Teratogenic Tinospora Cordifolia And Carica Papaya On White Mouse Fetuses (Mus Musculus)

Bagas ardiyantoro [1*], Noor arniwati mat daud[2]
Universitas Duta bangsa Surakarta, [1], Institute of medical science technology[2]
*Correspondence Email : bagas_ardiyantoro@udb.ac.id

ABSTRACT

Tinospora cordifolia and Carica papaya in the production of traditional herbal medicines, specifically in formulations known as tinospora cordifolia and Carica papaya herbal mixtures. Extracts from Tinospora cordifolia stems and Carica papaya contain bioactive flavonoid antioxidants, which can inhibit phosphodiesterase enzymes that hydrolyze cAMP. Elevated cAMP concentrations can impede growth acceleration. This research aims to assess the teratogenic effects on the fetuses of white mice after administering tinospora cordifolia and carica papaya extracts. The study involved 25 Mus musculus divided into five groups. The first group, serving as the negative control, received 0.5% Na CMC, while the second group, serving as the positive control, was injected with intravenous Cyclophosphamide at 50 mg/kg. The other groups were administered various doses: ET Dose 1120, ECP Dose 1120, Dose 1 ETCP 2:1, Dose 2 ETCP 1:2, and Dose 3 ETCP 1:1. The extracts were given during the organogenesis period, and on the 18th day, the mice underwent laparotomy to collect the fetuses. The research findings indicate that tinospora cordifolia and carica papaya extracts can cause teratogenic effects, particularly at Dose 3 ETCP 1:1.

KEYWORDS

Traditional Medicine, Tinospora Cordifolia And Carica Papaya, Teratogenic Test

INTRODUCTION

In modern times, many individuals favor natural treatments because they believe these remedies, when applied properly, tend to have fewer side effects compared to chemical or synthetic treatments. This perception has resulted in the expanded development of traditional medicines.(Mohamad et al., 2019). Traditional medicine is being advanced and refined by prioritizing Indonesia's available resources, including the study, research, and testing of various traditional treatments. The efficacy of traditional medicine as a healthcare measure still requires validation, especially regarding its potential side effects when used by pregnant women. This is particularly important given the high rates of mortality and morbidity during pregnancy, where the impacts of drug use on the fetus often outweigh the short-term and long-term risks to both the mother and child.(Elba et al., 2022).

The stem of Brotowali (Tinospora crispa L) is a climbing plant that is cultivated for medicinal purposes and also grows wild in fields and forests. Brotowali flourishes in tropical climates and is particularly common in Southeast Asia, including Thailand and Indonesia. It is recognized for its potential to treat diabetes mellitus, stimulate appetite, enhance metabolic processes to reduce fever, lower blood sugar levels, and improve respiratory function. The plant contains various compounds such as berberine alkaloid, soft resin, starch, glycosides, pikroretoside, resin, bitter substance pikroretin, tinokrisposide, palmatin, columbine, and flavonoids. Meanwhile, Curcuma domestica Val (Curcuma xanthorrhiza) is composed of compounds like curcumin, demethoxycurcumin, glucose, potassium oxalate, protein, fiber, starch, and essential oil. (Amalia Choironi, 2016). Brotowali (Tinospora crispa L) contains flavonoids and alkaloids that exhibit estrogenic properties. Additionally, the alkaloids in Brotowali are antiproliferative against cancer cells and show toxic properties. Alkaloids with antiproliferative properties can inhibit the oogenesis process in mice, affecting the proliferation phase. Furthermore, alkaloids, aside from antiproliferative effects, also exhibit embryotoxic and teratogenic properties.(Amalia Choironi, 2016; Basu et al., 2020)

A substance is classified as teratogenic if it impacts skeletal development, reproductive traits, morphometric characteristics, and histopathological features. These factors are vital for assessing teratogenic effects, highlighting the need for research on fetal morphometric characteristics. Previous studies have shown that oral administration of Brotowali extract from days 0-16 of gestation tends to reduce the number of corpus luteum, live fetuses, dead fetuses, and embryo reabsorption, without significantly affecting implantation. Carica papaya (papaya) has raised some concerns regarding its potential teratogenic effects, especially when consumed in certain forms during pregnancy. Studies suggest that unripe papaya and the latex present in papaya skin contain compounds like papain and chymopapain that may act as uterotonic agents, potentially leading to uterine contractions. This could pose a risk of miscarriage or preterm labor in pregnant women, especially in the early stages of pregnancy. However, these effects are more prominent when consuming unripe or semi-ripe papaya rather than fully ripe fruit(Santana et al., 2019)

