

A Study on Entrapment Efficiency of Earthworms (*Lumbricus rubellus*) Extract in the Ethosomal Drug Delivery System

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Abstract

Earthworms (*Lumbricus rubellus*) extract is known to contain bioactive protein as antibacterial compounds. One of the disadvantages of polar compounds is slow penetration into the skin layers which can be solved by formulating it in the form of ethosomal drug delivery system. The aims of this research was to find out information about ethanol concentration that provide the highest entrapment efficiency of the ethosome. Earthworms powder was macerated using 50% ethanol for 3 days. The extract was formulated into ethosome with variation of ethanol concentration that are 20%, 30%, 40%, and 50%, respectively. The measurement of entrapment efficiency was conducted by measuring the amount of active protein of earthworms extract that was entrapped in ethosome vesicles using spectrophotometer UV-Visible. The results showed that the entrapment efficiency was directly proportional to ethanol concentration in formula.

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Keyword

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Introduction

Earthworms (*Lumbricus rubellus* L.) had been used as China traditional medicine and empirically used by people to treat thypoid fever caused by *Salmonella thyposa* and acne vulgaris caused by *Propionibacterium acnes* (Immanita, 2012; Sun, 2015). This treatment was supported by previous studies which showed that earthworms has antibacterial and antioxidant activity (Aldarraji, 2013; Mathur, 2011). Earthworm extract in concentration 7.5% could inhibit the growth of *Propionibacterium acnes* and treat inflammation due to acne (Asmawati, 2016). Earthworm *L. rubellus* was made in a solid dosage form proved to have broad spectrum antimicrobial against Gram-positive bacteria *S. aureus*, Gram-negative bacteria *E. coli*, and fungi *C. albicans* (Damayanti *et al.*, 2008). Besides, earthworms also contained phenolic compound which has antioxidant activity (Aldarraji, 2013).

The disadvantages of earthworms extract are having bad smell and slow absorption into skin layer caused by polar bioactive compounds. Previous research has conducted the formulation of earthworm extract in the gel preparation, but it was not able to cover the smell up (Asmawati, 2016). Another formula containing earthworm extract, maltodextrin, distilled

water (2 : 5 : 50) was made by encapsulating extract in maltodextrin and tested for its antibacterial activity (Istiqomah, 2012).

The ethosome is a carrier of a kind of soft vesicle contained phospholipid, high concentrations of alcohol, and water (Tiwari, 2010 ; Patel, 2013). Ethosomal drug delivery system has many advantages such as good delivery of protein molecules, containing non toxic materials, increasing drug permeation into skin layers, and convenient to use.(Razavi, 2015; Sujatha, 2014). Good penetration of ethosome is caused by two mechanisms. Firstly ethanol takes a role as penetration enhancer which is penetrate into intercellular lipid, increase lipid fluidity of cell membrane, and decrease multilayer lipid of cell membrane. Secondly, ethosome increases skin permeability so that it is easily permeated into inner layer of skin and release the drug (Parashar, 2013).

Entrapment efficiency is the most important thing in characterization study of ethosome formulation. It indicates the amount of drug or bioactive compounds which is entrapped in the vesicles. It will absolutely affect the amount of drug that reaches the target of therapy (Barupal,2010). In order to find out the highest entrapment efficiency of earthworms extract, the measurement of entrapment efficiency was done to four ethosome formula with variation of ethanol concentration (20%, 30%, 40% and 50%). The aims of this research was to find out information about ethanol concentration that provide the highest entrapment efficiency of the ethosome.

Materials and Methods

Earthworm Preparation

Dried earthworms (*Lumbricus rubellus* L.) that were used in this research obtained from Makassar, South Sulawesi, Indonesia.

Extraction of Earthworms

Earthworms extraction was done by using maceration method. A total of 500 g dried earthworms was macerated using 50% solvent ethanol for 3 days. The solvent was evaporated using rotary evaporator and continued with lyophilization to obtain viscous extract.

Measurement of Total Protein Content of Earthworms Extract

A total amount of 50 mg extract was dissolved in distilled water to 10 ml (5 000 ppm). Taken 200 µl of the solution and then added with reagent C, incubated for 10 minutes and reagen D, incubate for 20 minutes. Distilled water was added up to 5 ml (200 ppm). The absorbances were measured using spectrophotometer UV-Visible at 702 nm.

Preparation of Ethosomal Dispersion

The composition of ethosome formula of earthworms extract is shown in Table 1.

