

Determination of Acetaminophen in Jamu Pegal Linu by High Performance Liquid Chromatography

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ABSTRACT: Traditional medicine is very popular in Indonesia, so it is necessary to do this research to analyze the presence or absence of chemical substances contained in traditional medicine. This research aims to determine acetaminophen content on jamu pegal linu circulating in Makassar city by High Performance Liquid Chromatography (HPLC). The jamu were obtained from several drugs stores near Terong traditional market. Five brands of jamu pegal linu detected their acetaminophen content by HPLC method using a UV-Visible SPD 20A detector with a wavelength of 254 nm. The result showed that chromatogram on jamu pegal linu is the same as the acetaminophen at the retention time of 5.28 minutes. The whole of jamu pegal linu analyzed positively contain acetaminophen and prohibited for consumption in accordance with the regulations of National Agency of Drug and Food Control (POM) Republic Indonesia No.KH.00.01.43.2773/2008.

KEYWORDS: Traditional medicine; jamu pegal linu; acetaminophen; High Performance Liquid Chromatography.

1. INTRODUCTION

Traditional medicine is one of Indonesia's cultural heritages that has been used for centuries, for the maintenance and improvement of health and prevention and treatment of diseases. Herbal medicine in Indonesia commonly called "Jamu". There are many types of jamu in Indonesia based on therapeutic benefits that can be generated, one of them is jamu pegal linu to overcome muscle pain (Banureah, 2009).

Seeing the large demand of herbal medicine, many manufacturers are taking advantage of this opportunity by producing their excellent range of products. In addition, many new herbal manufacturers are emerging. Herbal products produced including jamu pegal linu with various brands. Jamu pegal linu is one of the favorite products by the community, especially the livelihood as a field worker (Firdaus and Utami, 2009). Based on empirical evidence on the use of medicinal plants, the use of medicinal plants as traditional medicine proved relatively safe. Correct use rarely leads to side effects as reflected by the community's assumption that traditional medicine is a safe drug with no side effects. That opinion is not entirely true because it can happen that traditional medicine becomes unsafe due to several causes like mixing with chemicals (Handayani, 2001).

The abundance of herbal products makes the government difficult to supervise regularly. A World Health Organization (WHO) survey indicated that about 70–80% of the world's population who still rely on the use of herbal medicine as the main treatment (Akerele, 1993). This gives a gap to the possibility of cheating by some poor producers such as the addition of chemical drugs with the aim that the herbal consumed immediately felt the effect by consumers that will lead to high demand (Firdaus and Utami, 2009).

The addition of chemicals contradicts the Indonesian Law No.36 / 2009 on health which states that traditional medicines are ingredients or ingredients in the form of plant materials, animal materials, mineral materials, galenic or a mixture of such ingredients has been used for treatment, and can be applied in accordance with the norms prevailing in the community (Wahyuni and Sujono, 2004; Ditjen POM RI, 2004). The above rule is contrary to the addition of chemicals such as acetaminophen drugs added in some herbal preparations as traditional medicine including jamu pegal linu. Thus, needs to be done research on the chemical content of drugs in this case acetaminophen on jamu pegal linu circulating in the city of Makassar.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Standard Solution

Acetaminophen as standard reference was purchased from Sigma Chemie GmbH Aldrich. Pro analysis grades of iron (III) chloride (FeCl₃) and methanol was purchased from Merck. The mobile phase was prepared by mixing distilled water and methanol (3:1), then filtered with a 0.2 µm membrane filter then sonication. The stock solution of acetaminophen (c = 100 µg/L) was prepared by dissolving 5 mg with 50 ml of the mobile phase and homogenized by sonication for 10 minutes. The result then filtered with a 0.2 µm membrane filter. The obtained filtrate was measured in HPLC (Nasution, 2009).

2.2. Sample Preparation and Chemical Test

There are five samples of jamu pegal linu were purchased randomly at traditional market in the city of Makassar. Samples were weighed 5 mg, each inserted into a measuring flask, and added a 25 ml mobile phase, then homogenized, respectively. The mixture was filtered, and the solution was taken on HPLC system for measuring (Saragih, 2010). The qualitative test of acetaminophen was performed with the addition of distilled water and FeCl₃ solution resulting a purplish blue of sediment (Naid, 2011).

