

THE EFFECT OF 96% ETHANOL EXTRACT OF TURMERIC (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) AGAINST ESTROGEN HORMONE

Rizka Angrainy¹, Asita Elengoe², Rathimalar Ayakannu³,
Berliana Irianti⁴, Manisha⁵ and Aida Fitria⁶

^{1,2,3,4,5,6} Faculty of Applied Science, Lincoln University College,
Petaling Jaya, Selangor, Malaysia.

DOI: 10.5281/zenodo.14271988

Abstract

Background: During a woman's reproductive period (puberty to pre-menopause) the structure and function of the reproductive organs are maintained by the hormone estrogen. When entering menopause, estrogen hormone levels drop, causing the symptoms of menopause. To overcome menopause symptoms, hormone replacement therapy (HRT) is widely used with HRT where the hormone estrogen has been proven to reduce menopause symptoms, although it can increase the risk of breast, endometrial, and ovarian cancer. With these risks from HRT, it is necessary to find alternatives to overcoming menopause symptoms. One alternative that is widely developed is the herbal ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) **Objectives:** This study aims to analyze the effects of turmeric on estrogen hormones in rats. **Methods:** This study is an experimental study. The study was conducted for 30 days using 15 female Sprague-Dawley rats. The treatment consisted of 5 groups with a dose of 0 mg/Kg BB as a control, 10 mg/Kg BB Contraceptive Pills, a dose of 25 mg/Kg BB, a dose of 50 mg/Kg BB, a dose of 100 mg/Kg BB given orally via 1 ml of vial. On the 31st day, surgery and serum were taken from the rats. Estrogen hormone levels were tested using the Enzyme Linked Immunosorbent Assay (ELISA) method. **Results:** The average estrogen levels in all treatment groups were higher than the Control group with an average estrogen hormone ELISA result of 647 ng/L, P1 (given turmeric ethanol extract (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) as much as 25 mg) from the results of the ELISA test of estrogen hormone, an average of 691 ng/L was obtained, P2 (given ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) as much as 50 mg) from the results of the ELISA test of estrogen hormone, an average of 709 ng/L was obtained and P3 (given ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) as much as 100 mg) from the results of the ELISA test of estrogen hormone, an average of 617 ng/L was obtained. There was no significant difference in the average estrogen levels between the control group and the treatment group. **Conclusion:** 96% turmeric ethanol extract at a dose of 100 mg/kg BB has the potential to be a natural antifertility agent by reducing ovarian weight in rats.

Keywords: Ethanol 96%, Turmeric, Estrogen Hormone.

INTRODUCTION

According to Amanda J Welton, *et al* in the article "Health related quality of life after combined hormone replacement therapy" 2008, stated that women who use combination therapy of conjugated equine estrogen 0.625 mg plus medroxyprogesterone acetate 2.5 / 5.0 mg orally once a day for one year showed a decrease in menopausal symptoms in the form of hot flushes, night sweats, joint and muscle pain, insomnia, vaginal dryness so that it can improve the quality of life of postmenopausal women, but there are complaints of breast tenderness and vaginal discharge. The quality of life of postmenopausal women increases along with the length of use of combination therapy for years¹.

Research conducted by Judith K. Ockene, PhD, MEd entitled "Symptom Experience After Discontinuing Use of Estrogen Plus Progestin" 2005 stated that postmenopausal women after stopping combination therapy of conjugated equine estrogens plus

medroxyprogesterone for 8-10 months experienced vasomotor symptoms, pain, and joint stiffness ².

During a woman's reproductive years (puberty to pre-menopause) the structure and function of the reproductive organs are maintained by the hormone estrogen. When entering menopause, estrogen hormone levels drop, causing menopausal symptoms. To overcome menopausal symptoms, hormone replacement therapy (HRT) is widely used. HRT with estrogen has been shown to reduce menopausal symptoms, but increases the risk of breast, endometrial and ovarian cancer ³. With the risks of HRT, it is necessary to find alternatives to overcome menopausal symptoms. One alternative that is widely developed is using herbs, one of which is the turmeric plant (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) .

Turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) is a perennial herbal plant and has rhizomes (tubers) that are included in the ginger family (Zingiberaceae). Turmeric plant (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) is a tropical plant native to Asia and has now spread to subtropical areas around the world ⁴. The dark yellow powder produced from turmeric rhizomes is known as turmeric powder, in India, China and Asia it has long been widely used for food and medicine⁵

Tumerone has a synonym name, namely 2-Methyl-6-(4-methylcyclohexa-1,3-dien-1-yl)hept-2-en-4-one;2-Methyl-6-(4-methyl-1,3-cyclohexadien-1-yl)-2-hepten-4-one;α Turmerone; Turmerone. This compound is a group of sesquiterpenoid compounds. Here are some of the benefits of the turmerone compound, namely as an anti-inflammatory, turmerone has anti-inflammatory properties that can help reduce inflammation in the body, which can help reduce the risk of chronic diseases such as arthritis. Then as an antioxidant, this compound also has antioxidant properties, which can help fight cell damage due to free radicals and oxidative processes in the body and as brain protection, several studies have shown that turmerone can help protect the brain from degenerative diseases such as Alzheimer's and can improve cognitive function. And tumerone is abundant in turmeric ethanol extract ⁶.

Research on turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) has been widely conducted for medicinal purposes, one of which is a study conducted by Kusuma Dewi that there is an effect of giving turmeric rhizome extract (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) On the body temperature of white rats given the DPT vaccine ⁷. Other studies state that turmeric rhizome (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) has antipyretic effect on male white rabbits of New Zealand strain ⁸.

In a study conducted on the antibacterial effectiveness test of turmeric extract (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) On *Bacillus* sp (Gram positive) and *Shigella dysenteriae* (Gram negative) bacteria, it was shown that the inhibition zone of Gram positive bacteria was larger than Gram negative. This is due to the difference in wall structure between Gram positive and Gram negative bacteria ⁹. Wijayanto's research (2014) also showed that ethanol extract of turmeric rhizome (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) against *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) has greater antibacterial activity against the growth of *Staphylococcus aureus* than *Escherichia coli* ¹⁰. Research proves *in vitro* that turmeric rhizome extraction (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) able to inhibit the growth of both Gram-positive and Gram-negative bacteria, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. Aureus* ¹¹.

Ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) is obtained by maceration process. The solvent used in the extraction process greatly determines the results of the identification of the extracted bioactive compound components. Ethanol solvent is used based on its polarity level, this solvent has the strongest group than nonpolar and is able to extract more bioactive components that have higher polarity compounds.

In the female reproductive system, turmeric can inhibit ovulation, implantation, and the production of Follicle Stimulating Hormone, Luteinizing Hormone, estrogen, and progesterone. In addition, turmeric can inhibit the estrus cycle and cause biochemical changes in uterine fluid. It can be concluded that turmeric has the potential to control fertility, especially as an antifertility agent for men and women. The antifertility effects of turmeric are reversible.

RESEARCH METHODS

Tools and materials

Preparation of Test Extracts

The simple drug is extracted by maceration with 96% ethanol solvent.

Phytochemical Screening

Phytochemical screening is carried out to determine the presence of secondary metabolites, including Alkaloid, Flavanoid, Tannin, Saponin, Triterpenoid, and Steroid compounds.

ELISA examination

The tools used in this study were the Bio-Rad xMark Microplate Absorbance Reader, equipped with Microplate Manager software, 27.3 °C Incubator, Absorbent Paper, Precision Pipettes, and disposable pipette tips.

The materials used in this study were the ELISA Kit Bioassay Technology Laboratory, which consisted of Standard estrogen solution, ELISA plate coated with antibodies, Standard diluent solution, Streptavidin-HRP, Stop Solution, Substrate A solution, Substrate B solution, Wash buffer solution, Plate sealer, and Blood serum taken from Female Rats, 96% Ethanol Turmeric Extract

Preparation

All reagents in the ELISA Kit are placed at room temperature, then the standard solution is prepared. The working standard solution to be used is 6 different concentrations. The Estrogen Standard is diluted and made into several concentrations, namely 2400, 1200, 600, 300, 150, 75 ng/L. In addition, a washing solution is also prepared because this solution is available in concentrated form and must be diluted. Each plate requires 5 washes with a washing solution of 0.35 mL.

Research Procedures

Treatment

This research is an experimental research. The sample used is turmeric powder (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) obtained from the Riau turmeric area. The powder is then sieved using a 60 mesh sieve to obtain a fine and homogeneous powder. Making turmeric (*Curcuma longa* Linn. syn. *Curcuma*

domestica Val.) using the maceration or soaking method. Then given to rats with a predetermined dose to observe changes in estrogen hormones after being given 96% ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) .

