The effect of 96% ethanol extract of turmeric (*Curcuma longa L.* syn. *Curcuma domestica* Val.) on estrogen hormone levels

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Abstract

Estrogen is essential for maintaining the structure and function of the female reproductive system, and its decline during menopause triggers various symptoms. While Hormone Replacement Therapy (HRT) is commonly used, long-term use increases the risk of certain cancers. Consequently, natural alternatives like turmeric (*Curcuma longa* L. syn. *Curcuma domestica* Val.) are being investigated for their phytoestrogenic potential. This study aimed to investigate the effect of 96% ethanol extract of turmeric on estrogen hormone levels in female rats. An experimental study was conducted using 15 female Sprague-Dawley rats over 30 days, divided into five groups: control (0 mg/kg BW), contraceptive pill (10 mg/kg BW), and turmeric extract at doses of 25, 50, and 100 mg/kg BW. The extract was administered orally. On day 31, blood serum was collected, and estrogen levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA). Estrogen levels increased in all treatment groups compared to the control (647 ng/L), with levels of 691 ng/L (25 mg/kg), 709 ng/L (50 mg/kg), and 617 ng/L (100 mg/kg). However, these differences were not statistically significant. Turmeric extract showed a dose-dependent effect, with moderate doses indicating phytoestrogenic benefits. The decline at high doses suggests a dual action-beneficial at moderate levels, inhibitory at excess. Despite non-significant results, the trends support turmeric's potential as a natural HRT alternative, warranting further studies with larger cohorts and mechanistic evaluations to define optimal dosage, safety, and reproductive implications.

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Introduction

Estrogen refers to a group of steroid hormones that are fundamental to the development, regulation, and maintenance of the female reproductive system and secondary sexual characteristics.[1] Although primarily associated with female physiology, estrogen is also present in males, albeit in significantly lower concentrations.[2] Hormones in general are endogenous biochemical messengers synthesized by glands to control and coordinate functions across various tissues and organs.[3] They are critical in regulating growth, metabolism, reproduction, and mood.[4] A comprehensive understanding of hormonal roles, especially that of estrogen, is essential for women to navigate the complex physiological transitions across their lifespan.[5] Estrogen exists in three primary forms estradiol, estriol, and estrone each dominant during distinct life stages.[6] Estradiol predominates during the reproductive years, estriol during pregnancy, and estrone during the premenopausal transition.[7]

Physiologically, estrogen performs a multitude of vital functions. It assists in thermoregulation, enhances memory, and modulates neural circuits associated with sexual and reproductive behavior. It also plays a role in lipid metabolism, thereby reducing cardiovascular risk by controlling cholesterol levels. [8] Estrogen facilitates ovarian maturation and menstrual cycle initiation, supports uterine development for implantation, promotes breast development and lactation readiness, and helps maintain skeletal

integrity.[9] Puberty-related estrogen surges contribute to breast growth and the development of pubic and axillary hair[10] During pregnancy, elevated estrogen levels support angiogenesis and nutrient transport to the fetus.[11-13] Additionally, increased estrogen may cause vaginal secretions in early gestation. As women approach menopause, hormonal fluctuations culminate in a steady decline, resulting in various physiological and psychological symptoms.[14]

To alleviate menopausal symptoms resulting from estrogen deficiency, Hormone Replacement Therapy (HRT) is frequently employed. A study by Amanda J. Welton et al. demonstrated that daily oral administration of conjugated equine estrogen (0.625 mg) with medroxyprogesterone acetate (2.5/5.0 mg) over a one-year period significantly reduced vasomotor symptoms, joint pain, sleep disturbances, and vaginal dryness, thereby enhancing post-menopausal quality of life, although some patients reported side effects such as breast tenderness and discharge.[15] However, research by Judith K. Ockene, PhD, MEd, indicated that cessation of this combination therapy led to the recurrence of symptoms including vasomotor instability and joint stiffness.[16] Despite its therapeutic benefits, long-term HRT use is associated with increased risks of breast, endometrial, and ovarian cancers.[17] This risk profile underscores the importance of exploring safer alternatives, including phytotherapy, with turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) being a prominent candidate.[18]

Turmeric is a perennial rhizomatous herb from the Zingiberaceae family, indigenous to South Asia and now cultivated globally in subtropical climates.[19] The dried rhizome is processed into turmeric powder, which has been traditionally utilized across Asia for culinary and medicinal purposes.[20] Among its active constituents is turmerone a sesquiterpenoid compound also known as α-turmerone that exhibits anti-inflammatory, antioxidant, and neuroprotective activities.[21] These properties may contribute to reducing systemic inflammation, mitigating oxidative cellular damage, and enhancing cognitive resilience.[22] Turmerone, particularly concentrated in ethanol-based turmeric extracts, is of growing interest in therapeutic research.