Some animal studies indicate that papaya latex might have an effect on the reproductive system, possibly leading to abnormalities in embryo development or affecting pregnancy outcomes. While the fully ripe papaya is generally considered safe for consumption, caution is recommended regarding the consumption of papaya in unripe or concentrated forms, especially during pregnancy(Kong et al., 2021). Pregnancy is a critical period for both the mother and the embryo concerning environmental conditions. Ensuring the embryo's safety is essential, especially during organogenesis, a phase marked by significant cell polarization, differentiation, mobilization, and organization. Embryos are highly susceptible to teratogenic effects during this time (Alafiatayo et al., 2019).

Research across multiple countries shows that pregnant women often require medications for issues such as muscle pain, gastrointestinal discomfort, flu, infections, and additional supplements like vitamins. Traditional medicine is frequently preferred because it is perceived to have fewer side effects compared to modern medicine. Research by (Illamola et al., 2019) found no significant difference in the teratogenic risks between modern and traditional medicines. Pregnant women in indonesia often use herbal remedies like tinospora cordifolia and carica papaya to manage issues such as increased appetite. it's vital to conduct safety evaluations, including these herbal medicines, for pregnant women. the teratogenicity test is a valuable tool for assessing how substances affect fetal development, identifying those causing a range of effects from lethality to malformations and growth retardation, known as embryotoxicity. substances leading to fetal malformations, termed terata, fall under the category of teratogens or teratogenic substances. (Vargesson, 2019).

Teratogenic impacts of traditional medicines are widely reported due to the ability of these compounds to cross the placenta, thereby negatively impacting fetal growth. Although scientific evidence regarding the use of tinospora cordifolia and carica papaya extracts in pregnant rats is limited, teratogenicity testing is essential. They offer valuable information regarding safety considerations and potential risks of using these extracts during pregnancy, particularly regarding fetal congenital abnormalities(Yuandani et al., 2021a). Cyclophosphamide is often used as a positive control in teratogenic studies due to its well-documented teratogenic effects. As a chemotherapeutic agent, it works by cross-linking DNA, which can lead to cell death. However, it also has the potential to cause mutations and developmental abnormalities in a developing embryo or fetus. Mechanism of Action Cyclophosphamide interferes with DNA replication and repair, leading to mutations and other genetic damage. These effects can manifest as structural abnormalities in the developing fetus, making it an effective agent for testing the teratogenic potential of other substances(Bagas Ardiyantoro, 2024a; Yuandani et al., 2021b).

The researcher identified three main problems: whether tinospora cordifolia and carica papaya extract cause teratogenic effects in white mouse fetuses based on biometric, gross morphological, and skeletal parameters. This study aims to evaluate these teratogenic effects by measuring these parameters. The benefits include providing information on the teratogenic effects of these extracts, determining a safe dosage to avoid fetal harm, and contributing to pharmaceutical knowledge and drug development.

RESEARCH METHOD

This study is a pure experimental research using mice to determine the teratogenic effects at certain doses. Sampling was conducted periodically during the first, second, and third trimesters of mouse pregnancy. The study was carried out from January to July at the Natural Materials Laboratory and the Pharmacology Laboratory of Universitas Duta Bangsa Surakarta. The population consisted of *Tinospora cordifolia and carica papaya* from the Medicinal Plants Research Center in Tawangmangu, Karanganyar, Central Java. The samples used were extracts of *tinospora cordifolia and carica papaya*, produced in the laboratory of Universitas Duta Bangsa.

