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BSLT TOXICITY TEST OF TEMULAWAK ETHANOL EXTRACT (CURCUMA XANTHORRHIZA) FROM FIVE DIFFERENT REGIONS AND DETERMINATION OF CURCUMIN AND XANTHORHIZOL COMPOUND LEVELS

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Abstract: *The BSLT toxicity test is a preliminary test to look for ingredients that have the potential to act as anticancer from natural ingredients. A natural ingredient that has the potential to be an anticancer candidate is ginger. The aim of this research is to determine the level of toxicity against Artemia salina and look for anticancer candidates from ginger originating from different regions based on their toxicity, establish quality standards and determine the levels of curcumin and xanthorrhizol from extracts that have the highest toxicity. This research made 50% ethanol extract of ginger from the Cirebon, Tembalang, Wonogiri, Jambi and Southwest Sumba areas using the kinetic maceration method. The fifth extract was subjected to a phytochemical screening test, toxicity test using the BSLT method. The ginger extract which had the highest toxicity was tested for non-specific parameters and determined the levels of curcumin and xanthorrhizol. The results of phytochemical screening showed positive flavonoids, saponins, quinones, triterpenoids. The LC50 results obtained from the five extract samples were 48.34 mg/L, 41.85 mg/L 64.58 mg/L, 105.32 mg/L, 82, 17mg/L. The results of determining non-specific parameters met the specified requirements and obtained curcumin and xanthorrhizol levels of 72.83 and 117.07 ppm. Based on these results, it can be concluded that the 50% ethanol extract of Javanese tumeric from Tembalang meets the extract quality requirements, contains curcumin and xanthorrhizol compounds and has cytotoxic activity against Artemia salina.*

Keywords: *Toxicity, BSLT, Javanese tumeric, Curcumin, Xanthorhizol.*

INTRODUCTION

The use of active compounds from natural ingredients is one of the potential opportunities as an alternative to the search for new anticancers. The biodiversity of medicinal plants owned by Indonesia stretching from Sabang to Merauke is a large enough resource to be utilized and developed. Based on this, the development of alternative sources of anticancer derived from natural materials has been intensively carried out lately. One of the efforts is to explore plants that are commonly used as medicine by local people (1).

An ingredient can be known for its anticancer potential by approaching it using the BSLT method. The BSLT method is an initial screening of potentially correlated anticancer ingredients based on their toxicity value. The BSLT method is often chosen as a method of finding anticancer candidates because testing is relatively fast, inexpensive, and high in confidence (2). An extract, if declared toxic, based on BSLT testing, can be developed into further research by cytotoxic testing in cancer cell cultures to validate its effects as an anticancer that is selective, does not kill normal cells and does not cause resistance (3).

One of the plants that has the potential as a medicinal plant commonly used by the public is a plant from the genus curcuma (4) . Plants of the genus curcuma are found plants that produce rhizomes that have a distinctive smell. Curcuma belongs to the main genus of the *zingiberaceae family* and is known to be rich in secondary metabolite compounds (5). In research that has been done, it is known that plants of the genus curcuma have potential as anticancer through preliminary tests of BSLT seen from its toxic effects on *Artemia salina* (4). Some plants from the genus Curcuma that have known toxic effects on *Artemia salina* are Temuhitam *(Curcuma aeruginos*), white temu *(Curcuma zedoaria),*

Temu mango (Curcuma mango) (6)(7)(8). Based on this, researchers are interested in examining plants from other curcuma genera, namely Temulawak.

Temulawak has been widely used by local communities such as being used as a kitchen spice, health-enhancing drink, and as traditional medicine (9). Empirically, Temulawak is widely used by the community as a traditional medicine to treat digestive tract disorders, bile inflammation, rheumatism, high cholesterol, and increased appetite (10). Its various benefits in medicine are inseparable from the content of secondary metabolites contained in ginger such as alkaloids, flavonoids, saponins, steroids, triterpenoids, and essential oils (11). These compounds are known to have activities that inhibit growth and kill cancer cells. As in the content of secondary metabolite compounds from the flavonoid group isolated from various plants are known to have an effect on inhibiting the proliferation of cancer cells (12). Saponin compounds are known to have the effect of inhibiting the formation of overexpressed BCl-2 (13). Based on these results, ginger has the potential to be a candidate for anticancer ingredients. This potential can be tested preliminarily as providing information on the potential of ginger as an anticancer herbal medicine.

