Quality Control of Turmeric Rhizome (*Curcuma domestica* Val) as Traditional Medicine from Wonogiri, Central Java

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Abstract: Turmeric is one of the plants that can be used as traditional medicine. To improve the quality of turmeric as a traditional medicine, turmeric must be free from contamination of pesticide residues, aflatoxin, pathogen bacteria, and curcumin content contained therein. The aim of this research was to investigate the contamination of endosulfan and malathion pesticides, aflatoxin B1, *Escherichia coli* microbial contamination, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as to know the content of curcumin contained in turmeric rhizomes. The sample in this research was taken from Wonogiri region of Central Java, Indonesia by random sampling. The methods used were HPLC for Aflatoxin B1 analysis and curcumin and Gas Chromatography for residual pesticide analysis of Endosulfan and Malathion pesticides. Microbial testing included the establishment of Total Plate Count, AKK, MPN *Coliform*, and analysis of *Escherichia coli* microbial contamination, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results showed that the samples were not contaminated by Aflatoxin B1 and Endosulfan pesticides, but contained a residual malathion with levels of 0.014 mg/kg. Microbial test results showed that the turmeric samples from the Wonogiri market did not meet the quality requirements due to contamination of *Salmonella* sp. and the chopped AKK exceeded the specified limits.

1 INTRODUCTION

Turmeric plant (*Curcuma longa* L.) is a plant of biopharmaceutical, a plant that is useful in medicine and consumed as an effort to overcome health problems. Treatment using traditional medicine of turmeric rhizome is one of the alternative therapy which is done from generation to generation. Turmeric rhizome contains the active compounds such as curcumin, essential oils capable of inhibiting the growth of gram-negative and gram-positive bacteria. Curcumin in turmeric is an active compound that gives the yellow colour to turmeric rhizome, curcumin is produced naturally from turmeric rhizome together with two other curcumin analogue compounds that is demethoxycurcumin and bisdemethoxycurcumin (BPOM, 2011). Traditionally turmeric is used for the treatment of itching, tingling, swollen gums, abdominal pain, ulcers, jaundice, and gastrointestinal.

Turmeric rhizome (*Curcuma longa* L.) is used extensively for food, beverage, medicine, cosmetics and textiles. Standard quality of turmeric to be used for raw materials of the drug should also be considered. The quality standard of turmeric should be highly regarded, the standard quality of turmeric as a traditional medicine is free of pesticide contamination, aflatoxin, heavy metals, microbial, and curcumin levels (BPOM, 2016). Turmeric contamination of pesticides occurs in turmeric plants attacked by pests then farmers will spray turmeric with pesticides. The pesticides which commonly used are the type of *malathion* and *endosulfan*. Both of these insecticides have a broad spectrum and non-systemic. The content of pesticides allowed in turmeric is 0.05 mg/kg for the type of organophosphate. In addition to using pesticides to control pests, farmers are also use manure pile for turmeric growth. Manure pile is derived from animal waste and can contaminate turmeric, because bacteria or pathogenic microbes like to live on the faeces (Paramitasari, 2011). Microbes and pathogenic bacteria can be *coliform, Escherichia coli, Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The wrong technique of post-harvest turmeric processing, temperature conditions in the tropics,

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and high humidity cause the turmeric rhizome to be easily overgrown by *Aspergillus flavus* toxigenic strain, *A. parasiticus* and *A. nonius* (Wrather and Sweet, 2016). The product produced by this toxigenic strain is aflatoxin. Aflatoxin is myotoxic, as a secondary metabolic outcomes of these A*spergillus* strains, that can affect immunity, acute necrosis, cirrhosis and liver carcinoma.

According to WHO, countries in Africa, Asia and Latin America use herbal medicine as a complement to the primary treatment they receive. The traditional medicine failure for certain diseases such as cancer and the extensive information about herbal medicine around the world (Sukandar, 2006). Traditional herbal medicine is a mean of traditional medicine which is very important for the distribution of public health. It is seen that these herb has a huge potential and the prospect to be developed to be an opportunity for herbalists to develop their business.