2.3. HPLC Determination

Quantitative analysis of the acetaminophen standard (100 µg/mL) and extracted samples (200 µg/mL) were conducted using a HPLC system (SHIMADZU®). The samples were conducted sonication for 20 minutes filtered through disposable syringe filter (0.45 µm cellulose nitrate); the filtrates were directly infused into HPLC system. An octadecylsilane column (C₁₈) was used for the separation of the acetaminophen that using methanol : water (7:3 v/v) as the mobile phase. The flow rate was fixed at 1.0 ml/min into a column. The injection volume was 20 µL and ultraviolet SPD 20A detector wavelength of 254 nm was applied. The separation of active substance occurs by chromatographic mechanism, separation result is read by detector, recorded by recorder, calculated area (y) main peak of each standard solution and sample solution (Nasution, 2009).

2.4. Data analysis

The qualitative parameter is used retention time by comparing the retention time of chromatogram of the sample solution with reference standard solution of genistein in the same HPLC conditions (Fawwaz and Baits, 2016). A calibration standard curve for genistein was obtained by running on HPLC and then plotting area against concentrations. The best fit of the line curve was calculated by equation of line. Linearity was evaluated through the correlation coefficient (R²). The correlation coefficient, intercept and slope of calibration curve were calculated. The best fit of data was determined by linear regression using the following equation: $Y = bx + a$, where:

- Y = Absorbance
- b = Slope
- x = Concentration
- a = Intercept

3. RESULTS AND DISCUSSION

Contamination of harmful substances in food and beverages needs to be a concern because of the large amount of poisoning that causes death caused by contamination of hazardous chemicals. In recent years the analysis of contamination of food and beverage ingredients has continued to increase, including the determination of the levels of hazardous metals and residues of drugs either intentionally added or incidentally (Fawwaz, 2019).

Herbs are actually included in the food supplement category, made from natural ingredients as part of the plant, such as roots, leaves, and bark. Some also use materials from animal bodies, such as goat bile. The customer also not directly feels the effect, because it is supplement. It should be suspected if the effect of herbs is instantly (Banureah, 2009). The mistake made by herbal manufacturers is to use acetaminophen as a mixture in the manufacture of traditional medicine. Acetaminophen is deliberately added to make the herb more effective instantly. Manufacturers choose acetaminophen because of generic drugs that have a fairly cheap price, but manufacturers ignore the side effects caused when consumed in excessive doses and in the long time.

The results showed that all samples contain acetaminophen; this is evidenced from the results of chemical testing using FeCl₃, which showed a positive result that is the formation of purplish-blue sediment. HPLC detection use column C₁₈ as stationary phase, it most widely used because the ability to separate the compounds with various polarity. While the mobile phase used is water and methanol (3:1), it means isocratic elution, with a wavelength of 254 nm UV detector, previously running on a spectrophotometer.

The result illuminates that there is a certain amount of acetaminophen in all samples characterized by the same retention time at 5.28 minutes between standard reference and samples (Table 1), as evidenced by chromatogram data of acetaminophen in Figure 1 and the samples can be examined in Figure 2.

Table 1. Characteristics of acetaminophen standard reference and level of acetaminophen in sample jamu pegal linu

Samples (100 µg/mL)	Area (y)	Concentration (µg/mL)	Retention Time (min)
Acetaminophen	14894106	-	5.28
Sampel A	139117	0.93	5.26
Sampel B	150796	1.01	5.24
Sampel C	101413	0.68	5.23
Sampel D	70611	0.47	5.25
Sampel E	58239	0.39	5.37

The other research explain that herbs can be harmful, due to the contamination, inclusion of toxic species, allergens, pollen, insect parts, heavy metals such as lead, mercury and arsenic and scheduled poisons (drugs), whether intentional or unintentional, have been cited as the causes of herbal adverse reactions or toxicities (Hussin, 2001).

Other problems when mixing Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) into herbal medicine products, especially aspirin can lead to serious interactions if mixed with herbs that have antiplatelet activity such as ginkgo, garlic, ginger, bilberry, dong quai, feverfew, ginseng, turmeric, meadowsweet and willow. If aspirin is mixed with herbs containing tamarind, then it can increase bleeding. Similar to acetaminophen, it can also interact with ginkgo and some of the previously mentioned herbs, with the effect of increasing bleeding. Furthermore, the occurrence of hepatotoxicity and nephrotoxicity can occur when interacting with *Echinacea* and kava herbs, and with herbs containing salicylate (willow, meadowsweet), respectively (Abebe, 2002).

It should be noted that there are medicinal plants that have an analgesic, antipyretic, and anti-inflammatory effect, like *Cyperus rhizoma* (root puzzle) as analgesic, *Zingiberis rhizoma* as anti-inflammatory, *Kaempferia rhizoma* (kencur) as analgesic-antipyretic, *Carryphylli flos* (clove flower) as analgesic. *Rhizoma corydalis* processed with vinegar has greater analgesic effect (Chan, 2003).