Temulawak as a plant from the genus curucuma produces secondary metabolites that are commonly contained in plants from other curcuma genera, namely curcumin compounds. Curcumin compounds are compounds from the phenolic group that can function as antioxidants. Antioxidant compounds have links to the mechanism of capturing free radicals that can cause cancer (14). In addition to containing curcumin compounds, ginger is also rich in essential oils. Based on the results of research that has been done, Temulawak contains 18 kinds of essential oil fractions and one of them is xanthorhizol compounds which have the second largest percentage of essential oil fractions (15). Xanthorhizol is a specific compound that is only contained in ginger. Xanthorhizol compounds belong to terpenoid compounds from the sesquiterpene group. It is known that compounds from the terpene group can have the ability to inhibit tumor growth (16). Curcumin and Xanthorhizol compounds are the main bioactive compounds contained in Temulawak, therefore it is necessary to determine the presence of these two compounds contained in Temulawak extract which was used as a sample in this study both qualitatively and quantitatively (17).

Along with the times, there has been a lot of research using natural materials for the discovery of new anticancer ingredients. However, there is still little research into natural ingredients in finding candidates that have anticancer potential based on toxicity based on planting sites in different areas. The content of secondary metabolites produced by a plant can be influenced by internal factors and external factors. Internal factors are genetic factors of the plant while external factors include planting environmental conditions such as temperature, weather, light intensity, and soil height of the planting site (18). The bioactive content produced by plants can be influenced by genetic factors and the growing environment of ginger plants. The bioactive content of Temulawak will determine the bioactivity or efficacy of Temulawak. This has been tested by research that has been done that there are differences in the antimicrobial activity of temulawak extract from different planting sites (19).

Based on this background, this research will be screened on temulawak extracts from five different planting areas in Indonesia, the five regions, namely Cirebon, Tembalang, Wonogiri, Jambi, and Southwest Sumba. The method to be used in this study is to use the BSLT method to see the toxicity profile of temulawak extract against *Artemia salina* Leach larvae. Furthermore, temulawak rhizome extracts from areas that have the highest toxicity profile will be tested for curcumin and xanthorhizol compounds. Both are components that have sufficient content in ginger and are believed to be compounds that have strong biological activity against cancer cells.

Based on this background, the problem in this study was formulated as follows: (1) Does temulawak rhizome extract have a toxic effect on Artemia salina Leach larvae? (2) How is the difference in the toxicity results of temulawak extract from five different regions against Artemia salina Leach

larvae? (3) Does temulawak extract that has the highest toxicity contain curcumin and xanthorhizol compounds?

The purpose of this study was to determine the toxicity profile of Temulawak ethanol extract against Artemia salina Leach larvae. To determine the influence of temulawak planting areas seen from the toxicity value on Artemia salina Leach larvae, and determine the potential of temulawak ethanol extract from five different regions, namely Cirebon, Tembalang, Wonogiri, Jambi, Sumba based on the highest toxicity results. To determine the content of curcumin and xanthorhizol compounds from Temulawak ethanol extract which has the highest toxicity as a secondary metabolite compound produced is quite high in Temulawak and is believed to be an anticancer compound.

This study is expected to provide scientific information related to the potential of Temulawak rhizomes as anticancer based on the results of toxicity tests on Artemia salina Leach larvae using the BSLT method and determine the best Temulawak rhizome extract in terms of toxicity profiles based on differences in planting locations.