Based on the Regulation of BPOM, traditional medicine used as an internal medicine should be aware of pesticide content, aflatoxin, heavy metals and the presence of microbes such as *Salmonella, Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. These microbes should not be contained in traditional medicine (BPOM RI, 2014). *Escherichia coli* bacteria is used as an indicator of contamination, its presence in processed products indicates contamination of human or animal faeces through the water used. *Staphylococcus aureus* bacteria is a normal flora found in the skin and human lining membrane. While *Salmonella* sp. is a bacteria that causes infection. If swallowed into the body, it will cause symptoms that called *Salmonellosis.*

Based on the preface above, it is necessary to conduct a research about the quality of turmeric rhizome originating from Wonogiri, Central Java, because the region became the centre of traditional herbal medicine industry including turmeric (Sakti, 2009). The method used to determine the pesticides types, malathion and endosulfan, is by gas chromatography for analysis of Aflatoxin B1 and curcumin using HCV microbial testing conducted include the determination of Total Plate Count, AKK MPN Coliform as well as analysts.

2 METHODOLOGY

The instruments used in this research were: HPLC, GC (*Variant 450GC*), column C18. The materials used were: Turmeric, *Peptone Dilution Fluid* (PDF), *Plate Count Agar* (PCA), *Potato Dextrose Agar* (PDA), *Lactose Broth* (LB), *Brilliant Green Lactose Bile Agar 2%* (BGLB 2%), Eosin Methylene Blue Agar (EMBA), *Mac-Conkey broth* (MCB), *Nutrient Agar* (NA), *Tryptone Broth* (TB), *methyl red-Voges proskauer* (MR-VP), Simmon's Citrate *Agar*, *Trypticase Soy Broth* (TSB), *Baird Parker Agar* (BPA), *Brain-Heart Infusion Broth* (BHIB), *Tetrathionate Brilliant Green Broth* (TBGB), *Selenite Cystine Broth* (SCB), *Salmonella-Shigella Agar* (SSA), *Triple Sugar Iron Agar* (TSIA), *Cetrimide Agar.* The turmeric (*Curcuma longa* L.) rhizomes used were from Wonogiri, Central Java and in the form of powder and dry sliced

2.1 Simplicia Characteristic Tests

The macroscopic examination was performed by observing the morphology of turmeric rhizomes by considering the colour, shape, size, and texture. Microscopic examination of the rhizomes was by putting the simplicia powder on the object glass that is dripped with distilled water and covered with a cover glass, and then being observed under a microscope.

2.2 Pesticide Test

A total of 10 grams of turmeric rhizome powder was added with 75 ml of acetone mixture: 1: 1 v/v dichloromethane and left for one night for static extraction process. The powder is filtered with Whatman no. 40 filter paper, then concentrated with a vacuum rotary evaporator until only 1 mL remaining. The sample was then purified by passing it to a chromatographic column containing anhydrous sodium sulfate. Samples were then ready to be injected into gas chromatography (Deptan, 2006).

2.3 Aflatoxin Test

As total of 10 grams of turmeric rhizome powder was added with methanol and aqua bikes mixture (80:20), and left for one night for static extraction process. The powder was then filtrated with Whatman no. 40 filter paper, then concentrated by vacuum rotary evaporator, then rinsed with methanol gradually, and collected in test tube up to 10 mL. Samples are then ready to be injected into high performance liquid chromatography (Deptan, 2006).

2.4 Identification and Determination of Curcumin Levels

2.4.1 Determination of Curcumin Levels with HPLC

A total of 100.0 mg of turmeric extract samples was added into a 100.0 ml measuring flask of 15.0 ml of 0.01 N H_2SO_4, then in the ultrasonic and added 96% ethanol until the limit mark. The solution was filtered with 0.2 µm membrane into the VCC vial and injected as much of 50 µl for 12 minutes. Identification of active substance were done by comparing between sample retention time and standard curcumin retention time.

2.4.2 Qualitative Identification of Curcumin with Thin Layer Chromatography Method

Qualitative analysis of the curcumin was performed using thin layer chromatography method using stationary phase silica gel silicone 60 F254 and the mobile phase used was chloroform: methanol (95: 5) v / v., then observed on 366 nm UV rays.

2.5 Microbial Contamination on Turmeric Sample Test

2.5.1 Homogenization and Sample Dilution

A total of 10 grams of turmeric samples were dissolved in 90 ml of Peptone Dilution Fluid (PDF) media, resulting from 10^{-1} dilution. The resultant dilution was piped as much as 1 ml and inserted into the first tube and dilution of the PDF, resulting 10^{-2} 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (Radji, 2011).

2.5.2 Total Plate Count Determination

Each result of turmeric sample was piped as much as 1 ml, then inserted into a Petri dish containing 15-20 ml of Plate Count Agar (PCA) medium. After the media froze, the Petri dish is incubated at 37°C for 24-48 hours with the position reversed. (Radji, 2011).