This research examines the combined effects of *tinospora cordifolia and carica papaya* extract at various doses on *Mus musculus* during the organogenesis period, specifically from days 6 to 15 of gestation. The key variables include the identification and classification of extracts at a dose of 1120 mg/kg body weight per day, and the monitoring of physical conditions of the test animals, such as body weight and age. The doses were administered orally in ratios of 2:1, 1:2, and 1:1. The biometric aspects of the fetus involved the assessment and analysis of biological features to detect the potential teratogenic effects of a substance on the fetus, such as reabsorption (examining the presence and condition of the fetus). Lost or undeveloped fetuses (showing signs of reabsorption) were identified and counted. Body weight differences among fetuses, body length measured using calipers, and the number of defects were the focus of data analysis. The Kolmogorov-Smirnov test was used to check for normal distribution, and the results were analyzed with one-way ANOVA, followed by a post-hoc test to identify group differences. The aim of this study is to assess the teratogenic potential of this combination and to enhance understanding of its impact on fetal development in mice.

RESULT AND DISCUSSION

A. Results of Teratogenicity Test in Trimester

1. Biometrics

   This observation involves the number of live fetuses, dead fetuses, resorption, length, and weight of the fetuses. The results of these observations can be seen in the following tables:

2. Observation of Mouse Fetus Weight

   The fetal weight observation, performed post-extraction from the maternal abdomen, indicated that fetuses in the Cyclophosphamide group and the groups treated with Tinospora cordifolia and carica papaya extract (Dose 3 ETC 1:1) had lower weights than those in the normal control group. The results are shown in Table 1.

Table 1. Fetal weight

Group	Average weight (gram) ± SD
Normal Control	2,25 ± 1,42 [b]
Cyclophosphamide	0,73 ± 0,16 [a]
Dose ET 1120	1,88 ± 0,47 [b]
Dose EC 1120	2,21 ± 0,28 [b]
Dose 1 ETC 2:1	2,05 ± 0,35 [b]
Dose 2 ETC 1:2	1,08 ± 0,08 [b]
Dose 3 ETC 1:1	0,84 ± 0,72 [b]

Note: ETC: Tinospora cordifolia and carica papaya extract

a: Significantly different from normal control group

b : Significantly different from the *cyclophosphamide group*

Statistical analysis in Table 1 reveals a significant difference between the groups treated with Tinospora cordifolia and carica papaya extract (Dose ET 1120, Dose EC 1120, Dose 1 ETC 2:1, Dose 2 ETC 1:2, and Dose 3 ETC 1:1) and the Cyclophosphamide group. This difference may result from a reduction in cAMP concentration, which is often linked to increased growth activity. Flavonoid compounds inhibit the activity of the phosphodiesterase enzyme, thereby increasing cAMP concentration in fetal cells and tissues (Bagas Ardiyantoro, 2024) .Consequently, some fetuses experience weight changes.

3. Mouse Fetus Length Observation.

   Fetal length observations were made using a ruler, which was not very effective due to the small size of the fetuses. Therefore, measurements were made using millimeter block paper. The results of all fetal length examinations in all groups can be seen in Table 2.

Table 2. Fetal length

Group	Average weight (cm) ± SD
Normal Control	3,79 ± 1,02[b]
Cyclophosphamide	2,32 ± 0,29[a]
Dose EB 1120	3,11 ± 0,42[b]
Dose ET 1120	3,12 ± 0,33[b]
Dose 1 ETC 2:1	3,25 ± 0,30[b]
Dose 2 ETC 1:2	3,08 ± 0,44[b]
Dose 3 ETC 1:1	2,38 ± 0,36[b]

Note: ETC: Tinospora cordifolia and carica papaya
a: Significantly different from the normal control group
b : Significantly different from the *cyclophosphamide group*

Statistical analysis in Table 2 shows differences between groups treated with Tinospora cordifolia and carica papaya extract (Dose ET 1120, Dose EC 1120, Dose 1 ETC 2:1, Dose 2 ETC 1:2, and Dose 3 ETC 1:1) and the Cyclophosphamide group. Furthermore, there is a significant difference between the groups treated with these extracts and the normal control group. These differences might be due to dwarfism, and it is suspected that flavonoids possess anti-proliferative properties. (Ismail et al., 2017)

4. Percentage of Mouse Fetus Death

The percentage of mouse fetus death was observed by comparing the number of dead fetuses to the total number of live fetuses. The results are shown in Table 3.