The study was conducted for 30 days using 15 female Sprague-Dawley rats. The treatment consisted of 5 groups with a dose of 0 mg/Kg BB as a control, 1.8 mg/Kg BB Contraceptive Pills, a dose of 25 mg/Kg BB, a dose of 50 mg/Kg BB, a dose of 100 mg/Kg BB given orally via 1 ml of vial. On the 31st day, surgery and serum were taken from the rats. Estrogen hormone levels were tested using the Enzyme Linked Immunosorbent Assay (ELISA) method.

The prepared standard solutions were each added into the plate in the amount of 50 µL precisely and quantitatively. 50 µL of Streptavidin-HRP was added to each plate and then mixed well then covered with a sealer and incubated for 60 minutes at a temperature of 27.3 ° C. While waiting, the washing solution was prepared. After incubation, the sealer was removed and then the plate was washed with washing solution 5 times, each amounting to 0.35 mL. After washing and the washing solution had been drained, substrates A and B were added successively in the amount of 50 µL. Then covered with a sealer again and incubated for 10 minutes at a temperature of 27.3 ° C and in the dark.

Observation Using Microplate Absorbance Reader

After incubation for 10 minutes, the plate was removed and stop solution was added until the color changed from blue to yellow. Immediately after the stop solution was added, the optical density was determined by reading the absorbance using a Microplate Absorbance Reader at a wavelength of 450 nm.

RESULTS

Phytochemical Screening Results

Table 1: Phytochemical Screening Results

No	Identification of Compound Groups	Criteria	Test Results	Information
1	Alkaloid	White precipitate formed	There is White Precipitate plus Meyer's Reagent	Positive
2	Flavonoid	Orange, pink, red	The orange color turns red	Positive
3	Tannin	Blackish Green/dark blue	The orange color becomes blackish-green	Positive
4	Saponins	Foam forms in 5 minutes	There is a foam that is durable and stable	Positive
5	Triterpenoid	A brownish or violet ring forms at the solution border.	A brownish ring forms at the border of the solution.	Positive
6	Steroid	A greenish-blue ring is formed	No blue-green ring is formed	Negative

In Table 1. It can be seen that the Turmeric Ethanol Extract (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) contains compounds namely Alkaloids, Flavanoids, Tannins, Saponins, Triterpenoids.

Table 2: Results of Level vs Optical Density

No.	Standard Level Estrogen (ng/L)	OD (Optical Density)
1.	2400	0.075
2.	1200	0.181
3.	600	0.503
4.	300	0.855
5.	150	1.106
6.	75	1,240
7.	0	1.395

In Table.2, the standard curve was made with 7 graded concentrations, namely 0 ng/L, 75 ng/L, 150 ng/L, 300 ng/L, 600 ng/L, 1200 ng/L and 2400 ng/L. used to obtain a linear standard curve with $r = 0.998$ and used to measure estrogen hormone levels.

Table 3 and Graph: Results of ELISA Test of Estrogen Hormone in Rats

Data Analysis Report:

Sample ID	Well	OD	Mean	Conc (ng/L)
BK-01	H3	0.572	0.572	512.313
BK-02	A4	0.390	0.390	754.369
BK-03	B4	0.644	0.644	443.676
K+01	D2	0.444	0.444	668.677
K+02	E2	0.420	0.420	704.911
K+03	F2	0.466	0.466	637.728
K-01	A2	0.537	0.537	549.994
K-02	B2	0.379	0.379	773.815
K-03	C2	0.489	0.489	607.425
P1.01	G2	0.417	0.417	709.638
P1.02	H2	0.424	0.424	698.679
P1.03	A3	0.445	0.445	667.226
P2.01	B3	0.409	0.409	722.473
P2.02	C3	0.548	0.548	537.803
P2.03	D3	0.341	0.341	847.344
P3.01	E3	0.369	0.369	792.168
P3.02	F3	0.691	0.691	404.090
P3.03	G3	0.453	0.453	655.769