2.6 Total Number of Mold and Yeast Examination

The petri dish which contained 15-20 Potato Dextrose Agar (PDA) medium was prepared in advance. As much as 0.5 ml diluted sample of turmeric was put into the surface of the PDA medium, then it was incubated at 20-25°C for five days in a reversed position. The colonies growing on the media were observed and counted on the fifth day (Radji, 2011).

2.7 Coliform Examination

Coliform examination was performed using Most Probable Number method, 5 tubes system. This method includes:

2.7.1 Presumptive Test

The samples were taken into tubes containing Lactose Broth Media (LB). Next, the samples were inserted into second 5 tubes containing 5 ml of LB medium of single concentration. The area of inverted Durham tube. 0.1 ml of the sample was inserted into a row containing 5 ml of single nutritional LB medium and inverted Durham tube (Radji 2011).

Then the tubes were incubated at 37 °C for 24 - 48 hours. The gas-filled tube proceeded with the assertion test. This estimate test was to detect the presence or absence of bacteria capable of fermenting lactose that indicates the presence of colibacteria (Radji, 2011).

2.7.2 Confirmative Test

A total of 1 culture from the number of tubes which were forming the gas in the LB media estimator test was transferred into a tube containing 10 ml of Brilliant Green Lactose Bile Broth 2% (BGLB 2%), with an inverted Durham tube in it and 2 tubes of the same for sample and control (Radji, 2011).

All tubes were incubated at 37 °C for 24 - 48 hours, until the gas was formed in tubes. A tube with gas was tested with *Eosin Methylene Blue Agar* medium. Using an inoculation needle, the gas-formed tube was inoculated on the EMBA plate by scraping it and then incubated at 37 °C for 24 hours (Radji, 2011).

2.7.3 Complete Test

The gas-containing tube in the assertion test was taken using Ose, then it was scraped onto EMBA media. The tubes were incubated at 37 °C for 24 - 48 hours (Radji, 2011).

2.7.4 Gram Staining

A small amount of microbial growth was taken using the Ose tip, then spread onto droplet of water

over the object glass and dried by fixating on a small flame. The main paint solution (Crystalline violet) then washed with running water and dried. Then the preparation was stained with Lugol solution (I_2 + KI solution) and allowed for 45-60 seconds. The color disappears by dipping the preparations into 96% alcohol while being shaken for 30 seconds or until no more dyestuff flows from the preparation. Then the safranine was dropped into the preparation and be left for 1 minute, and rinse with running water and then dry. The object was then examined under the microscope (Radji, 2011).

2.8 Microbial Pathogen Contamination Analysis

2.8.1 Sample Homogenization and Dilution

- Contamination Analysis of *Escherichia coli*

The total of 10^{-1} dilution was taken and inoculated into three tubes containing the Mac Conkey broth medium (MCB) and there was a Durham tube in it. While contamination analysis of *Staphylococcus aureus*, homogenized and diluted with a PDF solution until 10^{-1} dilution. Then the sample was taken and added into a test tube containing 18 ml *Trypticase Soy Broth* (TSB).

- Contamination Analysis of *Salmonella thypi*

This analysis used Lactose Broth (LB) solution, by transferring 25 ml samples aseptically into a bottle containing 225 mL of LB media sample.

- Contamination Analysis of *Pseudomonas aeruginosa*

This analysis used enrichment medium of 100 ml of Tetrathionate Brilliant Green Broth (TBGB) medium and 100 ml of Selenite Cystine Broth (SCB) medium, each then incubated at 37 °C for 24 hours (Radji, 2011).

2.8.2 Growing into Selective Use

The tube which was positive of *E. coli*, the gas on the Durham, was taken into a selective solid medium *Eosin Methylene Blue Agar* (EMBA). The next step was confirmatory testing, IMVIC test, by inoculating bacterial culture on NA media into *indole, methyl red, Voges Proskauer, and citrate (IMVIC)*.

Staphylococcus aureus was inoculated into Brain-Heart Infusion Broth (BHIB) media. As much as 1 ml of each culture in BHIB was piped and transferred into a sterile test tube. Furthermore, 0.3 ml plasma was added in each tube. Plasma clotting showed positive *Staphylococcus aureus* coagulase.