Table 3. Percentage of Mouse Fetus Death

Group	Average of Fetus	Average of Dead Fetus	Average % Death
Normal Control	9,57 ± 1,90	0,00 ± 0,00	0,00 ± 0,00 [a,b]
Cyclophosphamide	8,00 ± 1,41	1,57 ± 0,53	19,64 ± 37,80 [a]
Dose EB 1120	8,43 ± 1,81	0,29 ± 0,49	3,39 ± 26,92 [b]
Dose ET 1120	8,29 ± 1,25	0,29 ± 0,49	3,45 ± 38,92 [b]
Dose 1 ETC 2:1	10,57 ± 0,98	0,29 ± 0,76	2,70± 77,46 [b]
Dose 2 ETC 1:2	7,57 ± 1,24	0,43 ± 0,53	5,66 ± 42,01 [b]
Dose 3 ETC 1:1	6.71 ± 1,24	1,00 ± 0,82	14,89 ± 59,16 [b]

Description: ETC: Tinospora cordifolia and carica papaya extract
a: Significantly different from the normal control group
b : Significantly different from the *cyclophosphamide group*

Data in Table 3 shows that the treatment group with Dose EB 1120 had an average mortality rate of 3.39 ± 26.92, Dose ET 1120 had 3.45 ± 38.92, Dose 1 ETC 2:1 had 2.70 ± 77.46, Dose 2 ETC 1:2 had 5.66 ± 42.01, and Dose 3 ETC 1:1 had 14.89 ± 59.16. These results indicate that groups treated with Tinospora cordifolia and carica papaya extract at various doses had lower average mortality rates compared to the Cyclophosphamide group. However, statistical analysis reveals no significant difference between the treatment groups and the normal control group.

5. Reabsorps

Reabsorption is observed by counting the number of fetuses that undergo Reabsorps. The results of these observations can be seen in the following table 4:

Table 4. Reabsorps

Group	Average of Fetus	Average of Reabsorps	Average % Reabsorps
Normal Control	9,57 ± 1,90	0,00 ± 0,00	0,00 ± 0,00 [a,b]
Cyclophosphamide	8,00 ± 1,41	3,70 ± 1,78	46,43 ± 127,24 [a]
Dose EB 1120	8,43 ± 1,81	0,85 ± 1,19	10,17 ± 67,03 [b]
Dose ET 1120	8,29 ± 1,25	1,13 ± 1,19	13,79 ± 96,92 [b]
Dose 1 ETC 2:1	10,57± 0,98	1,70 ± 1,48	16,22± 153,30 [b]
Dose 2 ETC 1:2	7,57 ± 1,24	1,28 ± 1,48	16,98 ± 117,57 [b]
Dose 3 ETC 1:1	6.71 ± 1,24	2,12 ± 1,66	31,91 ± 121,45 [b]

Note: ETC: Tinospora cordifolia and carica papaya
a : Significantly different from the normal control group
b : Significantly different from the *cyclophosphamide group*

Table 4 indicates the differences in reabsorption rates for various treatment groups compared to the Cyclophosphamide and normal control groups. Reabsorption, caused by fetal death in the womb, prevents normal development. The death percentages are as follows: Dose ET 1120 - 10.17 ± 67.03, Dose EC 1120 - 13.79 ± 96.92, Dose 1 ETC 2:1 - 16.22 ± 153.30, Dose 2 ETC 1:2 - 16.98 ± 117.57, and Dose 3 ETC 1:1 - 31.91 ± 121.45. The reabsorption rate increases with the dose. This may be due to the cytotoxic and anticancer properties of curcumin, which can act as teratogens, causing abnormalities or defects in the embryo. Curcumin's cytotoxic effects are associated with defects in the embryonic cell system and blastocytes, reduced oocyte maturation and fertilization, and embryonic development defects in vitro through cell apoptosis. (Fatikhurokhmah et al., 2022; Yuandani et al., 2021c)

6. Disability rate

The defective rate is observed by counting the number of deformed fetuses. The results of these observations can be seen in the following table 5:

Table 5. Disability numbers

Group	Average of Fetus	Average Disability Rate	Average % disability
Normal Control	9,57 ± 1,90	0,00 ± 0,00	0,00 ± 0,00 [a,b]
Cyclophosphamide	8,00 ± 1,41	2,86 ± 1,57	35,71 ± 111,27 [a]
Dose EB 1120	8,43 ± 1,81	1,29 ± 0,95	15,25 ± 52,47 [b]
Dose ET 1120	8,29 ± 1,25	1,57 ± 0,79	18,97 ± 62,76 [b]
Dose 1 ETC 2:1	10,57 ± 0,98	1,57 ± 1,40	14,86± 143,18 [b]
Dose 2 ETC 1:2	7,57 ± 1,24	0,86 ± 0,90	11,32 ± 70,71 [b]
Dose 3 ETC 1:1	6.71 ± 1,24	1,86 ± 0,90	27,66 ± 65,19 [b]

