International Journal of Science and Technology Research Archive

ISSN: 0799-6632 (Online)

Journal homepage: https://sciresjournals.com/ijstra/

(RESEARCH ARTICLE)

Check for updates

The effect of local white turmeric (*Curcuma Zedoaria*) on the uterus surface profile of white rats (*Rattus novergicus*) with ethinyl estradiol induction

Lina Sundayani [1,*], Lalu Srigede [2], Agrijanti [2] and Rita Sopiatun [1]

[1] *Department of Midwifery, Poltekkes Kemenkes Mataram, Jln. Prabu Rangkasari Dasan Cermen, Mataram, Indonesia.*
[2] *Department of Health Analyst, Poltekkes Kemenkes Mataram, Jln. Prabu Rangkasari Dasan Cermen, Mataram, Indonesia.*

International Journal of Science and Technology Research Archive, 2022, 03(02), 189–195

Publication history: Received on 12 October 2022; revised on 20 November 2022; accepted on 22 November 2022

Article DOI: https://doi.org/10.53771/ijstra.2022.3.2.0134

Abstract

Background: White turmeric rhizome (*Curcuma Zedoaria*) contains important compounds for traditional medicine and the pharmaceutical industry. The variation in the benefits of white turmeric is thought to be due to the compounds in the rhizome, significantly compounds with antioxidant activity.

Purpose: Determine the effect of local varieties of white turmeric in Sambori Bima Village, West Nusa Tenggara, Indonesia, on the uterine surface profile of white rats (Rattus Novergicus) induced by Ethinyl Estradiol.

Methods: This research uses 25 white rats divided into five treatment groups, namely control (-) not given Ethinyl estradiol or white turmeric extract, control (+) is given Ethinyl estradiol at a dose of 150mg/kg BW (Body Weight)/day without white turmeric extract. P1, P2, and P3 treatment were given Ethinyl estradiol at a dose of 150mg/kg BW/day and white turmeric extracts with diffferent concentration. P1 was given white turmeric extract 500mg/Kg BW/day, P2 was given white turmeric extract 750mg/Kg BW/day, P3 was given white turmeric extract 1000mg/kg BW/day. White turmeric extract was examined using GCMS, and uterine organs were taken for histopathological preparations.

Results: Methanol extract of white turmeric contains compounds including [2.2.1] heptan-2-ol, exo-β-element, sesquiterpene, Gremacrene, Epiglobulol, Spathulenol, Eudesmol and, Germacrone. Histopathological analysis shows the different distances between glands in the endometrial. Hyperplasia of the gland occurred on all treatments except for P3 (1000 mg/kg BW).

Conclusion: Administration of white turmeric extract with eight beneficial health compounds could stop the artificial proliferation of Ethinyl estradiol for 14 days plus 30 days.

Keywords: White turmeric; Uterus; White rats; Ethinyl estradiol

1 Introduction

Indonesia is a country that is rich in diversity and is known to have a diversity of ethnic/ethnic species with knowledge of traditional medicine used by some people from generation to generation and until now, which has been proven scientifically. Scientific knowledge has medicinal properties (1) (2).

Knowledge of medicine in West Nusa Tenggara has always been a cultural heritage of local wisdom. In the indigenous people of the Sambori tribe, Sambori traditional village, Lambitu Bima district, West Nusa Tenggara, white turmeric

* Corresponding author: Lina Sundayani
Department of Midwifery, Poltekkes Kemenkes Mataram, Jln. Prabu Rangkasari Dasan Cermen, Mataram, Indonesia.

(*Curcuma Zedoaria* Rosc) grows naturally. It is used as a raw material for traditional medicinal ingredients for tumor treatment. White turmeric has been cultivated, and the rhizome is processed into white turmeric powder as herbal medicine (3) (4).

White turmeric rhizome (*Curcuma Zedoaria*) contains important compounds for traditional medicine and the pharmaceutical industry. The variation in the benefits of white turmeric is thought to be due to the compounds in the rhizome, especially compounds that are antioxidants. Which can inhibit reactive oxygen species/reactive nitrogen species and free radicals to prevent diseases associated with free radicals, such as carcinogenesis, cardiovascular, and aging (5). Cervical cancer is one of the most common cancers suffered by women worldwide, especially in developing countries, including Indonesia. The number of sufferers reached 569,847 cases in 2018 (6). Hormonal imbalance is also associated with uterine cancer, which is why hyperplasia is considered a pre-cancerous condition.