Salmonella sp. bacteria, was identified by inoculating 1 tangle culture in a Petri dish containing *Salmonella-Shigella Agar* (SSA) medium. The alleged *Salmonella* sp. colony was characterized by a colorless colony to pink, clear to opaque (Radji, 2011). The next phase was confirmation test. In this test, 2-5 specific colonies of SSA selective medium were selected and inoculated on NA media. Then the colonies on NA medium were inoculated with puncture and scratching methods on the Triple Sugar Iron (TSIA) medium. If on the slant, *Salmonella* ferment lactose or sucrose then the color of the media turns yellow and if *Salmonella* does not ferment lactose or sucrose then the color of the media remains red or unchanged.

While *Pseudomonas aeruginosa* used the TSB cultures and inoculated on the surface of *Cetrimide Agar* (Cet.A) medium. Observed the growth of greenish colony (Radji, 2011). Further test on suspected colonies of *Pseudomonas aeruginosa,* a catalase test of the NA culture was tilted by taking the colony using an aseptic Ose needle. Placed on a glass object that was previously cleaned with 70% flattened alcohol, then added a drop of 3% hydrogen peroxide solution. Positive results are characterized by gas formation (Radji, 2011).

3 RESULT AND DISCUSSION

3.1 Materials

The materials used were rhizomes and turmeric powder (*Curcuma domestica* Val.) which were obtained from Wonogiri market region of Central Java, because Wonogiri is a central area of traditional medicine (herbal medicine), and in that area there are many small or medium industries that produce traditional medicine (herbal medicine) where one of the raw materials is turmeric.

3.2 Sample Identification

3.2.1 The Macroscopic Test

The Macroscopic examination aimed to observe the colour, shape, size and texture of turmeric rhizomes. As seen in table 1.

According to Pharmacopoeia Herbal Indonesia (2008), fresh turmeric colourized by yellow-orange, reddish orange-yellow to brownish orange-yellow and has a round shape up to rounded, sometimes branching, a width of 0.5 cm to 3 cm, length 2 cm to 6 cm. Based on the results of macroscopic tests, it

Table 1: Macroscopic test result of turmeric.

Macroscopic	Turmeric
Colour	yellow on the inside, brown on the outside
Shape	oval slighlty round

showed that the inside was yellow in colour, while the exterior was brownish and elliptical with a width of ± 2.5 cm, and length ± 4.5 cm.

3.2.2 Microscopic Test

This microscopic test was performed to see the anatomy of turmeric tissue, by putting the simplicia powder on the object glass that has been dripped with distilled water and covered with a cover glass, then viewed under a microscope. Microscopic test results from turmeric powder showed that it has epidermal tissue, stomata cells, bearing files and hair cover. It has one-layer epidermis, polygonal-shaped flat, cell wall pouring. Covering hair, conical, straight, or slightly crooked; length of 20 µm to 890 µm, thick wall. Perindrem consisted of 6 to 9 layers of cells in the shape of a long facet, the wall penetrates. Single grain starch is oval end having bulge or round to almost triangle with one side rounded (Farmakope Herbal Indonesia, 2008). The anatomy of tissue in turmeric has a characteristic that is the presence of parenkim, cell clumps and hair cover. The observable tissue anatomy included wood vessels, parenchyma and starch grains. It can be seen in the Figure 1.

The pictures above shows that the microscopic test using a microscope with 10x18 magnification, produced the anatomy of turmeric tissue namely xylem, covering hair, and starch grain periderm. This result was in accordance with the requirements of Pharmacopoeia Herbal Indonesia Issue 1 (2008).

Table 2: Results of chemical turmeric powder identification test.

Turmeric powder	Reagent	Result
2 mg	5 drop of sulfuric acid P	Blood red
2 mg	5 drop of chloric acid P	Brown
2 mg	5 drop of natrium dioxide solution 5% b/v	Red-orange
2 mg	5 drop of ammonia (25%) P	Red-orange
2 mg	5 drop of iron (III) chloride solution P 5% b/v	Brown
2 mg	5 drop of lead (II) acetate solution P 5% b/v	Pink

Table 3: Loss on drying test result.

Handling	Result
Loss on Drying	5,37%

3.2.3 Chemical Identification Test

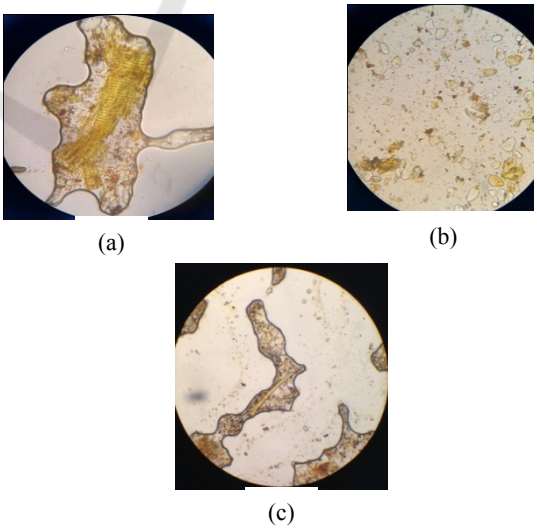
The identification of the turmeric sample was carried out using chemical reactions. Turmeric that had been dubbed and added with Materia Medika Indonesia volumes VI 1977. The resulting chemical identification test of turmeric rhizomes can be seen in Table 2.