A growing body of evidence supports turmeric's broad pharmacological potential. Kusuma Dewi's study found a significant reduction in body temperature in DPT-vaccinated rats treated with turmeric extract.[23] Similar antipyretic effects were observed in New Zealand white rabbits.[24] Antibacterial assays also reveal turmeric's greater efficacy against Gram-positive bacteria (e.g., *Bacillus* sp., *Staphylococcus aureus*) compared to Gram-negative strains (*e.g.*, *Shigella dysenteriae*, *E. coli*), likely due to differential cell wall structures.[25,26,27] Ethanol is recognized as an efficient solvent in turmeric extraction due to its high polarity, allowing for optimal isolation of active compounds.[28-30] In reproductive health, turmeric has shown the ability to inhibit ovulation and hormonal secretion (FSH, LH, estrogen, progesterone), disrupt the estrous cycle, and induce biochemical changes in uterine fluid – demonstrating reversible antifertility effects in both males and females.[31,32]

Materials and Methods

Tools and materials

In alignment with the experimental framework of this study, which involved quantifying estrogen levels in rat serum following turmeric extract administration, the tools and materials were selected to ensure precise biochemical measurement and controlled laboratory conditions. The tools utilized included a Bio-Rad xMark™ Microplate Absorbance Reader integrated with Microplate Manager software for accurate absorbance detection during ELISA analysis, a 27.3°C incubator to maintain stable environmental conditions during sample incubation, absorbent paper, precision micropipettes for accurate liquid handling, and sterile disposable pipette tips to prevent cross-contamination. The primary materials consisted of components from the Bioassay Technology Laboratory ELISA Kit specifically designed for estrogen quantification, including a standard estrogen solution, an ELISA plate pre-coated with specific antibodies, a standard diluent, Streptavidin-HRP conjugate, substrate solutions A and B, stop solution, wash buffer, and adhesive plate sealers. Supporting biological materials included blood serum obtained from female Sprague-Dawley rats and turmeric ethanol extract (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) prepared using 96% ethanol, with all tools and materials selected to ensure methodological consistency and accuracy in detecting potential phytoestrogenic effects of turmeric.

Preparation of turmeric extract

Turmeric rhizomes were obtained from the Riau region and processed into powder form. The powder was refined through a 60-mesh sieve to achieve a uniform particle size, ensuring extract consistency. Extraction was performed using the maceration technique, whereby the powder was soaked in 96% ethanol to allow the diffusion of bioactive compounds. The mixture was then filtered, and the filtrate was concentrated using appropriate methods before being stored under controlled conditions until use in the treatment phase.

Phytochemical screening

To determine the chemical profile of the turmeric extract, qualitative phytochemical screening was conducted to identify secondary metabolites with potential pharmacological activity. Alkaloids were detected using Dragendorff's reagent, producing an orange precipitate in positive samples. Flavonoids were identified via the Shinoda test, where the addition of magnesium powder and hydrochloric acid resulted in a red or pink coloration. Tannins were tested using ferric chloride ($FeCl_3$) solution, which produced a blue-black or greenish color in the presence of tannins. Saponins were assessed through a froth test, in which persistent foam formation indicated a positive result. Triterpenoids were identified using the Liebermann–Burchard reaction, showing a reddish-brown color change, while steroids were confirmed through acetic anhydride–sulfuric acid testing, yielding a blue or green coloration. These procedures provided a qualitative assessment of the key bioactive compounds in turmeric, many of which are associated with antioxidant, anti-inflammatory, and hormonal regulatory activities.

ELISA preparation

Before beginning the ELISA procedure, all reagents were allowed to reach room temperature to ensure optimal reaction performance. A standard curve was generated by serially diluting the estrogen standard solution into six concentrations: 2400, 1200, 600, 300, 150, and 75 ng/L. For each well, 50 µL of either standard solution or serum sample was pipetted, followed by the addition of assay-specific reagents as per the manufacturer's protocol. All standards and samples were analyzed in duplicate to enhance measurement reliability and reduce experimental variability. The wash buffer, provided in concentrated form, was reconstituted according

to the manufacturer's instructions. Each assay plate underwent five wash cycles, with 0.35 mL of diluted wash buffer per well, to minimize background interference and improve assay precision.

