RESEARCH ARTICLE

The development of temulawak *(Curcuma Xanthorrhiza)* herb production and pharmacological test as anti-hypercholesterolemia

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Abstract

Aim: This study aims to develop high-grade, safety test of temulawak herb production, and pharmacological test as anti-hypercholesterolemia. **Materials and Methods**: Capsules of temulawak were prepared from rhizome of temulawak herbs. The products were tested for its safety as capsule of temulawak. These included pathogenic microbe test (*Escherichia coli, Salmonella* sp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*), moldy/leavened test, ALT test, and heavy metal level (Hg, Pb, and Cd) test. The quality of the product concerning water content, weight similarity and product stability associated with curcuminoid content and the volatile materials were also tested. The activity as anti-hypercholesterolemia assay was finally tested by *in vivo* using male Wistar strain rats aged 50 days with a weight around 150 g which was fed with pellets and quail egg yolk. The treatment to the tested rats was performed to observe the rat blood lipid profile due to the treatment with capsule of temulawak herb products, compared to the negative control (without quail egg yolk) and the positive control which was given the quail egg yolk and simvastatin as an anti-hypercholesterolemia. **Results and Discussions**: The capsule of temulawak product satisfied the demand of safety and product quality standard. The capsule of temulawak does not contain pathogenic bacteria (*E. coli, Salmonella* sp., *S. aureus*, and *P. aeruginosa*), pathogenic mold, and leavened, whereas ALT and heavy metal level (Hg, Pb, and Cd) do not exceed the highest limit of standard; the water content and weight similarity of the capsule of temulawak meet the requirements, whereas the stability of the product measured on the curcuminoid content and its volatile materials are relatively consistent from the 1st to the 3rd month. The results show the effect of the dose and 4-week duration of the administration of temulawak capsule on the levels of cholesterol, triglyceride, high-density lipid, and low-density lipid in the rats' blood. **Conclusion**: The results obtained from the work suggest a potential application of temulawak rhizome for treatment to anti-hypercholesterolemic.

Key words: Anti-hypercholesterolemia, *Curcuma xanthorrhiza*, herb product safety, temulawak capsule herb quality

INTRODUCTION

As a tropical country, Indonesia has got rich naturally flora capital, particularly herbal plants. Some of them are *Curcuma xanthorrhiza roxb.* known as temulawak and *Curcuma mangga* or white turmeric, which are included to *Zingiberaceae* family. Temulawak is an originally Indonesian herb, which is traditionally used to cure hepatitis, increase stamina, and as an anti- hemorrhoids.[1,2] Several researchers who explored toward the pharmacological effect of curcuminoid compounds extracted from the *Zingiberaceae* family showed the typical properties of anticancer,[3-6] anti-inflammation,[7] antiosteoarthritis,[8] anti-Alzheimer,[9] anticholesterol, and reduce triglyceride levels in the blood.[10-14] The chemical

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content of volatile oil in those plants also showed the antibacterial nature.[15] The most reported researchers on secondary metabolic compound content of the plants in *Zingiberaceae* family are *Curcuma domestica; Curcuma longa; Curcuma xanthorrhiza; Curcuma zedoaria* plants.[2] In those several *Curcuma* plants, some of the species were reported to have been investigated and these contain diarilheptanoid derived phenol compound, curcuminoids, and sesquiterpene compound.[2]

Recently, a lot of herbal products or traditional medicines have been made of temulawak either as a whole or a mixture that can be easily found in market in the form of capsule, instant drink, or even bottled drink. Nevertheless, none of those products are standardized either by the safety or their merit. Research on bioactive compound of traditional herbs and herbal product development will be very advantageous economically, industrially, and even with other aspects which are in accordance with national autonomy and pride. The development of high-grade and safety test on temulawak herb products has been performed in this research. The standard measurement for the traditional medicine as established by the Directorate general of medicine and food control of the health department, Indonesia,[16] was applied. Temulawak herb products were tested pharmacologically as anti-hypercholesterolemia by *in vivo* method.

MATERIALS AND METHODS

Apparatus and Reagents

Glassware, analytical balance, water bath, shaker bath, microscope, camera, counter, desk glass, Eppendorf, object glass, drying cupboard, AAS-flame spectrophotometer, mercury analyzer, incubator, petri dish, halogen moisturizer balance, and Spectronic 20 Genesys were commonly used in this work. Temulawak rhizome, curcuminoid (E-Merk), ethanol, aquadest, NaCl 0.9%, microba (*Escherichia coli t*est, *Salmonella* test, *Staphylococcus aureus* test, and *Pseudomonas aeruginosa* test), cholesterol Stanbio Kit, triglyceride Stanbio Kit, high-density lipid (HDL) Stanbio Kit, standard cholesterol, standard triglyceride, positive control (simvastatin), heparin, aquadest, rats feed pellets, and quail egg yolk were involved in this work.