Note: ETC: Tinospora cordifolia and carica papaya
a: Significantly different from the normal control group
b : Significantly different from the *cyclophosphamide group*

Table 5 shows the differences in defect rates among various treatment groups compared to the Cyclophosphamide group. The average death percentages are as follows: EB 1120 - 15.25 ± 52.47, ET 1120 - 18.97 ± 62.76, Dose 1 ETC 2:1 - 14.86 ± 143.18, Dose 2 ETC 1:2 - 11.32 ± 70.71, and Dose 3 ETC 1:1 - 27.66 ±

65.19. The defect rate is determined by the ratio of deformed fetuses to the total fetuses in each group. Not all fetuses in a group exhibit morphological abnormalities, even those from the same parent, due to individual genetic susceptibility.

7. Grosmorphological observations

This observation is in the form of completeness and lack of legs, tail, hands, ears, lips, sky crevices and congestive. The results can be seen in the table as follows:

Table 6. Form of completeness organ

Group	Average of Fetus	Average Incomplete organs	Rerata % completeness organ
Normal Control	9,57 ± 1,90	0,00 ± 0,00	0,00 ± 0,00 [a,b]
Cyclophosphamide	8,00 ± 1,41	1,14 ± 0,69	14,29 ± 48,80 [a]
Dose EB 1120	8,43 ± 1,81	0,43 ± 0,79	5,08 ± 43,41 [b]
Dose ET 1120	8,29 ± 1,25	0,57 ± 0,79	6,90 ± 62,76 [b]
Dose 1 ETC 2:1	10,57± 0,98	0,57 ± 0,79	5,41± 80,62 [b]
Dose 2 ETC 1:2	7,57 ± 1,24	0,86 ± 0,69	11,32 ± 54,23 [b]
Dose 3 ETC 1:1	6.71 ± 1,24	1,14 ± 0,69	17,02 ± 50,00 [b]

Description: ETC: Tinospora cordifolia and carica papaya

a: Significantly different from the normal control group

b : Significantly different from the *cyclophosphamide group*

The statistical analysis indicates notable differences among the treatment groups: EB 1120 dose, ET 1120 dose, Dose 1 ETC 2:1, Dose 2 ETC 1:2, and Dose 3 ETC 1:1 compared to Cyclophosphamide. The EB 1120 dose group had an average mortality percentage of 5.08 ± 43.41, ET 1120 dose group - 6.90 ± 62.76, Dose 1 ETC 2:1 group - 5.41 ± 80.62, Dose 2 ETC 1:2 group - 11.32 ± 54.23, and Dose 3 ETC 1:1 group - 17.02 ± 50.00. These differences may stem from the antiproliferative properties of various compounds with potential anticancer effects, such as their ability to regulate the cell cycle by inhibiting cyclin-CDK activity and other protein kinases. Natural chemopreventive agents like flavonoids can induce cell cycle arrest at the G1 phase, while compounds like curcumin can impact the G0/G1 and G2/M phase transitions

8. Skeletal disorder

Alizarin staining was utilized to examine skeletal disorders in both normal and deformed fetuses. Abnormalities often involve inhibited bone ossification, affecting the process of bone formation and maturation. This disruption can lead to bones becoming brittle or imperfect, which affects their ability to be stained by alizarin dye. The results of observations can be seen in table 7.