Experimental design and procedure

This study was conducted based on the principles of true experimental design, which emphasizes random allocation, controlled interventions, and the use of comparison groups to establish causal relationships.[14] The dose–response concept guided the selection of turmeric extract concentrations, allowing evaluation of its potential phytoestrogenic effects across a range of doses.[15] Fifteen healthy female Sprague-Dawley rats were randomly assigned to five treatment groups (n=3 per group). Group 1 received no treatment (negative control), Group 2 was administered 1.8 mg/kg Body Weight (BW) of a standard contraceptive pill as a positive control, and Groups 3, 4, and 5 were given turmeric ethanol extract at doses of 25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW, respectively. All treatments were delivered orally in a volume of 1 mL daily for 30 consecutive days. On the 31st day, surgical procedures were performed to collect blood samples, and the serum was separated for estrogen hormone analysis.

ELISA procedure and observation

Estrogen levels in serum were quantified using ELISA, with all standards and samples run in duplicates to ensure reliability. Each well received 50µL of either standard or rat serum, followed by 50 µL of Streptavidin-HRP. After a 60-minute incubation at 27.3 °C, wells were washed five times with 0.35 mL of wash buffer. Subsequently, 50 µL each of Substrate A and Substrate B were added, incubated for 10 minutes in the dark, and the reaction was stopped with 50 µL of stop solution. Absorbance was measured at 450 nm, and estrogen concentrations were determined from the standard curve.

Results

Phytochemical screening of turmeric ethanol extract

Phytochemical screening was conducted to identify the presence of various secondary metabolites in the ethanol extract of turmeric (*Curcuma longa Linn. syn. Curcuma domestica Val.*). The qualitative analysis focused on six major compound groups, namely alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids. The results are summarized in Table 1. From Table 1, it is evident that the turmeric ethanol extract contains alkaloids, flavonoids, tannins, saponins, and triterpenoids, while steroids were not detected.

Standard curve of estrogen concentration vs optical density

To quantify estrogen hormone levels, a standard curve was generated using seven known concentrations of estrogen standard solutions. The Optical Density (OD) readings corresponding to each concentration were recorded to develop a calibration curve, as shown in Table 2. Based on Table 2, the standard curve exhibited a strong linear relationship with a correlation coefficient (r) of 0.998. This calibration was used to calculate estrogen concentrations in rat serum samples based on their OD readings.

Estrogen hormone levels in rat serum after treatment

The ELISA test was conducted to determine the estrogen hormone levels in the serum of female Sprague-Dawley rats after 30 days of treatment. Rats were divided into five groups: negative control (K-), positive control (K+), and three treatment groups receiving turmeric ethanol extract at doses of 25 mg/kg BW (P1), 50 mg/kg BW (P2), and 100 mg/kg BW (P3). An additional comparison group included female rats mated with males and given turmeric (BK). The results of the ELISA test are presented in Table 3 and visualized in Figure 1. From Table 3 and Figure 1, it can be observed that estrogen levels increased in rats treated with turmeric ethanol extract at 25 mg/kg BW (P1) and 50 mg/kg BW (P2), reaching 691 ng/L and 709 ng/L, respectively both higher than the control groups. However, the group receiving the highest dose (100 mg/kg BW, P3) exhibited a lower estrogen level (617 ng/L), suggesting a possible biphasic dose response. The positive control group (K+) and the BK group both showed similar estrogen levels (669 ng/L), while the untreated control group (K-) had the lowest baseline value at 647 ng/L.

Discussion

The results of this study indicate that the ethanol extract of turmeric (*Curcuma longa* Linn. syn. *Curcuma domestica* Val.) contains various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and triterpenoids, but not steroids. These active compounds may contribute to the observed increase in estrogen hormone levels in the tested female rats, as suggested by previous literature reporting that flavonoids can exhibit estrogenic activity by binding to estrogen receptors,[36] while certain alkaloids have been shown to influence endocrine function.[38] This is supported by the ELISA test results, which showed that treatment groups receiving turmeric ethanol extract at doses of 25 mg/kg BW and 50 mg/kg BW had higher average estrogen levels

Table 1. Phytochemical screening results of turmeric ethanol extract.