Curcuma zedoria, which, empirically in Indonesia and the Sambori Bima Tribe, in particular, has been used as a traditional medicine for medicine or herbal medicine in case of uterine bleeding, is local wisdom that is recommended to be investigated further as an anti-cancer ingredient.

Of the number of patients, as many as 70 percent of cases come at an advanced stage (IIB-IVA) which can only be treated with radiation. However, only a third of the cancer is gone. Therefore, to increase the success of treatment, it is necessary to add drugs. One of the herbal medicines that have been tested on cell cultures is curcumin. Curcumin is a rhizome extract, one of which comes from turmeric, which is widely grown in Indonesia. This research was presented by a specialist in obstetrics and gynecology, Faculty of Medicine, University of Indonesia, Dr.dr. Sigit Purbadi, SpOG(K), in his dissertation entitled Efficacy of Adding Biocurcumin (BCM-95) in the therapy of cervical cancer type IIIB squamous cell carcinoma: Phase Iib Clinical Trial.

The uterus exhibits both structural and functional cyclic changes in response to the ovarian hormones estrogen and progesterone. These changes prepare the uterus for implantation as well as a feeding ground for the embryo and fetus. If implantation does not occur, the blood vessels in the endometrium will weaken. Disorders of the uterus will affect embryo implantation so that it affects (7).

Administration of vitamin E can improve the histopathological structure of the uterus of white rats (*Rattus novergicus*) due to the side effects of Ethinyl estradiol, a synthetic form of estrogen. The results showed that administering vitamin E supplementation at a dose of 150 mg/kg BW/day could suppress the proliferation and necrosis of uterine endometrial epithelial cells in white rats (*Rattus norvegicus*). Thus, vitamin E could be an antioxidant in patients taking Ethinyl estradiol (8).

In this study, estrogen was given to female white rats to trigger proliferation in the uterus of white rats. Then a white meeting will be given to see if white turmeric can suppress the proliferation and necrosis. In addition, a test for the content of bioactive substances contained in the local variety of white meetings in the Sambori Bima area of NTB will also be tested using gas chromatography.

This study aimed to determine the effect of giving white turmeric (*Curcuma Zedoaria*) local variety in Sambori Village, Bima, NTB to the Uterus Surface Profile of White Rats (*Rattus novergicus*) induced by Ethinyl Estradiol.

2 Methods

2.1 Population and Sample

This research was experimental research which used a cross-sectional method. White turmeric extract preparation and analysis were conducted at the Faculty of Mathematics and Natural Sciences Laboratory, Mataram University, Indonesia. The treatment of testing animals and histological analysis were conducted at the Clinical Pathology Laboratory of the Islamic Hospital Mataram, Indonesia.

The testing animal used in this study was a female white rat (*Rattus Novergicus*) aged 2-4 months and weight 150-200 grams. The number of samples used in this study was calculated based on the Federer formula, so the total number of rats used was 25. The specimens used are uterine organs from experimental animals that have been given treatment.

2.2 Material and Equipment

The materials in this study are female Wistar white rats aged 2.5 months, weighing 150-200gram, from LPPT UGM Unit IV. Ingredients for extract: *C. zandoria*, aquadest, filter paper, aquadest,. The ingredients for preparing vaginal swabs are physiological NaCl solution, methanol, and Giemsa dye. Ingredients for making uterine histological preparations: uterus, formalin buffer solution (formalin 40% 100 ml, Sodium phosphate monobasic 4 g, Sodium phosphate dibasic 6.5 g, distilled water), absolute methanol, paraffin 59-60^{o}C, albumin, Albumin-Meyer adhesive reagent (egg albumin, glycerin, thymol), xylene, alcohol solutions of various concentrations (70%, 80%, 96%, and absolute alcohol), Canada balsam, Hematoxylin Erlich dye, 0.5% eosin solution in 70% ethanol. The equipment in this study is a Nikon Eclipse 50i type binocular microscope equipped with a USB sight digital camera type DS-U2. Testing animal equipment, including Animal cages, animal scales, and gastric probes. Tools for observing estrus: cotton bud, object glass, light microscope. Tools used to make uterine histological preparations: glassware, surgical equipment, oven, microtome, staining jar, hot plate, spirit lamp, spatula, brush, dropper, filter paper, cotton swab, tissue, slide, and coverslip (7).