Table 7. Skeletal disorder

Group	Average of Fetus	Average of skeletal disorder	Average % skeletal disorder
Normal Control	9,57 ± 1,90	0,00 ± 0,00	0,00 ± 0,00 [a,b]
Cyclophosphamide	8,00 ± 1,41	2,86 ± 1,57	35,71 ± 111,27 [a]
Dose EB 1120	8,43 ± 1,81	0,86 ± 0,96	10,17 ± 38,07 [b]
Dose ET 1120	8,29 ± 1,25	0,71 ± 0,76	8,62 ± 60,30 [b]
Dose 1 ETC 2:1	10,57 ± 0,98	1,14 ± 0,90	10,81± 92,20 [b]
Dose 2 ETC 1:2	7,57 ± 1,24	1,00 ± 0,82	13,21 ± 64,17 [b]
Dose 3 ETC 1:1	6.71 ± 1,24	2,29 ± 0,76	34,04 ± 54,77 [b]

Description: ETC: Tinospora cordifolia and carica papaya

a: Significantly different from the normal control group
b : Significantly different from the *cyclophosphamide group*

Statistical analyses, including the Kolmogorov-Smirnov One-sample test, ANOVA test, and Post hoc test, were conducted to compare groups. Brotowali stem and Curcuma domestica Val rhizome extracts at various doses showed significant differences compared to the Cyclophosphamide group. For instance, the EB 1120 dose group had an average mortality percentage of 10.17 ± 38.07, while the ET 1120 dose group had 8.62 ± 60.30. Alkaloid compounds in these extracts may affect skeletal abnormalities by stimulating uterine smooth muscle contractions during pregnancy, potentially altering bone growth direction. (Supriatin et al., 2018)

Cyclophosphamide, also known as cytophosphane, belongs to the alkylating agent family within the oxazophorin group. Alkylating antineoplastic agents have the capability to attach to alkyl groups within DNA. This compound induces cell death and halts tumor proliferation by creating cross-links between guanine bases at the N-7 position on the DNA double helix. These cross-links occur both between strands (interstrand) and within strands (intrastrand), leading to DNA fragmentation or breakage, thereby inhibiting cell division and promoting cell death.

From testing fetal length, fetal weight, fetal death, reasobsion, disability rate, organ completeness, and skeletal abnormalities showed a significant difference with the Cyclophosphamide group and a significant difference with the normal control group. The dose of Tinospora cordifolia and carica papaya is at risk of causing teratogenic effects because there are differences with normal controls and the higher the dose has a heavier effect, occurring in all tests this can potentially cause teratogenic effects if the dose used is more than the test dose of 1.04 ml. This is inversely proportional if only the carica papaya herbal medicine the effect will not potentially cause teratogenic

CONCLUSION

The research on the teratogenic effects of Tinospora cordifolia and carica papaya extract on mouse fetal development concluded both extracts potentially cause teratogenic effects on fetal development as measured by biometric parameters, gross morphological parameters, and skeletal abnormality parameters, particularly at doses exceeding 1120 ml.

.

REFERENCES

Alafiatayo, A. A., Lai, K. S., Syahida, A., Mahmood, M., & Shaharuddin, N. A. (2019). Phytochemical Evaluation, Embryotoxicity, and Teratogenic Effects of Curcuma longa Extract on Zebrafish (Danio rerio). *Evidence-Based Complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/3807207

Amalia Choironi, N. (2016). QUALITY STANDARDIZATION OF BROTOWALI (Tinospora crispa) STEM EXTRACT STANDARDISASI KUALITAS EKSTRAK BATANG BROTOWALI (Tinospora crispa). *Traditional Medicine Journal*, *21*(1), 2016.

Bagas Ardiyantoro. (2024a). TERATOGENIC TEST OF BROTOWALI STEM EXTRACT (Tinospora crispa) AND TEMULAWAK RHIZOME EXTRACT (Curcuma xanthorrhiza) ON WHITE MOUSE FETUSES (Mus musculus). *Journal Eduvest - Journal of Universal Studies*, *4*(1). https://doi.org/https://doi.org/10.59188/eduvest.v4i1.999

Bagas Ardiyantoro, A. M. P. M. (2024b). *Teratogenic Test Of Brotowali Stem Extract (Tinospora Crispa) And Temulawak Rhizome Extract (Curcuma Xanthorrhiza) On White Mouse Foetuses (Mus Musculus)*. https://doi.org/https://doi.org/10.59188/eduvest.v4i1.999

Basu, P., Meza, E., Bergel, M., & Maier, C. (2020). Estrogenic, antiestrogenic and antiproliferative activities of euphorbia bicolor (Euphorbiaceae) latex extracts and its phytochemicals. *Nutrients*, *12*(1). https://doi.org/10.3390/nu12010059