No	Compound group	Test criteria	Test result	Interpretation
1	Alkaloid	Formation of white precipitate with Mayer's reagent	White precipitate observed	Positive
2	Flavonoid	Color change to orange, pink, or red	Orange color turned red	Positive
3	Tannin	Color change to blackish-green or dark blue	Orange color turned blackish-green	Positive
4	Saponins	Persistent foam within 5 minutes	Stable foam formation observed	Positive
5	Triterpenoid	Formation of a brownish or violet ring at phase interface	Brownish ring observed at the border of the solution	Positive
6	Steroid	Formation of greenish-blue ring	No greenish-blue ring observed	Negative

(691 ng/L and 709 ng/L, respectively) compared to the negative control group (647 ng/L). However, at the highest dose (100 mg/kg BW), estrogen levels decreased to 617 ng/L, suggesting a possible toxic or inhibitory effect at this concentration.

According to Suprihatin, flavonoids and alkaloids in plants can act as abortifacients, potentially causing miscarriage or disturbances in reproductive processes.[33] This is further supported by findings from Ningsih,[34] stating that flavonoid and alkaloid compounds can disrupt cell membranes, alter membrane components, and inhibit cell division by impairing the formation of membranes responsible for nutrient transport and cellular energy metabolism. In reproductive terms, the permeability of egg and embryo cell membranes is crucial for embryonic growth and development (cleavage).[35]

The impact of alkaloids on the membranes of egg and embryo cells results in membrane shrinkage, leading to decreased membrane integrity, which impairs the development of the egg and embryo cells, potentially resulting in embryonic death. Moreover, saponins, which are characterized by their foaming properties, also negatively impact animal reproduction. They are known to act as abortifacients, inhibit zygote formation, and prevent implantation. Saponins exhibit cytotoxic effects, particularly on developing cells such as those undergoing oogenesis.[36]

This study also aligns with Ockene, who states that disturbances during pregnancy can be caused by both internal and external factors. Internal factors include chromosomal abnormalities, while external factors may involve exposure to viruses, radiation, malnutrition, and chemical substances such as alkaloids, steroids, and alcohol.[16] Therefore, the active compounds in turmeric may exert reproductive effects through both hormonal and cellular pathways. Interestingly, although the treatment groups receiving turmeric extract showed increased estrogen levels compared to the negative control, these levels remained lower than those in the positive control group receiving contraceptive pills (average 669 ng/L). This suggests that while turmeric extract may act as a natural phytoestrogen, its potency is still lower than that of synthetic estrogen found in oral contraceptives. Overall, these data suggest that turmeric ethanol extract has the potential to enhance estrogen hormone levels in animal models, particularly at moderate doses (25-50 mg/kgBW). The decline in estrogen levels at the highest dose (100 mg/kgBW) may indicate toxicity or a physiological compensatory response to excessive exposure to active compounds. Therefore, based on these findings, the researchers recommend further studies using higher dose variations and long-term observations to determine the optimal effect and potential toxicity of turmeric ethanol extract.[33]

Conclusions

The administration of 96% ethanol extract of turmeric (*Curcuma longa Linn.* syn. *Curcuma domestica Val.*) was found to effectively increase estrogen hormone levels, with the highest elevation observed at a dose of 50 mg/kg body weight, surpassing even the levels found in the positive control group that received birth control pills. This suggests that turmeric extract at moderate doses may have phytoestrogenic activity capable of enhancing endogenous estrogen production. However, at a higher dose of 100 mg/kg body weight, a significant decline in estrogen levels was observed, falling below both the positive control and the untreated negative control group. This indicates that excessively high doses may exert an inhibitory or potentially toxic effect on hormone regulation. Therefore, it can be concluded that while turmeric extract

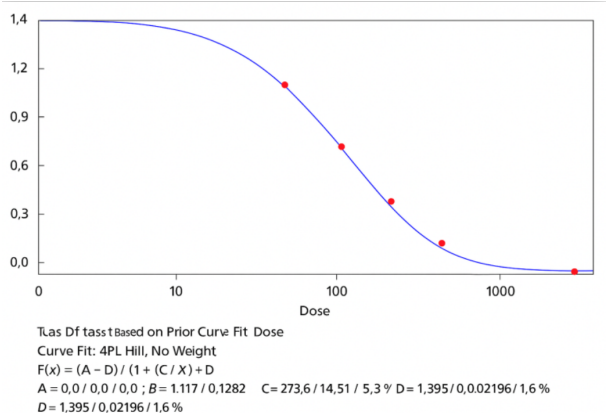

Figure 1. Estrogen hormone levels based on ELISA test in rat serum.