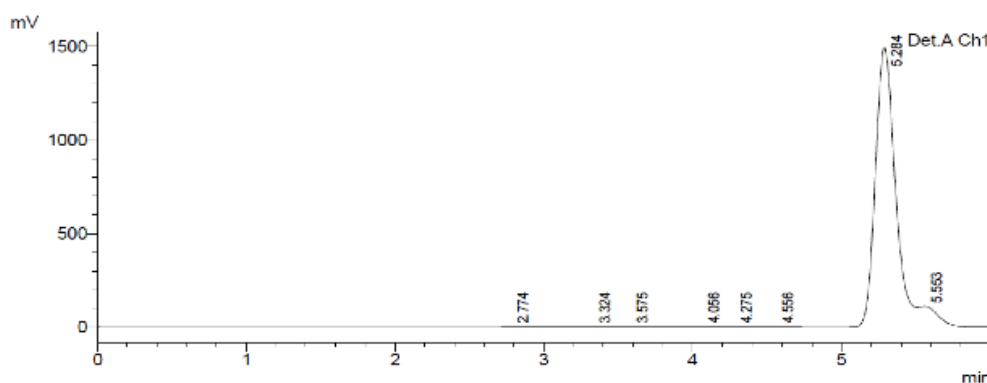


Figure 1. Chromatogram of acetaminophen standard reference

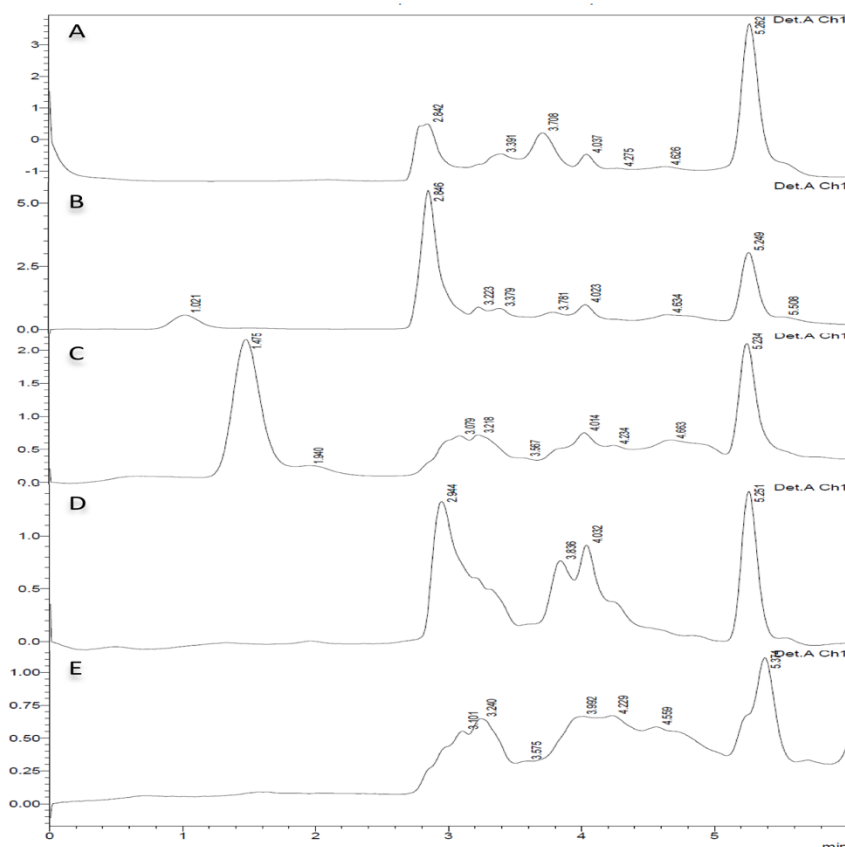


Figure 2. Chromatogram of sample A, B, C, D, and E

It's good the manufactory produces traditional medicine like jamu pegal linu by adding ingredients of medicinal plants that have the effect as above, even if it does not provide an instant effect. However, it can meet the standard of traditional medicine and is not harmful to people consumed. Finally, strict supervision of government is required in the production of herbal medicine with reference to Good Manufacturing Practice (GMP). The result illuminates that there is

a certain amount of acetaminophen in all samples characterized by the same retention time at 5.28 minutes between standard reference and samples (Table 1), as evidenced by chromatogram data of acetaminophen in Figure 1 and the samples can be examined in Figure 2.

4. CONCLUSION

Based on the results of the analysis conducted on 5 types of jamu pegal linu circulating in the city of Makassar, it turns out all the samples contains acetaminophen. According to the regulation of the POM RI No.KH.00.01.43.2773/2008 on traditional medicine containing medicinal chemicals, acetaminophen should not be present in traditional medicine.

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