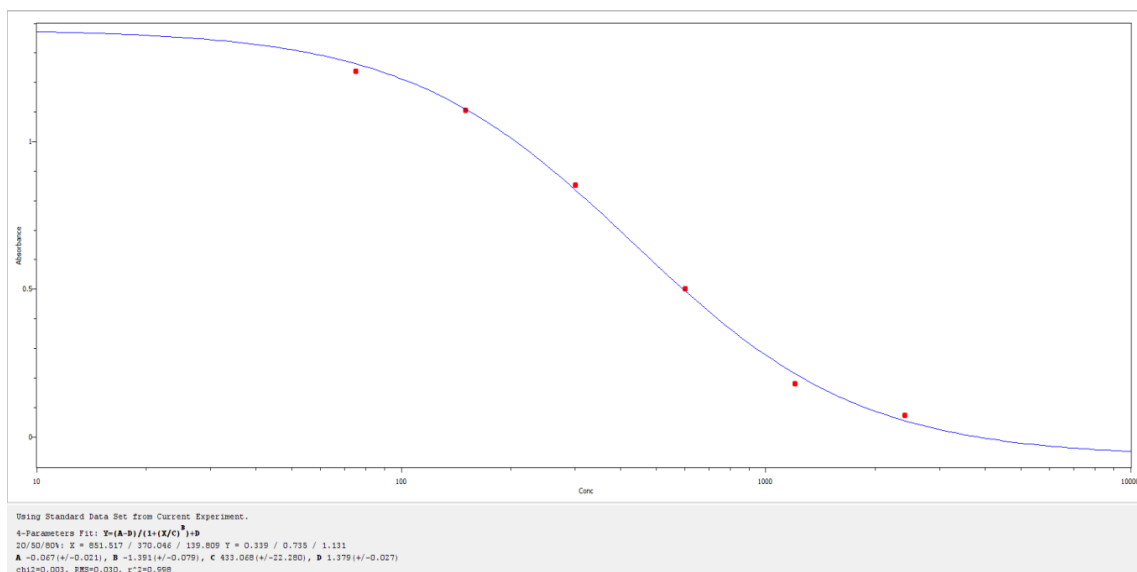


Figure 1: ELISA graph in rats

From Table 3 and Graph 1 above, the results of the research that has been carried out obtained the results of BK (Female Rats mated with Males given turmeric), K+ (Positive control given birth control pills) obtained the results of the ELISA estrogen hormone on average of 669 ng/L, K- (negative control) is the treatment of rats that were not given anything as a comparison obtained the results of the ELISA estrogen hormone on average of 647 ng/L, P1 (given ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) as much as 25 mg) from the results of the ELISA test of estrogen hormone, an average of 691 ng/L was obtained, P2 (given ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) as much as 50 mg) from the results of the ELISA test of estrogen hormone, an average of 709 ng/L was obtained and P3 (given ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) as much as 100 mg) from the results of the ELISA estrogen hormone test, an average of 617 ng/L was obtained.

DISCUSSION

According to Nigg and Seigler (1992) in Suprihatin (2020), flavonoids and alkaloids in plants can cause abortion. Furthermore, according to Gill et al. (2000) and Juneja et al. (2001) in Suprihatin (2020), the flavonoid and alkaloid groups of plants can cause disruption to cell membranes, resulting in changes in the components of the membrane and can inhibit cell division due to inhibition of the formation of cell membranes that play a role in transporting nutrients needed for cell metabolism in producing energy.

The permeability of the egg and embryo cell membranes is closely related to the process of embryo growth and development (cleavage).¹²

The effect of alkaloids on the membranes of egg cells and embryos causes membrane shrinkage so that membrane integrity will decrease and affect the development of egg cells and embryos (cleavage) resulting in the embryo dying.

Saponin is a foamy solution. The negative effects of saponin on animal reproduction are known as abortivum, inhibiting zygote formation and antiimplantation. Saponin is cytotoxic to cells especially those undergoing development such as during oogenesis.¹³

According to Fadhilah H (2021), the factors that cause disturbances during pregnancy are internal factors and external factors. Internal factors, for example, include chromosomal abnormalities, while external factors can be caused by viruses, radiation, malnutrition, various chemicals such as alkaloids, steroids and alcohol.¹⁴

The estrogen hormone levels given by birth control pills are higher than the estrogen hormone levels of negative control. The estrogen hormone levels given by turmeric ethanol extract (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) and this indicates that the ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) can increase estrogen hormone levels in rats. Based on the results of the study, it is recommended to continue the study using higher dose variations.¹⁵

CONCLUSION AND RECOMMENDATIONS

96% ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) can increase the highest estrogen hormone levels, namely the administration of 96% ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) with

a dose of 50 mg/Kg BB compared to the positive control of birth control pills. However, with a dose of 100 mg/Kg BB, the estrogen hormone levels were lower than the positive control group of birth control pills and the control group that was not given anything. So a dose of 100 mg/Kg BB can reduce estrogen hormone levels.