2.3 Preparation of methanol extract

The section was made from 200 grams of material heated with 500 ml of water at 100^{o}C for one hour, calculated after the water bath began to boil while stirring evenly. The material is cooled and filtered using a Buchner funnel and a vacuum pump. The filtrate was rinsed with hot water (500 ml) several times. The filtrate is concentrated with a rotary evaporator so that the volume becomes 100 ml. Furthermore, the water extract was dried with a freeze-dryer (5).

2.4 Treatment of testing animals

The test animals were kept in individual cages made of plastic with a roof in the form of wire rams. Feed and drink for the test animals were provided ad libitum. The test animal feed was in the form of BR2 pellets, while the drinking water was PAM water. Test animals are maintained in a laboratory under controlled and constant conditions. This study used 25 female white rats, which were divided into five treatment groups, namely control (-) (KN) not given Ethinyl estradiol or *C. zandoria* (-). Positive Control (KP) was given Ethinyl estradiol at 150 mg/kg BW/day without C. zandoria, P1, P2 P3 was given *C. zandoria* I, II, and III were given aqueous extract at a dose of 500 mg/kg BW, 750 mg/kg BW, and 1000 mg/kg BW orally with a frequency of 2x a week for eight weeks (9). Testing animals were ovariectomized for 20 days using a gastric probe. Ethinyl estradiol was given at 150 mg/kg BW/day. The rats were ovariectomized for 20 days using a gastric probe. Treatment was for 30 days, and on the thirty-one day, all experimental animals were necropsied. Uterine organs were taken for histopathological preparations using the hematoxylin-eosin (HE) staining technique. The variables examined were necrosis and the proliferation of uterine epithelial cells (10).

2.5 Data analysis

Local white turmeric compounds were analyzed with Gas Chromatography-Mass Spectrometry (GCMS). Histological preparations were made in series, then observed under a microscope and analyzed quantitatively by comparing the histological structure between treatments and analyzing the effect of administration of white turmeric (*C. zedoaria*) local variety in Sambori village, Bima, NTB on the uterine surface profile of *R. Novergicus* induced by Ethinyl estradiol.

3 Results and discussion

White turmeric (*C. zedoaria*) rhizome was extracted using methanol because readings using Gas Chromatography mass spectrometry (GCMS) required a compound that was easily vaporized (<35^{o}C) as a solvent (11). The identification of methanol extract of *C. zedoaria* using GCMS has detected a total of 30 shown in table 1. However, only eight compounds have potential health-beneficial effects. That compounds are Bicyclo[2.2.1]heptan-2-ol (peak 1), beta.-Elemente (peak 2), Germacrene-D (peak 3), Curzerene (peak 4), Epiglobulol (peak 5), Spathulenol (peak 7), beta-eudesmol (peak 8), Germacrone (peak 10).

Eight compounds observed from local white turmeric extract in this study have various health benefits. [2.2.1] heptan-2-ol has a fresh characteristic and *camphor* and a spicy taste with anticonvulsant, anti-cancer, antitumor, antibacterial, and antifungal. Beta Element is the essential oil of Curcuma which has anti-cancer properties. Germacrene is a candidate anti-lung adenocarcinoma drug. Curzerene can cure DMBA-induced pre-cancerous mouse model, suggesting it can cure breast cancer (12). Epiglobulol is an antibacterial, especially for bacteria that cause gastrointestinal infections (13). Spathulenol is a tricyclic alcohol sesquiterpene that can inhibit proliferation in lymphocytes, inducing cell apoptosis via a caspase-3-independent pathway of inhibitory effects (14). beta-eudesmol inhibits tumor growth effect (15). Germacrone inhibits tumors and cancer by blocking the estrogen receptor mechanism (16).