Table 2. Standard estrogen concentration *vs* optical density.

No.	Estrogen Level (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

Table 3. Estrogen hormone levels in female rats after treatment.

Group	Description	Mean Estrogen Level (ng/L)
BK	Female rats mated with males and given turmeric	669
K+	Positive control (contraceptive pill 1.8 mg/kg BW)	669
K-	Negative control (no treatment)	647
P1	Turmeric extract 25 mg/kg BW	691
P2	Turmeric extract 50 mg/kg BW	709
P3	Turmeric extract 100 mg/kg BW	617

has the potential to increase estrogen levels, its effectiveness is dose-dependent, with 50 mg/kg body weight being the most optimal dose identified in this study.

References

1. Alnahdi AS, Idrees M. Nonlinear dynamics of estrogen receptor-positive breast cancer integrating experimental data: A novel spatial modeling approach. Math Biosci Eng 2023;20:21163-85.
2. Herniyatun H, Andriani G. Perbedaan kualitas seksual wanita dengan kontrasepsi hormonal dan non hormonal di Desa Kamulyan Kecamatan Tambak. Lentera J Ilm 2021. Available from: https://www.academia.edu/download/113511655/786.pdf
3. Moisand A, Madéry M, Boyer T, et al. Hormone receptor signaling and breast cancer resistance to anti-tumor immunity. Int Immunol 2023;35:473-88.
4. Pu H, Wen X, Luo DX, Guo Z. Regulation of progesterone receptor expression in endometriosis, endometrial cancer, and breast cancer by estrogen, polymorphisms, transcription factors, and epigenetic mechanisms. J Steroid Biochem Mol Biol 2023;229:106215.
5. Irizarry VCT, Jiang Y, He Y, Xu P. Hypothalamic estrogen signaling and adipose tissue metabolism in energy homeostasis. Front Endocrinol (Lausanne) 2022;13:898139.
6. Hornung RS, Benton WL, Tongkhuya S, et al. Progesterone and allopregnanolone rapidly attenuate estrogen-associated mechanical allodynia in rats with persistent temporomandibular joint inflammation. Front Integr Neurosci 2020;14:26.
7. Setiawan R, Iryanti I, Muryati M. Efektivitas media edukasi audio-visual dan booklet terhadap pengetahuan premenopause, efikasi diri dan stres pada wanita premenopause di Kota Bandung. Perilaku Promosi Kesehatan 2020;2:1-12.
8. Casarini L, Lazzaretti C, Paradiso E, et al. Membrane estrogen receptor (GPER) and follicle-stimulating hormone receptor (FSHR) heteromeric complexes promote human ovarian follicle survival. iScience 2020;23:101812.
9. Cetinkaya A, Kilinc E, Camsari C, Ogun MN. Effects of estrogen and progesterone on the neurogenic inflammatory neuropeptides: implications for gender differences in migraine. Exp Brain Res 2020;238:1679-89.
10. Mohanty SS, Sahoo CR, Padhy RN. Role of hormone receptors and HER2 as prospective molecular markers for breast cancer: an update. Genes Dis 2022;9:404-21.
11. Azizi-Lalabadi M, Pirsaheb M. Investigation of steroid hormone residues in fish: a systematic review. Process Saf Environ Prot 2021;147:1172-81.
12. Lan KC, Lai YJ, Cheng HH, et al. Levels of sex steroid hormones and their receptors in women with preeclampsia. Reprod Biol Endocrinol 2020;18:97.
13. Pagano MT, Ortona E, Dupuis ML. A role for estrogen receptor alpha36 in cancer progression. Front Endocrinol (Lausanne) 2020;11:506.
14. Wang C, Tran DA, Fu MZ, et al. Estrogen receptor, progesterone receptor, and HER2 receptor markers in endometrial cancer. J Pathol Transl Med 2020;54:29-34.
15. Welton AJ, Vickers MR, Kim J, et al. Health related quality of life after combined hormone replacement therapy: randomised controlled trial. BMJ 2008;337:a1190.
16. Ockene JK, Barad DH, Cochrane BB, et al. Symptom experience after estrogen plus progestin in the Women's Health Initiative randomized controlled trial. JAMA 2005;294:183-93.