Ethical Consideration

This research complied with the declaration of Pharmaceutical Laboratory ethical principles for medical research involving animals subjects. The study was approved by the Ethics Review Board of the University of Andalas, Indonesia (No.22/UN.16.2/KEP-FK/2024)

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria

Author Disclosure

All authors declared no conflict of interest

Funding Source

None

The Study Conducted

Mei 2024

Bibliography

- 1) Welton AJ, Vickers MR, Kim J, et al. Health related quality of life after combined hormone replacement therapy: Randomised controlled trial. *Bmj.* 2008; 337(7669): 550-553. doi:10.1136/bmj.a1190
- 2) Ockene JK, Barad DH, Cochrane BB, et al. of Estrogen Plus Progestin. 2005; 294(2):183-193.
- 3) Paszkowski T, Bińkowska M, Dębski R, Krzyczkowska-Sendrakowska M, Skrzypulec-Plinta V, Zgliczyński W. Menopausal hormone therapy in questions and answers - A manual for physicians of various specialties. *Przegląd Menopauzalny.* 2019; 18(1):1-8. doi:10.5114/pm.2019.84150
- 4) Santander B. Formularium ramuan obat tradisional indonesia. *Jurnal Ilmu Farmasi.* Published online 2017.
- 5) Indonesia DR. Kebijakan obat tradisional nasional. Jakarta: Departemen Kesehatan Republik Indonesia. Published online 2007.
- 6) Goozee, K. G., Shah, T. M., Sohrabi, H. R., Rainey-Smith, S. R., Brown, B., Verdile, G., ... & Martins RN. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. *British Journal of Nutrition.* Published online 2016:115(3), 449-465.
- 7) Kusuma D MITA. Aktivitas antibakteri ekstrak daun Majapahit (*Crescentia cujete*) terhadap pertumbuhan bakteri *Ralstonia solanacearum* penyebab penyakit layu. *LenteraBio.* Published online 2014:3 (1).
- 8) Kusumaningrum YI. Hubungan antara Pengetahuan Ibu dan Faktor-Faktor Sosial ekonomi Orangtua dengan Praktik Pemberian Makanan Pendamping ASI (MP-ASI) pada Bayi Usia 6-12 Bulan di Desa Kemuning Kecamatan Ampel gading Kabupaten Pemalang. (Doctoral dissertation, Universitas Negeri Semarang). Published online 2018.
- 9) Yuliati Y. Uji efektivitas ekstrak kunyit sebagai antibakteri dalam pertumbuhan bacillus sp dan shigella dysenteriae secara in vitro. *Jurnal Profesi Medika.* Published online 2016:10(1):26-32.
- 10) Wijayanto W. Uji aktivitas antibakteri ekstrak etanol rimpang kunyit putih (*curcuma mangga* Val) terhadap staphylococcus aureus ATCC 6538 dan eschericia coli ATCC 11229 secara in vitro. [Skripsi]. Surakarta: Fakultas Kedokteran Universitas Muhammadiyah Surakarta. Published online 2014.

- 11) Hidayati E, Juli N ME. Isolasi enterobacteriaceae patogen dari makanan berbumbu dan tidak berbumbu kunyit (*curcuma domestica* val.) serta uji pengaruh ekstrak kunyit (*curcuma domestica* val.) terhadap pertumbuhan bakteri yang diisolasi. *Jurnal Matematika Dan Sains*. Published online 2002:7(2):43–52.
- 12) Suprihatin T, Rahayu S, Rifa'i M, ... Senyawa pada serbuk rimpang kunyit (*Curcuma longa* L.) yang berpotensi sebagai antioksidan. *Buletin Anatomi dan....* Published online 2020. <https://ejournal2.undip.ac.id/index.php/baf/article/view/9107>
- 13) Ningsih AW, Nurrosyidah IH. Pengaruh perbedaan metode ekstraksi rimpang kunyit (*Curcuma domestica*) terhadap rendemen dan skrining fitokimia. ... *Medika (J-Pham* Published online 2020. <http://jurnal.stikesrsanwarmedika.ac.id/index.php/jpcam/article/view/27>
- 14) Fadhilah H, Rachmani K, Hajaring N. Aktifitas Kunyit (*Curcuma domestica* Val.) Sebagai Antiinflamasi Ditinjau Dari Berbagai Literatur. *Edu Masda Journal*. Published online 2021. <http://openjournal.masda.ac.id/index.php/edumasda/article/view/120>
- 15) Titisari N, Firmawati A, Fauzi A, Ayu M, Masnur I, ... Reproductive Cycle of Female Javan Langur (*Trachypithecus Auratus*) Based on Estrogen and Luteinizing Hormone Levels. *cabidigitallibrary.org*; 2021. doi:10.5555/20210366207