Table 1 Results of GC-MS analysis of the chemical components of the active fraction extract *Curcuma Zedoaria*

Peak	Time Area	Area%	Name
1	8,685	7832793	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-,
2	9.91	17765303	(-)-.beta.-Elemente
3	10.335	14932481	GERMACRENE-D
4	10.42	36008374	Curzerene
5	10.81	8748600	EPIGLOBULOL
6	10. 845	14929489	RT:10.825
7	10.975	23065833	SPATHULENOL
8	11.095	29780496	.beta.-Eudesmol
9	11.215	30474258	
10	11.255	40494559	Germacrone
11	11.315	17299193	RT:11.280
12	11.34	8525652	
13	11.415	+	RT:11.36086: 1491 RT
15	11.4505	1.45E	
:	1491	11.3605	:11,470
16	11.55	55314129	
17	11,595	1.68E+08	RT:11,585
18	11.63	80833526	RT:11,605
19	11,745	94075886	RT:11,725
20	11,815	58221097	RT:11,800
21	11,865	33195084	RT:11,830
22	11,905	15155530	RT:11,885
23	11.97	16907989	, RT:197 16907989, RT:1 JHFC
24	12.08	T20368943	
25	12.295	10063281	RT:12.270
26	12.34	11624809	RT:12.325
27	12.385	26768389	RT:12.365
28	12.485	13742617	RT:12.465
29	12.705	62213481	9-Octadecenal, (Z)- (CAS) CIS-OCTADEC-9-OCTADEC-9-
OCTADEC	14.07	7989095	RT:14.035

Each treatment has resulted in the vary of uterus surface profile. Negative control treatment (without the administration of Ethinyl estradiol extract *C. zedoaria* in white mice (*R. novergicus*, the observation results show that the endometrium with proliferative glands is lined with monotonous rounded epithelium. Histopathological observations of KP (Positive Control) uterus white rats (*R. novergicus*) given Ethinyl Estradiol 150 mg/KG BW without *C. zedoaria* consisting of 5 replication is visible endometrium with mild hyperplasia with The glandular hyperplasia is quite densely lined with monotonous rounded epithelium, visible foci of atypia cells, the distance between the glands is close. Uterine histopathological results of white rats (*R. novergicus*) P1 (Treatment 1) given *Ethinyl estradiol* 150mg/ KG B and extract of *C. zedoaria* 500mg/kg BW 5 replications, Endometrial appearance with mild hyperplasia of glands

with densely packed glands lined with monotonous rounded epithelium, fairly close distance between glands. Histopathological results of rat uterus (*R. novergicus*) P2 (Treatment 2) administration *Ethinyl Estradiol* 150mg/KG Band *C. zedoaria* 750 mg/kg BW 5 replications is visible endometrium with proliferative glands partially mild hyperplasia lined with epithelial cells monotonous circle the distance between the glands is quite close. Histopathological results of the uterus of white rats (*R. Novergicus*) P3 (Treatment 3) given Ethinyl estradiol 150mg /KG B and extract of C. zedoaria 1000 mg/kg BW 5 replications Endometrial with proliferative glands lined with monotonous round epithelium, the distance between the glands is quite far, no hyperplasia appears. From the readings of the preparations, the treatment of giving *Curcuma Zedoaria* 1000 mg/kg BW 1 time a day for 30 days can stop the artificial proliferation of Ethinyl estradiol for 14 days plus 30 days.

