Dominant chemical compound of chicken bile extract

Tuty S*, Fidrianny I, Sukrasno

Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung-40132, Indonesia

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**ABSTRACT**

The purpose of this research is to study the dominant chemical compounds of chicken bile extract. Chicken bile, which is usually disposed of as useless waste, is made a choice. The study began with material collection from native chicken farmers, Kluwut Village, Bulakambah sub-district, Brebes Regency, Central Java. The determination was carried out at Bandungense Herbarium School of Biological Science and Technology-ITB. The choice of animals were native chickens. They belonged to the family Phylum: Chordata, Class: Aves, Nation: Galliformes, Tribe: Phasianidae, Surname: Gallus, Type: Gallus gallus Linn, The common name: native chicken (Indonesia), and domestic fowl in English. The bile portion was cut into small pieces and dried using a freeze dryer. The reflux method was then extracted using solvents with a different polarity, which are n-hexane, ethyl acetate, and ethanol. It gave out n-hexane extract, ethyl acetate extract, and ethanol extract. The extracts were evaporated using a rotary evaporator. The percentage of the obtained yield was n-hexane extract 7.63%, ethyl acetate extract 8.61%, and ethanol extract 34.91%. Selected extract ethyl acetate extract was fractionated by Vacuum Liquid Chromatography (VLCI) and was monitored by a thin layer of Chromatography (TLC). Then fraction 5-6 was continued to sub-fractionation by Classical Column Chromatography (CCC). Nuclear Magnetic Resonance (NMR) investigated isolate X and found that isolate X appeared to be cholesterol. From the second fractionation (VLCII), fraction 2-7 proceeded to the VLCIII, and then subfraction 7 was evaluated using GC-MS. The dominant chemical compounds of subfraction 7 were oleic acid 38.72%, n-hexadecanoic acid (35.6%), octadecanoic acid (17.94%), palmitoleic acid (1.53%).

*Corresponding Author

Name: Tuty S
Phone: 0812 1435 3933
Email: tuti.slamet@yahoo.co.id

**INTRODUCTION**

The study aims to study the local chicken bile of Java, which is usually considered as a waste. There are different types of chicken - local chicken and broiler chicken. Studies have been conducted on other animals like - bovine (Gomez et al., 2017), bear (Feng et al., 2009), and pig bile (Ipharraguerre et al., 2018). Also, there have been studies conducted on the antioxidant levels of chicken (Tuty et al., 2019). But, there hasn't been any specific research on the utility of chicken bile by humans. Based on chicken population data in West Java Province, it can be seen the number of chicken bile that becomes waste is very much compared to the population of native chickens in Central Java amounting to 40,753,808. Gall bladder in chicken functions as a reservoir of various poisons (toxins, antibiotic waste, etc.) that enter through chicken feed and drink into the digestive tract which is then filtered by the liver to be...
further discharged through the small intestine with chicken manure. Thus the accumulation of poisons or toxins in body cells can be avoided. One way to find active compounds by using the guided fractionation bioassay method. By knowing the activity of a group of chemical compounds contained in the fraction, isolation of compound can be performed so that the active compound obtained. Components in chicken bile may be similar to bear bile. UDCA (Ursodeoxycholic acid) has many effects, such as immune-modulators (Yoshikawa et al., 1992) and cyto-protection (Heuman et al., 1991). Studies on chemical compounds in chicken bile extracts (i.e. n-hexane, ethyl acetate, and ethanol) have not been reported. This study aims to evaluate the dominant chemical compounds of chicken bile extracts.