The Production of Temulawak Capsule

The production of the capsule of temulawak was done as the following steps. About 10 kg of temulawak rhizome was peeled, washed in flowing water, sliced in a very thin piece, and dried in vacuum cupboard drying (temperature 30–40°C) until dried rhizome was obtained. It was then grounded and resulted in 1450 g of powder. About 200 g of the powder was then packed into 500 mg/capsules which were piled in capsule board. It was then packed in plastic bottles.

Safety Test on the Capsule of Temulawak

Safety test on the product of temulawak in capsule was performed according to the standard of Traditional Medicine, established by the Directorate general of medicine and food control of the health department, Indonesia.[16] These include pathogenic bacterial tests, such as *E. coli* test, *Salmonella* test, *S. aureus* test, and *P. aeruginosa* test, moldy and leavened tests.

Bacterial, Moldy, Leavened, and ALT tests

About 2 g of capsule of temulawak was mixed with 10 mL of NaCl (0.9% sterile) to produce a Fortex suspension of medicinal substance. For fungi plate number: About 1 ml of the suspension was flattened on and spread throughout a media which contains chloramphenicol to block the bacterial growth; it was then incubated at room temperature for 7 days. The fungi growth was observed and the number of colonies was counted.

For bacteria total plate number: The suspension was diluted on scale of 1/10, 1/100, 1/1000, and1/10,000. About 1 mL of each dilution was taken and flattened on a media surface so that the blood on the petri dish. After that, the petri dish was placed sideways so that the liquid could be assembled in one place, then after the residue of the liquid was thrown away using pipette it was incubated in 37°C for 48 h. Finally, the number of bacterial colonies was counted.

Heavy Metal Level Test

The heavy metal level test of Pb and Cd was conducted using AAS-flame spectrophotometry, while of Hg it was using mercury analyzer.

Product Quality, Weight Similarity, and Product Stability Tests

The water content test was conducted using toluene distillation (azeotropic method) into the flask was put 4 g sample of temulawak and added toluene 60 mL. Then, distillation at 60°C min for 45 min, then raised at 90°C for 15 min, and at 100°C for 30 min. Next is calculated the volume of distilled water. Water content can be calculated by formula:

Water content (V/B) = Volume water measured/initial weight of temulawak × 100%.

The weight similarity measurement was conducted by picking randomly of 20 capsules taken from each capsule board that contains 500 mg/capsules. It was then measured one by ones. The products stability test was done by measuring the curcuminoid level using chromatography and spectroscopy, while the level of vaporized materials was measured using Halogen Moisture Balance.

Determining Content of Curcuminoid

The curcuminoid analysis was performed according to the modification of Zhang method.[17] The standard solutions were prepared by dissolving about 10 mg of standard curcuminoid (E-Merk) in ethanol to 10 mL. The solution was diluted until resulted in the following content: 10; 15; 20; 25; and 30 µg/ mL. The spectrum was recorded in 400–800 nm amplitude by Spectronic 20 Genesys. Of sample preparation, 20 capsules of temulawak products were emptied, homogenized, and all the contents were measured by dissolving it about 10 mg in ethanol up to 50 mL. The filtrate was separated using filter and its spectrum was then recorded in 400–800 nm amplitude by Spectronic 20 Genesys.

Pharmacological Test as Anti-hypercholesterol

Pharmacological test against anti-hypercholesterolemia was conducted by *in vivo*. The test was treated on male Wistar strain rats of 50-day-old, weight 150 g, and all in good health. For about 4 weeks, the rats were then fed on an experimental diet containing 5% of either carboxyl methyl cellulose (CMC) as a control or Indonesian plants which were added to the basal diet. The rats were fed with pellets, given drink of tap water, and also given Quail egg yolk every day, and the weight of each rat, the feed and water intake were recorded daily. The rats were divided into nine groups, and each group was composed of five rats. The contents of cholesterol levels, triglyceride, and HDL were recorded 4 times: (1) Before giving the Quail egg yolks, (2) after giving the Quail egg yolks, (3) after a 2-week administration of temulawak, and (4) after a 4-week administration of temulawak. More detail of anti-hypercholesterolemia test procedure has been published previously.[18]

RESULTS AND DISCUSSIONS

Temulawak, which is produced by the farmer in Kulon Progo, Yogyakarta, Indonesia, and then processed to be the product of temulawak capsules. The product was then treated with the series of herbal tests following the quality and safety tests, and the results were collected in Table 1.