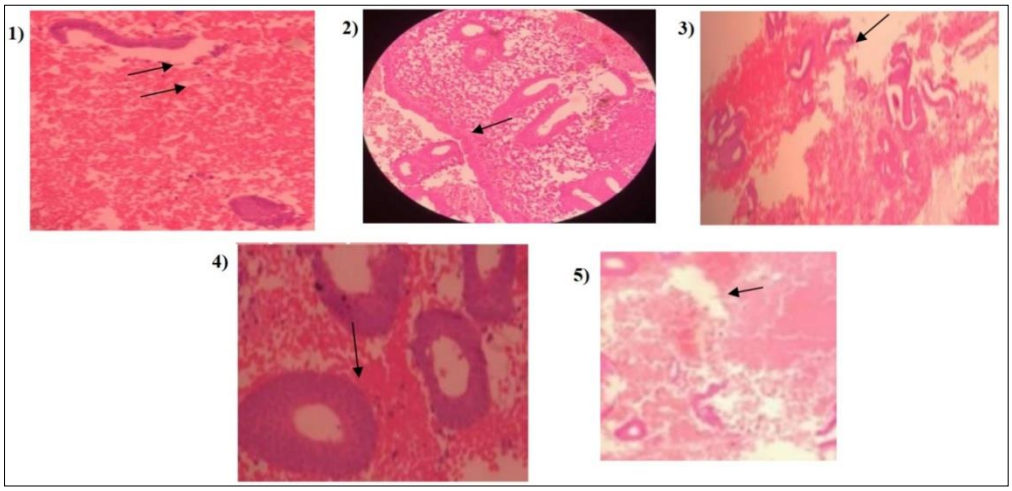

Figure 1 Histopathological Results of White Rats Uterus Surface Profile. 1) Negative control, 2) Positive Control, 3) P1 (given Ethinyl Estradiol 150mg/KG B and *Curcuma Zedoaria* extract 500mg/kg BW), 4) P2 (given Ethinyl Estradiol 150mg/KG B and *Curcuma Zedoaria* 750 mg/kg BW, 5) P3 (given Ethinyl estradiol 150mg/KG B and Curcua zedoaria extract 1000 mg/kg BW)

Ether extract of *C. Zedoaria* has the effect on the proliferation of the triple-negative human breast cancer cell line MDA-MB-231 and Epirubicin used as a control in this study resulted in MDA-MB-231 cells being inhibited by the extract ether *C. zedoaria* (17). Meanwhile, white turmeric (*C. zedoaria*) has an anti-cancer mechanism by inducing apoptosis of cancer cells without damaging other surrounding cells and inhibiting cancer cell proliferation (18). *C. zedoaria* contains chemicals such as curcuminoids, RIP (Ribosome Inacting Protein), isocurcumenol, demothxycurcumin, bisdemethoxycurcumin, epicurzerenone, curdione, and ethyl p-methoxycinnamate which function to inactivate cancer cell development and inhibit cancer cell growth. In addition, white turmeric also contains chemicals such as curzerenone, zedoaron, essential oils, diferuloylmethane, flavonoids, curcumin, trimetoxyflavones, tetramethoxyflavone, tetrahydrodemethoxycurcumin, dihydro curcumin, and polyphenols as pharmacologically beneficial (19).

Other studies have isolated, identified, and characterized antitumor principles from the rhizomes of *C. zedoaria* to assess cytotoxic effects on human and murine cancer cells. It is essential to determine apoptosis-induced capacity in cancer cells and to evaluate tumor-reducing properties in an in-vivo mouse model. A tumor reduction study at Vivo revealed that a dose of 35.7 mg/kg body weight significantly reduced pericardial tumors in experimental animals (18).

From several research results that have been carried out, it is proven that crude extracts in the form of ethanol and ethanol extracts and even aqueous extracts of *C. Zedoria* can be proven to inhibit artificial proliferation in experimental rats (*R. novergicus*) (11)(19)(20). This simulation was the closest simulation of human uterine proliferation.

Some evidence suggests that this condition is caused by an excess of estrogen. This hormonal imbalance is also associated with uterine cancer, which is why hyperplasia is sometimes considered a pre-cancerous condition (21). Thus, the use of *C. zedoaria* in Indonesia, particularly in the Sambori Bima Tribe, as a traditional medicine in case of uterine bleeding is local wisdom recommended for further research as an anti-cancer ingredient.

4 Conclusion

Local white turmeric extract from Bima has eight compounds with health-beneficial compounds, which could stop the artificial proliferation of Ethinyl estradiol for 14 days plus 30 days at a concentration of 1000 mg/kg BW. These compounds could be used as medication for uterine bleeding and has potential anti-cancer activity.

Compliance with ethical standards

Acknowledgments

The authors would like to acknowledge the LPPM Poltekkes Kemenkes for the fund support of this research.

Disclosure of conflict of interest

All co-authors have seen and agree with the contents of the manuscript and there is no conflict of interest.

Statement of ethical approval

The research was carried out based on ethical clearance from The Politeknik Kesehatan Kemenkes Mataram Health Research Ethics commission with Number:207/UN18.F7/ETIK/2019.

References

[1] Kurniawan E. Use Value of Plant Species as Traditional Medicine by the Tenggerese Community in Probolinggo Village – East Java. J Science and Arts ITS. 2015;4(1):1–4.

[2] Jadid N, Kurniawan E, Himayani CES, Andriyani, Prasetyowati I, Purwani KI, et al. An ethnobotanical study of medicinal plants used by the Tengger tribe in Ngadisari village, Indonesia. PLoS One. 2020;15(7):1–16.