MATERIALS AND METHODS

This research is an experimental research that first collected Temulawak rhizome material from five different regions in Indonesia. The temulawak rhizomes obtained are dried into simplisia, then the temulawak rhizomes are made into powder. The powder obtained was extracted using the kinetic maceration method using 50% ethanol solvent and concentrated with a rotary vacuum evaporator until a concentrated extract was obtained.

The research was conducted at the Thesis Laboratory of the Faculty of Pharmacy, Pancasila University, Jl. Srengseng Sawah, South Jakarta. Plant determination in this study was carried out to ensure the correctness of the plants used before the study began. Plant determination was carried out at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia. This research stage is with plant determination, collection of research materials, the process of making simplisia, making ethanol extract of ginger rhizomes, concentration of ethanol liquid extract of simplisia of ginger rhizomes, organoleptic examination, examination of extract moisture content, phytochemical screening, BSLT toxicity testing, qualitative identification with KLT, examination of extract quality parameters that have the highest toxicity, determination of curcumin and xanthorhizol compound levels with KCKT.

RESULTS AND DISCUSSION

Toxicity test research using BSLT was conducted to test samples of temulawak rhizome extract in search of anticancer potential from a natural ingredient based on the results of toxicity to Artemia salina Leach. This research began with the collection of temulawak from five different regions, namely Cirebon, Tembalang, Wonogiri, Jambi, and Southwest Sumba. Temulawak that has been obtained is dried to be used as simplisia. The drying process is carried out using an oven with a temperature of 50□C, the dried Temulawak bachelor is then blended to obtain simplisia powder.

The five simplisia powders obtained were extracted with 50% ethanol solvent using kinetic maceration method. The solvent used for extraction was chosen 50% ethanol solvent because it is expected to attract compounds that are polar to nonpolar, this is based on the nature of ethanol solvents that can attract nonpolar to polar compounds because ethanol is composed of ethyl (CH3) and hydroxy (OH) groups so that it can dissolve compounds that are nonpolar to polar. 50% ethanol consists of a composition of 50% ethanol and the remaining 50% is water which is a strong polar solvent, besides that the selection of the two solvent compositions is based on BPOM regulations regarding the solvents allowed for medicinal materials are water and ethanol because they tend to have low toxicity when compared to other organic solvents. The selection of extraction methods using

the kinetic maceration method because maceration is a cold method of extraction that is difficult to attract compounds in simplisia, with the kinetic force given it can be an enhancer of the withdrawal of compounds contained in simplisia because kinetic stirring can help in breaking down cell walls from plant parts so that solvents can penetrate cell walls and dissolve the compounds contained. The selection of this method is expected to optimize the dissolved compounds into the solvent. The results of dissolved compounds in the solvent can be described from the yield results obtained, because the yield value is related to the amount of dissolved compounds. The higher the yield, the higher the dissolved substance content. The yield results of extracts from Temulawak originating from different regions of Cirebon, Tembalang, Wonogiri, Jambi, Southwest Sumba obtained yields of 17.80%, 14.52%, 16.16%, 16.40%, 13.34% respectively.

The five extracts from Cirebon, Tembalang, Wonogiri, Jambi, and Southwest Sumba were tested for phytochemical screening. Phytochemical screening is a qualitative test conducted as a preliminary test to determine the class of compounds contained in Temulawak rhizome extract from five different regions. The determination of the group of compounds contained is seen based on the color reaction produced. This study was conducted phytochemical screening of alkaloid groups, flavonoid groups, saponin groups, quinone groups, tannin groups, coumarin groups, steroid / triterpenoid groups, and essential oil groups. Based on phytochemical screening testing of the five extracts, the results of the group of compounds that were positively identified as contained in the five extracts were flavonoids, saponins, quinones, triterpenoids, and essential oils. 50% ethanol extract of Temulawak rhizomes from different regions was tested for toxicity using the BSLT method. This method uses Artemia salina L larvae that have been adapted since hatching for 48 hours as test animals. The larvae used for toxicity testing use larvae that have been 48 hours old because larvae at that age are the most active phase that has formed a perfect mouth and digestive tract, and have increased body resistance. The extract to be used for the test solution is evaporated first to ensure that the remaining solvent used has evaporated so that it can be ascertained that the death of shrimp larvae is due to the content of secondary metabolites contained. After the solvent evaporated, DMSO 1% was added as a solubility enhancer, the selection of DMSO 1% as a surfactant because it tends not to have a toxic effect so it does not have a deadly effect on Artemi salina larvae.