It can be seen from Table 1 that the product does not contain pathogenic bacteria, i.e., *E. coli, Salmonella, S. aureus*, and *P. aeruginosa*; pathogenic moldy, and leavened, ALT and heavy metal levels (Hg, Pb, and Cd) do not exceed the highest limit standard. The water content and the weight similarity concerning the safety of temulawak capsule meet the requirements do not exceed the highest limit standard, whereas the product stability measured on curcuminoid content and its volatile materials are relatively consistent from the 1st to the 3rd month. These data indicate that the product of temulawak capsules was saved as herbal medicine and by the laws of Traditional Medicine established by the Directorate general of medicine and food control of the health department, Indonesia.[16]

Pharmacological test against anti-hypercholesterolemia was conducted by *in vivo*. The treatment of each group of rats lasted for 50 days. The blood of all the rats was taken from sinus orbitalis for the lipid profile analysis which includes determining total cholesterol levels, triglycerides, HDL, and low-density lipid (LDL), before and after giving the Quail egg yolk, after a 2-week and a 4-week administration of temulawak capsule. The results show that there are effects of the dose and 4-week duration of the administration of temulawak capsule on the level of cholesterol, triglyceride, HDL, and LDL in the rats' blood. More detailed discussion has been reported in previous publications.[18]

Thus, the temulawak capsule developed in this work contains curcuminoid of 43.77 ± 2.34 µg/capsule and after storage, for 3 months it is of 41.07 ± 2.62 µg/capsule. According to the previous research,[13] curcuminoid is the most active and abundant compound of the rhizomes of temulawak. Besides, curcuminoid the essential oil of the rhizomes of temulawak also contains xanthorrhizol, but in this work, no analysis of the compound was done. Xanthorrhizol has antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive, antiplatelet, nephroprotective and hepatoprotective, estrogenic, and antiestrogenic properties.[19] The presence of curcuminoid and xanthorrhizol in temulawak may show an activity to lower cholesterol and triglyceride levels in mice. The results of this work are in accordance with some previous reports on the use of extract and active compounds from temulawak as antihyperglycemic and anticholesterol.[11-14]

CONCLUSION

The production of temulawak capsule resulted from a herbal product fulfills the safety and quality standard. The safety of the temulawak capsule showed that the resulted temulawak capsule does not contain pathogenic bacteria (*E. coli, Salmonella, S. aureus, and P. aeruginosa*); pathogenic moldy, and leavened, ALT and the heavy metal (Hg, Pb, and Cd) levels are less than the highest limit standard. The quality of the temulawak capsule showed that the water content and the weight similarity are less than the highest limit standard. The stability of the product measured on the curcuminoid content and the vaporized materials are relatively consistent from the 1st to the 3rd month. There is an effect of dosage and the 4-week duration of the administration of temulawak on the cholesterol, triglyceride, HDL, and LDL levels in the rat's blood. The results obtained from the work suggest a potential application of temulawak rhizome for treatment to anti-hypercholesterolemic.

Table 1: The result of safety and quality test of temulawak herb products

Test parameter	Result	Note
The pathogenic bacteria test		
Escherichia coli	Negative	
Salmonella	Negative	
Staphylococcus aureus	Negative	
Pseudomonas aeruginosa	Negative	
ALT test		
ALT bacteria	700 CFU/g	
ALT mold	5 CFU/g	
Mold and leavened test		
Mold	1 colony/g	Not pathogen mold
Leavened	Negative	
Heavy metal		
Hg	1.81 ppb	Method mercury analyzer
Pb	<0.096 ppm	SSA-flame
Cd	<0.01 ppm	SSA-flame
Product quality test consists of		
Water content test	7.78%/capsule	
Weight similarity measurement	409.55±20.74 mg/capsule	
Product stability		
a. Curcuminoids content		
The 1st month	43.77±2.34 µg/capsule	
The 3rd month	41.07±2.62 µg/capsule	
b. Determining the vaporized material (from the 1st to 3rd month counted)	13.11±0.55%	

ALT: Alanine aminotransferase, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa*

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