[3] Zulharman, Yaniwiadi B, Batoro J. Ethnobotany of Medicinal and Food Plants for the Sambori Tribe Community, Bima District, West Nusa Tenggara Indonesia. Nat B. 2015;3(2):198–204.

[4] Daughter of MS. White turmeric (Cucurma zedoaria): its chemical substance and the pharmacological benefits. J Major. 2014;3(7):88–93.

[5] Saefudin D, Syarif F, Chairul. Antioxidant potential and antiproliferative activity of white turmeric extract (Curcuma Zedoaria Rosc.) on hela cells. Widyariset. 2014;17(3):381–9.

[6] Ministry of Health. World cancer day 2019. 2019.

[7] Harlita H, Probosari RM, Ariyanto J. Histological Changes in Wistar Rat (Rattus norvegicus) Uterus: Antifertility Activity of Cashew Nut Shell Extract (Anacardium occidentale L.). Bioeducation J Bio Educator. 2017;8(2):1–4.

[8] Weight I.K. Effect of Vitamin E and Ethinyl Estradiol on Uterine Histopathology of White Rat (Rattus norvegicus). Indones Med Veterinus. 2018;7(3):243–51.

[9] Murwanti R, Meiyanto E, Nurrochmad A, Kristina SA. Effect of ethanol extract of Curcuma zedoaria Rosc. on post-initiation stage lung tumor growth in female mice induced by Benzo[a]pyrene. Maj Farm Indonesia. 2004;15(1):7–12.

[10] Sitasiwi AJ. Correlation between Estradiol 17-β Hormone Levels and Mice (Mus musculus l.) Uterine Endometrial Thickness during One Estrus Cycle. Bul Anat and Physiol. 2008;16(2):38–45.

[11] Silalahi M. Curcuma Zedoaria (Christm.) Roscoe (Benefits and Bioactivity). J Pro-Life. 2018;5(1):515–25.

[12] Wang Y, Li J, Guo J, Wang Q, Zhu S, Gao S, et al. Cytotoxic and Antitumor Effects of Curzerene from Curcuma longa. Planta Med. 2017;83(1):23–9.

[13] Singh V, Rana N, Ali M. GC-MS Analysis and Anti-Microbial Activities of Volatile Oil of Nardostachys jatamansi D.C. Rhizomes Obtained from Haridwar Region, Uttrakhand. Pharmacogn J. 2018;10(2):230–4.

[14] do Nasciemento KF, Moreira FMF, Santos JA, Kassuya CAL, Croda JHR, Cardoso CAL, et al. Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of Psidium guineense Sw. and spathulenol. J Ethnopharmacology. 2018;10(210):351–8.

[15] Ma EL, Li YC, Tsuneki H, Kao JF. β-Eudesmol suppresses tumor growth through inhibition of tumor neovascularisation and tumor cell proliferation. J Asian Nat Prod Res. 2008;10(1–2):159–67.

[16] Lim MS, Choung SY, Jeong KW. Germacrone Inhibits Estrogen Receptor α-Mediated Transcription in MCF-7 Breast Cancer Cells Phytother National Lybrary Medicine. Phyther Res. 2016;30(12):2036–43.

[17] Gao X, Li Q, Li H, Zhang H, Su J, Wang B, et al. Extracts from Curcuma Zedoaria Inhibit Proliferation of Human Breast Cancer Cell MDA-MB-231 In Vitro. Evid Based Complement Altern Med. 2014;730678.

[18] Lakshmi S, Padmaja G, Remani P. Antitumour Effects of Isocurcumenol Isolated from Curcuma Zedoaria Rhizomes on Human and Murine Cancer Cells. Int J Med Chem. 2011;2011(253962):1–13.

[19] Muflika SP. White turmeric (Curcuma Zedoaria): its chemical substance and the pharmacological benefits. Med J Lampung Univ. 2014;3(7):88–93.

[20] Sarmoko. Temu Putih (Curcuma Zedoaria Rosc) [Internet]. 2010 [cited 2022 Sep 10]. Available from: http://ccrc.farmasi.ug.ac.id/?page_id=104

[21] Shin Y, Lee Y. Cytotoxic Activity from Curcuma Zedoaria Through Mitochondrial Activation on Ovarian Cancer Cells. Toxicol Res. 2013;29(4):257–61.