The table above shows that the sample used is true turmeric powder because the results obtained are in accordance with the chemical identification test according to Materia Medika Indonesia (1977).

3.2.4 Loss on Drying Test

Loss on drying aimed to provide a maximum (range) of the number of compounds lost in the drying process (MOH RI, 1995). Loss in drying test results is seen on Table 3.

The result above showed that the sample meets the requirements because according to Pharmacopoeia Herbal Indonesia Edition I (2008), the limit of loss on drying is less than 12%.



(a) (b) (c)

Figure 1: The microscopic test result of turmeric: a) xylem with thickening of stairs and parenchyma with secretion cells, b) Starch grain periderm, c) Covering hair with irregular lumps colourized by yellow to brown.

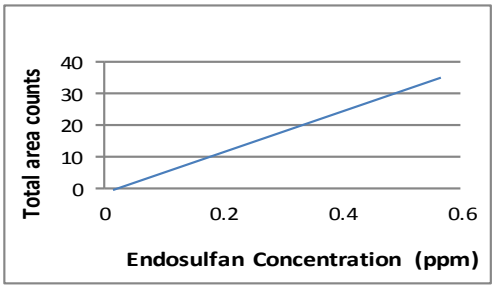


Figure 2: Results of endosulfan calibration.

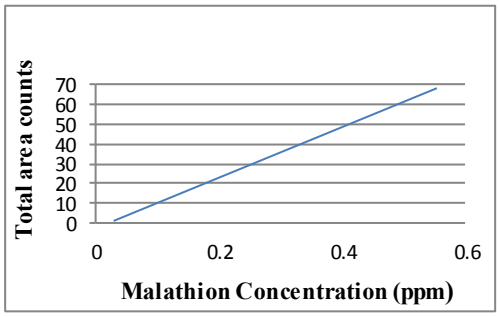


Figure 3: Results of malathion calibration curve.

3.3 Pesticide Test

3.3.1 Standard Normative Endosulfan Curve

Preparation of standard solution of Endosulfan by making five concentrations in acetone solution. Standard normative uptake measurements were performed using gas chromatography with an electron detector (ECD), an oven temperature of 150ºC. The raw solution was injected into the ECD for 20 minutes to get the peak. The result of the linear line equation of the curve is y = 64935x + 19368 with the correlation coefficient (r) = 0.991.

The value of (r) = 0.991 on the endosulfan calibration curve (figure 2) showed that the line formed between the endosulfan concentration with the area are in accordance with line linearity requirements, because the linearity test is done by making the calibration curve, which can produce the equation of the regression line with the correlation coefficient (r) ≥ 0.9990.

Table 4: Results of pesticide test of endosulfan and malathion types.

Tested subtance	Content (ppm)		Average (ppm)	SD
	1	2		
Organochorine	0	0	0	0
Lindan (ɤ-BHC)	0	0	0	0
Aldrin	0	0	0	0
Heptaklor	0	0	0	0
Dieldrin	0	0	0	0
DDT	0	0	0	0
Endrin	0	0	0	0
Endosulfan*	0	0	0	0
Organophosphate				
Diazinon	0	0	0	0
Fenitrotion	0	0	0	0
Metidation	0	0	0	0
Malathion *	0,028	0,0015	0,014	0,0187
Klorfiripos	0	0	0	0
Parathion	0,041	0,090	0,065	0,035
Profenopos	0	0	0	0

* Tested Substance

3.3.2 Standard Normative Malathion Curve

Standard normative uptake measurements were performed using gas chromatography with an electron detecting detector (ECD), an oven temperature of 150 ºC. The standard solution was injected into the apparatus for 20 min to get the peak in appendix 4. The result of the linear line equation of the curve is y = 12666x + 4449 with the correlation coefficient (r) = 0.992 (figure 3).