The toxic effect can be determined from the results of observations by calculating the percent mortality of Artemia salina larvae at each test concentration. The percent mortality is obtained from the calculation of the ratio of the number of dead larvae divided by the number of initial larvae multiplied by 100% for each concentration. The results of the calculation of the percent of mortality obtained were searched for probit values from each group of test animals through the probit table, then a graph was made with a straight-line equation of the relationship between the probit value and the concentration log until the equation y = bx + a was obtained. The next stage calculates each obtaining LC50 value from each toxicity test of temulawak rhizome extract from different regions. The way to get the LC50 value can be calculated from the equation by entering the value 5 (probit of 50% of Artemia salina deaths) as y so that x is produced as the concentration log value. Furthermore, the x value is antilogged, to obtain the LC50 value. According to Meyer the toxicity level of an extract is as follows: LC50 $\leqslant$ 30 mg/L = highly toxic; LC50 $\leqslant$ 30 -1000 mg/L = toxic; $\geqslant$ 1000 mg/L = non-toxic.

Based on the results of LC50 calculations using the BSLT method, the toxicity results of 50% ethanol extract of temulawak rhizomes from the Cirebon area of 48.34 mg / L are included in the toxic category, temulawak rhizome extract from the Tembalang area of 41.85 mg / L is included in the toxic category, temulawak rhizome extract from the Wonogiri area of 64.58 mg / L is included in the toxic category, Temulawak rhizome extract originating from the Jambi area amounting to 82.17 mg / L is included in the toxic category, and temulawak rhizome extract originating from the Southwest Sumba area of 105.32 mg / L is included in the toxic category. The difference in toxicity to Artemia salina L

larvae is thought to be influenced by differences in planting areas of Temulawak plants. Differences in planting areas are one of the factors that affect differences in toxicity values due to differences in environmental conditions from each region. such as, temperature, weather, climate, soil height, where these differences affect secondary metabolites because it is known that secondary metabolites are compounds produced by plants as a form of self-defense from the surrounding environment (50). Based on several studies that differences in growing places affect the content of secondary metabolite compounds and biological activities with previous studies that compared the content and antioxidant activity and toxicity of temulawak rhizome extract from different planting sites (18).

Based on the results of toxicity calculations with probit, it is stated that the smaller the LC50 value, the greater the toxicity because bioactive compounds are higher, causing higher death of Artemia salina larvae. LC50 is a value that indicates the concentration of toxic substances that can cause death of test animals up to 50%. The content of secondary metabolite compounds of flavonoids, saponins, triterpenoids and essential oils contained in the sample is suspected to be the compound that causes the death of Artemia salina L. shrimp larvae. Artemia salina larvae have a skin membrane that is very thin so that the diffusion of substances from the environment that affect metabolism in the body. Another mechanism as (stomach poisoning) where secondary metabolites enter the body of larvae through the mouth so that it affects their digestive organs and matabolism system will be disrupted. The presence of flavonoids in the cell environment can cause rupture of cell membranes. uncontrolled influx of Na+ ions into the cell, leading to rupture of the cell membrane. This is because the OH- group in flavonoids binds to integral proteins of the cell membrane so that active transport of Na+ and K+ is contained. Stopped active transport leads to uncontrolled entry of Na+ ions into the cell, leading to rupture of the cell membrane. It is this rupture of the cell membrane that causes cell death (51).

