependent induction of β-galactosidase in the yeast two-hybrid assay (ERβ). The activity of *M. gagei* and *C. tora* was investigated by the authors and the activity was investigated by the authors and the activity was investigated by the authors and the activity was investigated by the authors and the activity was investigated by the authors and the activity was investigated by the authors and the activity was...
found to be due to their naphthoquinones, naphthopyrones, and acetyl naphthalene derivatives, respectively.\textsuperscript{19,20} While, the naringinase-treated extract of \textit{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.\textsuperscript{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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