3.3.3 Pesticide Content on Sample

The sample of turmeric was measured using gas chromatography. In this gas chromatography, the system was arranged with 1mL/minute flow velocity, using gas phase N_2 with 80 psi flow pressure, stationary phase VFRV 1701 Pesticide capillary with diameter 0.25 µm, 30 m long, ECD detector (Electron Capture Detector) and at a temperature of 300 ° C. The measurement results is seen on Table 4.

The results of pesticide residue analysis on turmeric rhizome showed that the sample was not detected containing pesticide residue organochlorine especially for endosulfan type, because the use of pesticides for the group of organochlorines has been banned by the Minister of Agriculture with Law No. 434.1/kpts/TP.270/7/2001 due to its persistent in the environment (Isnawati, 2005). The result of the test was the contamination of organophosphate pesticide residue of malathion type with concentration of 0.014 ppm. Judging from the resultant content of the sample, it still fulfilled the requirement because based on the quality requirement of turmeric simplistic of the Food and Drug Control Agency concerning the Maximum Limit of Pesticide Residue by 0,05ppm (BPOM, 2006). The test results also detected other types of pesticides group, other organophosphates Parathion with levels of 0.065 ppm. This is because the organophosphates in the

turmeric rhizome can be induced by the use of pesticides directly and indirectly (due to contamination of surrounding pesticides). Pesticides move from agricultural land to rivers and lakes carried by rain or evaporation left behind or dissolved in the flow of the surface, located on the soil layer and dissolved along with the groundwater flow. (Djojosumarto, 2008).

3.4 Aflatoxin Contamination Analysis

The result of analysis of aflatoxin contamination on turmeric rhizome by HPLC method. The prepared sample was then measured using HPLC with a wavelength of 365 nm.

Analysis of turmeric rhizome aimed to identify the absence of aflatoxin B1 in the sample. Aflatoxin B1 analysis on samples showed negative or undetectable results, this may be due to the aflatoxin content of the turmeric sample was small, so the test did not show results, because handing in pre and post-harvest was good, so that the turmeric was not contaminated by aflatoxin.

3.5 Curcumin Analysis

3.5.1 Qualitative and Quantitative Curcuminoid Analysis

The qualitative test of curcumin compound was done by Thin Layer Chromatography (TLC) method using chloroform: methanol (95: 5) v/v and the stationary phase used was silica gel 60 F254 (Pharmacopoeia Herbal, 2008). The result of curcuminoid identification by Thin Layer Chromatography can be seen in Figure 4.

The curcumin identification performed was observed with *UV cabinet* on 366 nm UV rays, the detection did not use spray reagent because curcuminoid was visible under UV light at 366 nm wavelength. The curcuminoid identification result shows that there are 3 rickshaws in which curcumin compound (X) is a constituent of turmeric, while desmethoxycurcumin (Y) and bidesmethoxycurcumin (Z) are the identifying compounds of curcuminoid. The result of identification of the spots obtained looks like standard spotted results with Rf value of 0.68 and the Rf value of the four samples is also equal to the standard. The spots are curcumin compounds because they have the same color and Rf values in each sample and the comparators used.

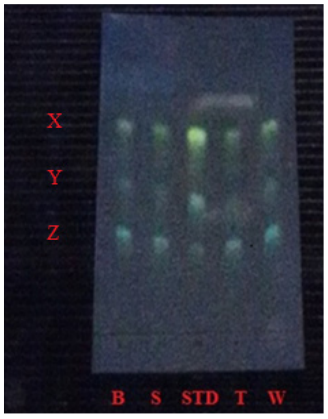


Figure 4: Result of curcumin content identification with TLC at wavelength 366 nm. (X): Curcumin; (Y): Demetoxicurcumin; (Z): Bisdemetoxicurcumin; (STD): Standard of Curcumin.

3.5.2 Curcumin Content Test with HPLC

After testing curcumin with TLC, High Performance Liquid Chromatography was performed. 96% ethanol was used as a solvent in the determination of curcumin content by using HPLC. Curcumin testing method with gradient technique using 0.1% Trifluoroacetic acid (TFA) motion phase and Acetonitrile at 425 nm wavelength for 12 minutes with a flow rate of 1.0 ml/min. Tests using a gradient system aimed to separate samples containing components with a very diverse polarity that can provide good results. Determination of the level of curcumin with this gradient system obtained 3 chromatograms at the standard and the first chromatogram sample is curcumin ranging from retention time 8.6 minutes, followed by chromatogram desmethoxycurcumin at retention time 9,2 minutes and bidesmethoxycurcumin at retention time 9.7 minutes.