**RESULTS AND DISCUSSION**

Antioxidant compounds have a vital role in health. Various scientific evidence showed that antioxidant compounds could ward off free radicals that can trigger multiple chronic diseases such as cancer, coronary heart disease and diabetes. In Chinese medicine formulars, bear bile has also been used for the treatment of diabetes, nephritis, haemorrhoids, hepatitis chronic (Wang et al., 2005). Other research shows that bear bile mainly consists of bile acids, amino acids, bile pigments, fats and some phospholipids (Feng et al., 2009). Bile consists of bile acids, protein, bile salts, calcium and fat. The function of bile is for the absorption of fats and vitamins A, D, E and K (Graha, 2010; Vítek and Haluzík, 2016). Other researchers (Murlin et al., 1975) examined the effects of micelle formation and absorption of neutral chicken fat and fatty acids; meanwhile, this study examined the chemical content of chicken bile extract. The study began with material collection from native chicken farmers, Kluwut Village, Bulakambah sub-district, Brebes Regency, Central Java. Then the chicken bile is cut into small sizes and dried using a freeze dryer to reduce the water content in crude drug to obtain a stock of chicken bile that is not damaged and can be stored for a long time without the need for refrigeration (Hariyadi, 2013). The extraction was carried out by the reflux method, using solvents with increasing polarity namely n-hexane, ethyl acetate and ethanol in sequence to separate compounds in three polarities. Most non-polar compounds will be extracted in n-hexane solvent; most semi-polar compounds will be separated in ethyl acetate solvent and finally most polar compounds in ethanol. Chemical screening of crude drug and chicken bile extract has also been done to determine the presence of secondary metabolites and gave positive results on phenols, flavonoids and steroids/triterpenoid. The ethyl acetate extract of chicken bile has been proven to have the highest antioxidant activity compared to n-hexane and ethanol extracts (Tuty et al., 2019). Antioxidant compounds have a significant role in health. The scientific evidence showed that antioxidant compounds could ward off free radicals that can trigger various chronic diseases such as cancer, coronary heart disease and diabetes. Bile consists of bile acids, amino acids, bile pigments, fats and some phospholipids (Feng et al., 2009). The function of bile is for the absorption of fats and vitamins A, D, E and K (Graha, 2010). The other research by (Murlin et al., 1975) exposed the effects of micelle formation and absorption of neutral fat and fatty acids in chickens, meanwhile this study examined the chem-

**MATERIALS AND METHODS**

**Materials**

Chicken bile, freeze dryer (Benchtop) a set of reflux apparatus, a collection of vacuum liquid chromatography apparatus, a set of classic column chromatography apparatus, TLC silica gel GF254, Nuclear Magnetic Resonance (Agilent). Gas Chromatography-Mass Spectrometry (Agilent). Other chemicals used were analytical grade.

**Preparation of sample**

Chicken bile was cut into small size and dried using a freeze dryer and then stored in a dry bottle.

**Extraction**

Extraction was done by reflux method using solvents with increasing polarity, namely n-hexane, ethyl acetate, and ethanol to obtain n-hexane extract, ethyl acetate extract and ethanol extract. The selected extract, namely ethyl acetate extract, was fractionated, then subfractionated to get the chemical compound of chicken bile.

**Isolation of active compounds**

The selected extract (ethyl acetate extract) was fractionated by vacuum liquid chromatography (VLC I) method (Gritter et al., 1991). The fractions obtained was monitored by thin-layer chromatography (TLC). Then fraction 5-6 was continued to subfractionation by classical column chromatography (CCC) and found the isolate X.

Isolate X, which was obtained then investigated using Nuclear Magnetic Resonance (NMR) (Creux et al., 1998). Meanwhile, from the second fractionation (VLCII), fraction 2-7 proceeded to the VLCIII and then subfraction seven was evaluated using GCMS.
Figure 1: Research flow

Crude Drug Chicken Bile

↓ Reflux (n-Hexane)

n-Hexane extract

Residue

↓ Reflux (ethyl acetate)