Based on the results obtained, the LC50 value of 50% ethanol extract of temulawak rhizomes originating from the Tembalang area is lower when compared to the four 50% ethanol extracts of temulawak rhizomes originating from Cirebon, Wonogiri, Jambi, and Southwest Sumba. 50% ethanol extract of temulawak rhizomes originating from the Tembalang area can be a potentially cytotoxic temulawak extract candidate compared to the four extracts from other regions.

Based on the results of toxicity tests with the BSLT method, it was determined that the extract had the highest toxicity seen from the smallest LC50 results. Extracts that have the highest toxicity are then tested for non-specific parameters consisting of determination of total ash content, determination of acid insoluble ash, determination of metal contamination, and determination of residual ethanol solvent. The establishment of these non-specific parameters is intended to ensure extracts that have the highest toxicity meet the standards as stated in the monograph. Assurance of the extract meets standards to maintain stability and safety, as well as maintain the consistency of the active compound content contained in the extract.

The determination of total ash is carried out with the aim of providing an overview of the internal and external mineral content derived from the initial process until the formation of the extract. Internal minerals are minerals derived from the plant itself such as calcium, phosphorus, and magnesium which are minerals needed by humans. The determination of acid insoluble ash reflects the presence of mineral or metal contamination from the beginning of the material obtained until the extract manufacturing process. Possible metal contamination is silicates derived from soil or sand, heavy metal elements. Based on the test results, the determination of total ash and acid insoluble ash was obtained at 5.41% and 0.32% respectively. The results meet the requirements listed in the extract monograph.

The determination of metal contamination is carried out by the AAS method which is a tool used for analysis for the determination of metal and metalloid elements whose measurement is based on

the absorption of light with a certain wavelength by metal atoms in the free state. This research was carried out to determine metal contamination aimed at ensuring heavy metal content that should not be present or below the predetermined metal contamination limit. The determination of metal contamination is carried out on extracts that have the highest toxicity in ensuring safety because heavy metal content can have a toxic effect on humans when it exceeds the threshold. Some symptoms of poisoning such as abdominal pain, nausea, vomiting, diarrhea, and if the case is severe can cause kidney failure and death. Based on this, metal contamination of extracts with the highest toxicity was determined. Based on the results of the determination of Pb, Cd, and As metal contamination, Pb metal contamination of 1.95 ppm, Cd metal 0.08 ppm, and As metal was not detected, where these results were included in the maximum limit of metal contamination determined.

Determination of residual solvent levels in ethanol extract of temulawak rhizomes was carried out using gas chromatography and obtained the result of the absence of residual ethanol solvent. Determination of residual solvent content is one of the non-specific extract quality parameters that aims to guarantee that the extract does not leave residual solvent beyond the specified limit. In accordance with the regulations of the Food and Drug Administration that the remaining solvent remaining in the extract is not more than 1%. In this study, 50% ethanol solvent was used, so from the remaining solvent test in this study to find out the remaining ethanol solvent contained in the extract. The purpose of determining the remaining solvent in this study is to ensure that the remaining solvent contained in the extract is below the specified limit, because it will affect the safety of the extract.

The next test is a qualitative and quantitative test of the active compounds curcumin and xanthorhizol contained in extracts that have the highest toxicity. The content test of curcumin and xanthorhizol compounds was carried out because to ensure that extracts originating from the Tembalang area that have potential as anticancer candidates contain active curcumin and xanthorhizol compounds, this is done because these two compounds are the most active compounds in ginger so it needs to be ensured that the extract that has the highest toxicity contains both compounds.

Qualitative determination was carried out using thin-layer chromatography with dichloromethane eluent: chloroform. The two types of eluents were chosen because of their solubility properties that can develop the compounds to be analyzed, namely curcumin and xanthorhizol. Based on qualitative tests using thin-layer chromatography, 3 stains were obtained with Rf1 0.21, Rf2 0.72, Rf3 0.88, of the three stains, it is suspected that Rf1 and Rf2 are curcumin and xanthorhizol, respectively. This refers to the book Atlas of thin-layer chromatography of plants in the identification of xanthorhizol using dichloromethane eluent: chloroform obtained a standard Rf value profile of curcumin 0.30 and standard Rf xanthorhizol 0.70. Based on previous studies using dichloromethane eluent: chloroform obtained KLT profile with Rf values of curcumin and xanthorhizol respectively 0.26 and 0.69 (46).