17. Paszkowski T, Bińkowska M, Dębski R, et al. Menopausal hormone therapy in questions and answers: a manual for physicians of various specialties. Prz Menopauzalny 2019;18:1-8.
18. Kim JW, Kim HA, Suh CH, Jung JY. Sex hormones affect the pathogenesis and clinical characteristics of systemic lupus erythematosus. Front Med (Lausanne) 2022;9:906475.
19. Santander B. Formularium ramuan obat tradisional Indonesia. J Ilmu Farmasi Indones 2017;15:89-96.
20. Departemen Kesehatan Republik Indonesia. Kebijakan obat tradisional nasional. Jakarta: Depkes RI; 2021.
21. Hirschberg AL, Bitzer J, Cano A, et al. Topical estrogens and non-hormonal preparations for postmenopausal vulvovaginal atrophy: an EMAS clinical guide. Maturitas 2021;143:91-102.
22. Goozee KG, Shah TM, Sohrabi HR, et al. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. Br J Nutr 2022;115:449-65.
23. Kusuma DM. Aktivitas antibakteri ekstrak daun Majapahit (Crescentia cujete) terhadap pertumbuhan bakteri Ralstonia solanacearum penyebab penyakit layu. LenteraBio 2024;3:15-21.
24. Kusumaningrum YI. Hubungan antara pengetahuan ibu dan faktor-faktor sosial ekonomi orang tua dengan praktik pemberian MP-ASI pada bayi usia 6-12 bulan di Desa Kemuning Kecamatan Ampelgading Kabupaten Pemalang [dissertation]. Semarang: Universitas Negeri Semarang; 2021.
25. Yuliati Y. Uji efektivitas ekstrak kunyit sebagai antibakteri dalam pertumbuhan Bacillus sp dan Shigella dysenteriae secara in vitro. J Profesi Medika 2022;10:26-32.
26. Wijayanto W. Uji aktivitas antibakteri ekstrak etanol rimpang kunyit putih (Curcuma mangga Val.) terhadap Staphylococcus aureus ATCC 6538 dan Escherichia coli ATCC 11229 secara in vitro [undergraduate thesis]. Surakarta: Fakultas Kedokteran, Universitas Muhammadiyah Surakarta; 2021.
27. Hidayati E, Juli NM. Isolasi Enterobacteriaceae patogen dari makanan berbumbu dan tidak berbumbu kunyit (Curcuma domestica Val.) serta uji pengaruh ekstrak kunyit terhadap pertumbuhan bakteri yang diisolasi. J Matematika dan Sains 2022;7:43-52.
28. Nair A, Amalraj A, Jacob J, Kunnumakkara AB, Gopi S. Non-curcuminoids from turmeric and their potential in cancer therapy and anticancer drug delivery formulations. Biomolecules 2021;11:9.
29. Suharsanti R, Astutiningsih C, Susilowati ND. Kadar kurkumin ekstrak rimpang kunyit (Curcuma domestica) secara KLT densitometri dengan perbedaan metode ekstraksi. J Wiyata 2020;7:86-93.
30. Sari IP, Nurrochmad A, Setiawan IM, et al. Effects of Costus speciosus ethanolic extract on male rats: The action mechanism and the ability to impregnate. Pak J Pharm Sci 2022;31:997-1001.
31. Ling W, Florenly F, Liena L, et al. Effectiveness of turmeric ethanol extract cream preparation (Curcuma longa) in speeding up wound healing in male Wistar rats. Int Res J Pharm Appl Sci 2022;12:55-62.
32. Nasution EZ, Harahap FM. Formulation and production of ethanol extract derived from black turmeric (Curcuma caesia Roxb) for use as an antibacterial hand sanitizer spray. J Chem Nat Resour 2024;6:80-7.
33. Suprihatin T, Rahayu S, Rifa'i M, et al. Senyawa pada serbuk rimpang kunyit (Curcuma longa L.) yang berpotensi sebagai

antioksidan. Bul Anat Fisiol 2020;5:101-8.
34. Ningsih AW, Nurrosyidah IH. Pengaruh perbedaan metode ekstraksi rimpang kunyit (Curcuma domestica) terhadap rendemen dan skrining fitokimia. J Pharm Med Sci 2020;2:22-9.
35. Fadhilah H, Rachmani K, Hajaring N. Aktivitas kunyit (Curcuma domestica Val.) sebagai antiinflamasi ditinjau dari berbagai literatur. Edu Masda J 2021;2:55-62.
36. Titisari N, Firmawati A, Fauzi A, et al. Reproductive cycle of female Javan langur (Trachypithecus auratus) based on estrogen and luteinizing hormone levels. CAB Rev 2021;16:1-12.