Result of curcumin with HPLC test found that the turmeric plants a good place to grow turmeric growth. Land in the Wonogiri area has alluvial soil type where this type of soil is good for turmeric growth (Raharjo and Rostiana, 2005). Alluvial soil is a soil formed from fine deposits. This type of soil is widely used in agriculture due to its nature which has a high nutrient content. Turmeric plants can grow well in rainfall ranging from 2000 to 4000 ml/year and has rainfall ranging from 2,790 mm.

Table 5: The result of tests of total plate count on powder and chopped.

Turmeric	Number of colonies (Colony/g)	Maximum Limit of Microbial Contamination	Information
Powder	1,17 X 10^4	≤ 10^6 colony/g	Qualified
Chopped	7 X 10^4	≤ 10^6 colony/g	Qualified

3.6 Bacteria Test

3.6.1 Total Plate Count Test Result

The powder and rizhomes turmeric samples were grown on Seed Plate Count Agar (PCA) hatching medium calculated bacterial growth. Bacteria that can be calculated ranges from 30-300 colonies, so the results obtained as in Table 5.

The total number or amount of aerobic bacteria from the turmeric sample powder and chopped is between 1.17 X 10^4 to 3.45 X 10^6 bacteria per ml of sample. According to the provisions stipulated by the Head of the Food and Drug Supervisory Agency No. 12 of 2014, on the requirements of traditional medicine that the total number of chopped plates and powder ≤ 106 then the turmeric sample from the eligible Wonogiri Market is set.

3.6.2 Total Number of Mold and Yeast Result

In this test, samples of turmeric powder and chopped were grown on Potato Dextrose Agar media (PDA) and calculated the growth of mold and yeast. Colonies that can be calculated ranging from 30-300 colonies, resulting in Table 6.

The fungi were a group of eukaryotic microorganisms that vary widely, so the ability to take advantage of nutrients from the environment and metabolic abilities of fungi also vary widely. The total amount of mould and yeast from the turmeric sample obtained was 8.85 X 10^3 to 4.6 X 10^4 mould and yeast per ml sample. The total amount of mould and yeast in the turmeric powder had fulfilled the requirements set by the Head of the Food and Drug Administration Law No. 12 of 2014 of traditional medicine that is 10^4, but the chopped sample did not meet it, maybe the case in tttthe process of storing the simplicia that have not met the criteria.

Table 6: Result of determination of total number of mold and yeast turmeric powder and chopped.

Turmeric Sample	Number of Colonies (Colony/g)	Maximum Limit of Microbial Contamination	Information
Powder	0,885 X 10^4	≤ 10^4 colony/g	Qualified
Chopped	1,13 X 10^4	≤ 10^4 colony/g	Not qualified

Table 7: Results of the *Coliform* MPN examination turmeric powder and chopped.

Turmeric Sample	*Lactose Broth*			Number of Postive Tubes	MPN/100 ml
	10 ml	1 ml	0,1 ml		
Chopped	2	3	0	2-3-0	9
Powder	0	0	0	0-0-0	<2

3.6.3 Most Probable Number (MPN) of Coliform Examination Result

Coliform examination results of turmeric (powder and chopped) showed a positive result of the presence of Coliform bacteria, thus the examination was followed by IMVIC test. The complete Coliform test consists of 3 stages: test of predictor, test of confirmation and complete test. In the LB (Lactose Broth) probe test, a positive result is seen by bubbles forming on the Durham tube. The formation of gas in Durham tubes as a result of *lactose* fermentation does not necessarily indicate the number of *Coliform* bacteria, because lactose can also be fermented by other microbes such as lactic acid bacteria. Therefore, the positive probable test should be continued with the assertion test, the BGLB (Brilliant Green Lactose Bile Broth) test containing bile salt is a component that can inhibit bacterial growth besides Coliform, and give Coliform bacteria chance to grow well.

The result of the research of turmeric samples in the form of powder and chopped on medium (BGLB) showed negative result, with MPN value 9/100 small on chopped and <2/100 ml on powder (table 7). Furthermore, the tube showed a positive in the assertion test, followed by a test by inoculating a dispute of the assay test results into a Petri dish containing *the Eosin Methylene Blue Agar* (EMBA) medium and incubated at 37 °C for 24-48 hour.

Table 8: Result of pathogen microbes.