Determination of curcumin and xanthorhizol levels was carried out using high-performance liquid chromatography. The mobile phase used is acetonitrile:methanol; acetic acid 0.1%. In this study, curcumin and xanthorhizol levels were determined using the stationary phase of column C18, flow rate of 1 mL/min, injection volume of 20 µL, and photo diode array detector. The determination of levels is carried out by plotting the area of peaks read in the system into the equation of the curcumin calibration standard curve and the xanthorhizol calibration standard curve resulting from the regression equation of the relationship between concentration and the peak area of curcumin standard solution and xanthorhizol standard solution. Based on the curcumin standard regression equation obtained y = 139576x + 140489 xanthorhizol standard regression equation and equation obtained y = 25746x – 63095 with R correlation values of 0.997 and 0.9967 respectively. Based on the correlation value, a value close to 1 indicates a linearity relationship between the concentration relationship and the read peak area.

The determination of curcumin and xanthorhizol levels was tested against extracts originating from the Tembalang area. Determination of the levels of these two compounds in extracts from the tembalang area because they have the highest toxicity profile of the other four regional origins. The determination of curcumin and xanthorhizol levels aims to see the content of curcumin and xanthorhizol compounds contained in temulawak extract which have potential anticancer ingredients based on the results of initial screening of potential toxicity to Artemia salina larvae using the BSLT method, determining the levels of these two compounds is done because curcumin has the largest content in the curcuminoid fraction and xanthorhizol is a typical phytochemical compound that is only contained in Temulawak so that the levels of this compound can also be used as a determinant of the quality of ginger rhizomes. Determination of curcumin and xanthorhizol levels from the results of the peak area of the extract sample that is read and plotted into the calibration curve equation formula obtained from linear regression of the concentration relationship with the area of the raw solution of each compound. Based on the determination of levels, curcumin levels were obtained 74.79 ppm and xanthorhizol 118.17 ppm. One of the main causes of cancer is the loss of balance between cell proliferation and cell death. When a cell goes through death due to the absence of a signal, apoptosis and uncontrolled cell proliferation leads to various types of cancer. Apoptotic signals are generated through two main signals, namely the intrinsic pathway and the extrinsic pathway.

The mechanisms involved in the inhibition of tumorigenesis by curcumin are diverse and involve a combination of anti-inflammatory, antioxidant, immunomodulatory, proapoptosis, and anti-angiogenic properties through plelotropic effects on genes and cell signaling pathways at various levels. Reactive Oxygen Species (ROS) inhibitors to inhibition at the level of redox homeostasis enzymes glutathione peroxidase and superoxide dismutase, occur in a complex, with multiple effects depending on time and concentration. This dual effect can be attributed to changes in oxidative stress and antioxidant gene expression levels leading to inhibition or promotion of cell death.

Curcuminoids influence tumor angiogenesis through various interdependent processes, namely mechanisms at the level of transcription factors NF-κB, AP-1 (associated with inflammatory processes) and early response of growth proteins, which weaken IL-8 expression in pancreatic and head and neck cancer cell lines and prevent the induction of VEGF synthesis, secondly inhibition of angiogenesis mediated by NO and iNOS, third is inhibition of COX-2 and 5-LOX, fourth is action at the level of angiogenic factors such as VEGF major factors for migration, cell survival and proliferation during angiogenesis, and basic fibroblast growth factors as well as actions at levels of stability and coherence of the extracellular matrix, including decreased regulation of metalloproteinase (MMP) namely MMP-2 and MMP-9, and increased regulation of metalloproteinase-1 tissue inhibitors. Turmeric also interferes with the release of angiogenic factors stored in the extracellular matrix.