Elba, F., Hilmanto, D., & Poddar, S. (2022). Factors influencing the use of herbal medications during pregnancy at Public Health Center, Indonesia. *Journal of Public Health Research*, *11*(4). https://doi.org/10.1177/22799036221139939

Fatikhurokhmah, H. M., Agustini, R., Kimia, J., Matematika, F., Ilmu, D., & Alam, P. (2022). Concentration Effect of Brotowali Stem (Tinospora Crispa (L.)) in Ethanol Extracts on the A-Glukosidase Enzyme Inhibition. In *Indonesian Journal of Chemical Science* (Vol. 11, Issue 3). http://journal.unnes.ac.id/sju/index.php/ijcs

Illamola, S. M., Amaeze, O. U., Krepkova, L. V., Birnbaum, A. K., Karanam, A., Job, K. M., Bortnikova, V. V., Sherwin, C. M. T., & Enioutina, E. Y. (2019). Use of herbal medicine by pregnant women: What physicians need to know. *Frontiers in Pharmacology*, *10*. https://doi.org/10.3389/fphar.2019.01483

Ismail, H. F., Hashim, Z., Soon, W. T., Rahman, N. S. A., Zainudin, A. N., & Majid, F. A. A. (2017). Comparative study of herbal plants on the phenolic and flavonoid content, antioxidant activities and toxicity on cells and zebrafish embryo. *Journal of Traditional and Complementary Medicine*, *7*(4), 452–465. https://doi.org/10.1016/j.jtcme.2016.12.006

Kong, Y. R., Jong, Y. X., Balakrishnan, M., Bok, Z. K., Weng, J. K. K., Tay, K. C., Goh, B. H., Ong, Y. S., Chan, K. G., Lee, L. H., & Khaw, K. Y. (2021). Beneficial role of carica papaya extracts and phytochemicals on oxidative stress and related diseases: A mini review. In *Biology* (Vol. 10, Issue 4). MDPI AG. https://doi.org/10.3390/biology10040287

Mohamad, T. A. S. T., Islahudin, F., Jasamai, M., & Jamal, J. A. (2019). Preference, perception and predictors of herbal medicine use among malay women in Malaysia. *Patient Preference and Adherence*, *13*, 1829–1837. https://doi.org/10.2147/PPA.S227780

Santana, L. F., Inada, A. C., Santo, B. L. S. do E., Filiú, W. F. O., Pott, A., Alves, F. M., Guimarães, R. de C. A., Freitas, K. de C., & Hiane, P. A. (2019). Nutraceutical potential of carica papaya in metabolic syndrome. In *Nutrients* (Vol. 11, Issue 7). MDPI AG. https://doi.org/10.3390/nu11071608

Supriatin, Y., Sari, N., Syafrullah, H., Jurusan,), Konsentrasi, K., Medis, A., Tinggi, S., Bakti, A., Bandung, A., Stikes,), Husada, D., Kunci, K., & Brotowali, B. (2018). *PEMANFAATAN EKSTRAK BATANG BROTOWALI (Tinospora Crispa) MENGGUNAKAN PELARUT METHANOL SEBAGAI LARVASIDA NYAMUK Aedes aegypti*.

Vargesson, N. (2019). The teratogenic effects of thalidomide on limbs. *Journal of Hand Surgery: European Volume*, *44*(1), 88–95. https://doi.org/10.1177/1753193418805249

Yuandani, Tarigan, K. S. A., & Yuliasmi, S. (2021a). Teratogenic effects of ethanol extract of Curcuma mangga Val. rhizomes in wistar rats. *Toxicological Research*, *37*(4), 429–434. https://doi.org/10.1007/s43188-020-00074-x

Yuandani, Tarigan, K. S. A., & Yuliasmi, S. (2021b). Teratogenic effects of ethanol extract of Curcuma mangga Val. rhizomes in wistar rats. *Toxicological Research*, *37*(4), 429–434. https://doi.org/10.1007/s43188-020-00074-x

Yuandani, Tarigan, K. S. A., & Yuliasmi, S. (2021c). Teratogenic effects of ethanol extract of Curcuma mangga Val. rhizomes in wistar rats. *Toxicological Research*, *37*(4), 429–434. https://doi.org/10.1007/s43188-020-00074-x