Turmeric Sample	Bacteria Identification Test			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella*	*Pseudomonas aeruginosa*
Chopped	Negative	Negative	Positive	Negative
Powder	Negative	Negative	Positive	Negative

3.6.4 Pathogen Microbial Contamination Analysis Result

The presence of pathogenic bacteria in food should be avoided so that product users are protected from adverse effects caused by consumed products. One cause of disease transmission and the cause of poisoning is contamination of microbes in a food. Microbes in terms of bacteria, such as *Escherichia coli*, *Staphylococcus aureus, Salmonella,* and *Pseudomonas aeruginosa* can contaminate food consumed by humans. The examination results (table 8) of pathogenic microbial contamination was obtained as follows:

Escherichia coli

The formation of gas in Durham tubes as a result of lactose fermentation does not necessarily indicate the number of *Coliform* bacteria, because lactose can also be fermented by other microbes such as lactic acid bacteria. The results of the *E. coli* test showed a negative on the chopped sample and the positive on the powder sample in MCB medium. The positive of the powder samples were scratched onto the EMBA media. The results of the casting on the EMBA media showed negative or no gloss, so the negative *E. coli* test results on both the turmeric sample were concluded.

Staphylococcus aureus

Staphylococcus aureus is a normal flora found in human skin. It is a type of pathogenic bacteria that can cause infection and abnormalities in the skin (Radji, 2011). Ecologically, *Staphylococcus aureus* is closely related to humans especially in the skin, nose and throat. Thus, food and drink are mostly polluted through management by humans. Overall, these organisms are not strongly competitive with others and consequently these bacteria have no important role in uncooked food ingredients.

However, in cooked or salted food, where existing organisms have been damaged by warming or growth inhibited by salt concentrations, *Staphylococcus aureus* cells may continue to progress to a dangerous level. Poisoning due to *Staphylococcus aureus* contaminated food is mostly related to food products that have been cooked especially those managed by humans. The symptoms of *Staphylococcus aureus* contaminated food are intoxicated. The growth of these organisms in food produces toxic enterotoxins, which when ingested may result in abrupt on slaught, stomach cramps and severe vomiting. Diarrhea may also occur (Buckle *et al*., 2007).

The examination result of pathogenic microbial *Staphylococcus aureus* planted into BPA media (Baird Parker Agar) showed negative result. This medium contained lithium chloride and tellurite to grow the microbes in the sample, as well as pyruvate and glycine that support the growth of *Staphylococcus aureus* bacteria. If the samples contain *Staphylococcus aureus* bacteria, colony will grow glossy black colour, as the results of the analysis did not show a shiny black result, turmerics from Wonogiri Market were negative from *Staphylococcus aureus* microbial pathogens.

***Salmonella* sp.**

Salmonella test is used to establish the presence of pathogenic microbial *Salmonella* sp. These microbial pathogens are Gram-negative microbes that are stick-shaped and cause typhoid, paralysis and foodborne diseases. *Salmonella* sp. consists of 2500 serotypes which are all pathogenic in both humans and animals. TSIA is rich in lactose, sucrose, dextrose, ferrous sulfate. The medium is used to sort out microorganisms that have the ability to degrade sulfur and ferment carbohydrates. With the fermentation of phenol red, if microorganisms can not ferment the three types of sugar (sucrose, lactose, glucose) present in the media then the media will turn yellow. If microorganisms can only ferment dextrose. The occurrence of dextrose fermentation by *Salmonella* will decrease the pH to acid condition. This condition causes phenol red (red medium) changes to yellow. That is what happened on examination of bacterial pathogen powder and turmeric samples from Wonogiri Market.

Pseudomonas aeruginosa

Medium Cet. A (*Cetrimide Agar*) is commonly used for the isolation of *Pseudomonas aeruginosa.* Cet. A is a quarternary ammonium compound that can inhibit the growth of other bacteria, but does not occur in *Pseudomonas aeruginosa*. The examination result of chrysanthemum and turmeric powder from Wonogiri are negatively contain *Pseudomonas aeruginosa*.

4 CONCLUSION AND SUGGESTION

4.1 Conclusion

Based on the research on turmeric rhizome from Wonogiri area, it can be concluded that the samples did not contain endosulfan type organochlorinated pesticide residues, but it contained pesticidal residue from organophosphate type that is malathion, with 0,014 ppm concentration level. The curcumin level met the requirements of Pharmacopoeia Herbs of ≤ 6.6% and the curcumin content was 7,8482%. Contamination of *Salmonella* sp. bacteria was found, and thus it did not meet the requirements of the Regulation of the Head of the Food and Drug Supervisory Agency No. 12 of 2014 about traditional medicine.

4.2 Suggestion

We recommend to test other quality standards such as the heavy metal test on turmeric samples from Wonogiri.

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