Selective echoexpression of cyclooxygenase-2 (COX-2) is seen in the process of carcinogenesis in many types of tumors. In contrast to COX-2 inhibitors, such as celecoxib which inhibit the catalytic activity of isoenzymes, curcumin inhibits the transcription of COX-2 proteins, reducing their levels in cells. Inhibition of the COX-2 gene may be curcumin's primary anti-inflammatory activity. An in vitro study of human cervical cancer cells showed downregulation of COX-2, induced nitric oxide synthase (iNOS) and cyclin D. This combination of anti-inflammatory and antioxidant activity led to angiogenesis-mediated inhibition of vascular endothelial growth factors in human intestinal microvascular, i.e. endothelial cells.

The mechanism of action of curcumin can occur both intrinsically (in mitochondria) and extrinsically (mediated through cell surface transmembrane death receptors). The intrinsic pathway generally begins with activation of the tumor suppressor p53, which is a regulator of the cell cycle, as well as through members of the B-cell lymphoma (Bcl-2) family. Overregulation of p53 activates Bcl-2 inactivating homologous antagonists (Bak) and Bcl-2-associated protein x (Bax), which are pro-

apoptotic members of the Bcl-2 family. Bak and Bax promote apoptosis by forming pores in the mitochondrial membrane that release cytochrome-c into the cytoplasm, thereby activating caspase. Nuclear factors (erythroid-derived 2) such as 2 (Nrf2) are transcription factors involved in primary defense pathways against the effects of oxidative stress. The transcription factor Nrf2 pathway is a regulator of genetic variation related to electrophile detoxification and ROS and repairs and removes products damaged by cancer cells. Curcumin has antiproliferative potential by activating the Nrf2 pathway, restoring p53, and modulating inflammatory molecules.

Curcumin is able to inhibit proliferation and induce apoptosis through extrinsic pathways mediated through activation of tumor necrosis factor receptors (TNF). The TNF pathway triggers activation of caspase-8 and caspase-3, which are pro-apoptotic, but also induce NF-kB, which regulates COX-2 gene expression. Curcumin also suppresses NF-kB activation. Using molecular studies, curcumin has been shown to bind to TNF-a through various van der Waals interactions and hydrogen bonding, which can inhibit the binding of TNF-a to its receptors, thereby preventing NF-kB activation (52).

Several studies have proven that xanthorhizol induces apoptosis through the activity of p53-dependent mitochondrial pathways. P53 is a gene that can induce apoptosis so that it can be the key cause of cancer cell death. Xanthorhizol can increase p53 and bax regulation but does not affect the antiapoptotic protein Bcl-2. Bax is the product of p53-induced apoptotic genes. It is assumed that p53 transcriptionally activates the proapoptosis bax gene and/or suppresses the Bcl-2 antiapoptotic gene. Bax expression stimulates apoptosis, and bax gene products weaken the effect of Bcl-2 protein (53)

CONCLUSION

50% ethanol extract of temulawak rhizomes originating from five regions has toxic properties against Artemia salina larvae. 50% ethanol extract of temulawak rhizomes from five regions had a toxic effect on Artemia salina larvae with LC50 values of 48.34 mg/L each temulawak extract from the Cirebon region, 41.85 mg/L temulawak extract from the Tembalang region, 64.58 mg/L temulawak extract from the Wonogiri region, 82.17 mg/L temulawak extract from the Jambi region, 105.32 mg/L temulawak extract from the Southwest Sumba region and temulawak extract which has the highest toxicity from the region tembalang with LC50 value of 41.85 mg / L. Temulawak rhizome extract which has the highest toxicity identified qualitatively and quantitatively contains curcumin and xanthorhizol with curcumin content of 74.79 ppm and xanthorhizol of 118.17 ppm. For further research, cytotoxic testing of temulawak rhizome extract against cancer cells can be carried out to ensure the toxic effects of BSLT screening results.

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