Ethyl acetate extract

VLC I

FR 1-20

FR 5-6

CCC

Isolate X

NMR

Cholesterol

VLC II

FR 1-20

VLC III

FR 2-7

Sub FR 7

GC -MS

Oleic acid
n-Hexadecanoic acid
Octadecanoic acid
Palmitoleic acid

Figure 2: H¹-NMR of isolate X
Table 1: Confirmation of isolate X with literature

| No | \( ^{13}C \) Isolate X | \( ^{13}C \) Cholesterol |
|----|------------------------|------------------------|
| 1  | 37.4                   | 37.5                   |
| 2  | 31.8                   | 31.6                   |
| 3  | 72.0                   | 71.3                   |
| 4  | 42.4                   | 42.4                   |
| 5  | 140.8 quarterner       | 141.2                  |
| 6  | 121.9                  | 121.3                  |
| 7  | 32.1                   | 32.0                   |
| 8  | 32.1                   | 32.3                   |
| 9  | 50.3                   | 50.5                   |
| 10 | 36.7 quarterner        | 36.5                   |
| 11 | 21.2                   | 21.2                   |
| 12 | 39.9                   | 40.0                   |
| 13 | 42.4 quarterner        | 42.4                   |
| 14 | 56.9a                  | 56.9                   |
| 15 | 24.5                   | 24.3                   |
| 16 | 28.4                   | 28.3                   |
| 17 | 56.3                   | 56.5                   |
| 18 | 12.0                   | 12.0                   |
| 19 | 19.6                   | 19.4                   |
| 20 | 35.9                   | 35.4                   |
| 21 | 18.9                   | 18.8                   |
| 22 | 34.3                   | 36.5                   |
| 23 |                        | 24.1                   |
| 24 | 39.7                   | 39.8                   |
| 25 |                        | 28.3                   |
| 26 | 23.0                   | 22.8                   |
| 27 | 22.7                   | 22.8                   |

Figure 3: C\(^{13}\)-NMR of isolate X
ical compounds of chicken bile extract. The fractionation of selected extracts (ethyl acetate extract) was carried out by vacuum liquid chromatography (VLC I) method, silica gel 60 as stationary and combination of n-hexane, ethyl acetate and methanol as mobile phase, and got 20 fractions. Then the fractions were monitored by TLC. Furthermore, fractions which had the same pattern were combined. The flow chart of this study can be seen in Figure 1. Then fractions 5-6 was subfractionated by classical column chromatography (CCC), silica gel 60 as stationary phase and the mobile phases using a combination of n-hexane and ethyl acetate, and found 95 subfractions then monitored by TLC. Isolates X was obtained from the subfractionation process. Furthermore, isolate X was characterized and identified by RMIH and C13 (Figure 2 and Figure 3). The result stated that isolate X was cholesterol. Confirmation results of isolate X can be seen in Table 1 (Kalinowski et al., 1988). According to (Anderson and Connor, 1994), cholesterol has a role as an insulator, a constituent of cell membranes, an essential component in the synthesis of vitamin D2, bile acids, adrenocortical hormones and reproductive hormones. The second fractionation (VLC II) was conducted, then fractions 2-7 was continued to VLC III, and subfraction seven was evaluated using GC-MS (Willet and Kealey, 1987). The result demonstrated that dominant chemical compounds of subfraction 7 were oleic acid (38.72%), n-hexadecanoic acid (35.6%), octadecanoic acid (17.94%), palmitoleic acid (1.53%) Figure 4. Oleic acid and palmitoleic acid are unsaturated fatty acids. The previous researches exposed that oils containing polyunsaturated fatty acids (PUFA) are known to reduce blood cholesterol and increase other health values (Praagman et al., 2019). Meanwhile, the other studies expressed that n-3 fatty acids (FA) are essential nutrients in early human development (Nettleton, 1993).

**CONCLUSION**

From this study, it can be concluded that chicken bile extract contains the dominant chemical compounds are cholesterol, oleic acid, n-hexadecanoic acid, octadecanoic acid and palmitoleic acid, which are very beneficial for human health.

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None

**Conflict of Interest**

None

| Peak | R time | %     | Name              |
|------|--------|-------|-------------------|
| 33   | 22,400 | 38.72 | Oleic acid       |
| 30   | 20,596 | 35.6  | n-Hexadecanoic acid |
| 34   | 22,574 | 17.94 | Octadecanoid acid |
| 29   | 20,328 | 1.53  | Palmitoleic acid |

Figure 4: GC-MS Spectrum of subfraction 7
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