Table 1. Composition of ethosome formula of earthworms extract

Compositions	Amount			
	F1	F2	F3	F4
Earthworms extract	0.5 %	0.5 %	0.5 %	0.5 %
Soya lecithin	2%	2%	2%	2%
96%Ethanol	20%	30%	40%	50%
Propyleneglycol	10%	10%	10%	10%
Distilled water up to	100%	100%	100%	100%

The ethosome was made using cold method. Earthworms extract was dissolved in ethanol and homogenized using magnetic stirrer for 5 minutes at 750 rpm, 40°C. After that, soya letichin was dispersed into the mixture. After 5 minutes, propyleneglycol was added into the mixture and homogenized for 5 minutes. Distilled water at 30°C was added at last. The size of ethosome vesicles was minimized by sonicating for 15 minutes.

Determination of Entrapment Efficiency

An amount of 1 ml of ethosome was added to effendorf tube, then centrifugated for 2 hours at 13.000 rpm, 4°C. Taken 250µl of the supernatant, then was added with reagen C, incubated for 10 minutes and reagen D, incubated for 20 minutes. Distilled water was added up to 5 ml. The absorbance was measured using spectrophotometer UV-Visible. Percentage of entrapment efficiency was calculated using the formula below:

$$EE = \frac{\text{amount of entrapped drug}}{\text{amount of total drug}} \times 100\%$$

Results and Discussion

Entrapment efficiency is an important thing in the characterization study in ethosomal formulation. It indicates the amount of drug entrapped in the prepared vesicles (Verma, 2011). Because of that, it is expected that the higher the entrapment efficiency, the higher amount of drug that reaches target of therapy. In this study, there are four concentration of ethanol that was used that are F1 (20%), F2 (30%), F3 (40%), and F4 (50%).

The results showed the percentage of entrapment efficiency of F1, F2, F3, and F4 that are 35.56%, 30.45%, 59.16%, and 72.58%, respectively as shown in Figure 1.

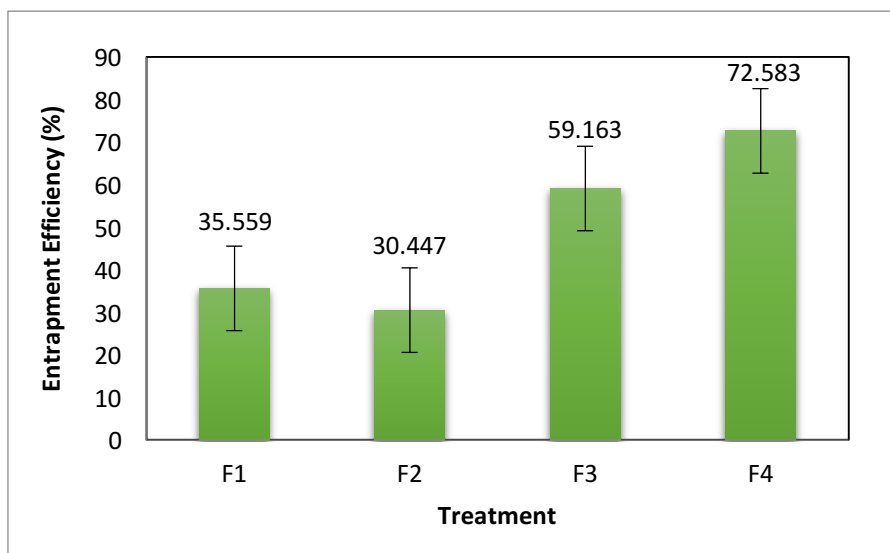


Figure 1. Entrapment Efficiency of Ethosome of Earthworms Extract

The results indicated that the entrapment efficiency was affected by ethanol concentration in formula. The highest entrapment efficiency was 72.58% given by F4 which containing 50% of ethanol. This is likely due to the solubility of extract in the ethosomal core was higher in F4 than other formulas. In this study, the entrapment efficiency of the formulation escalated along with the increasing of alcohol concentrations. Alcohol is a natural enhancer, which has the property to alter the skin permeability. However, transdermal permeability of

ethosomal formulations was found to be higher compared to hydroalcoholic drug solution which indicates that alcohol is not the only one contributor to increase the skin permeability.

Several studies have investigated the possible mechanism of enhancing skin permeability by lipid vesicular system. Vesicles can interact with the stratum corneum lipids and alter the permeability, which facilitates penetration of drug across stratum corneum. Enhanced permeation of drug with ethosomal formulations could be caused by the combination effect of alcohol and lipid vesicular system (Chourasia,2011). According to Vijayan (2015), entrapment efficiency was affected by molar ratio of lecithin and ethanol. In addition, in the formulation of serratiopeptidase enzyme ethosome, the highest entrapment efficiency was 75.37% given by formula containing lecithin 5% (w/w) : ethanol 20 ml, propyleneglycol 10 ml, active compound 0.05 mg (Vijayan, 2015). Another previous research showed that there are two factors influencing the entrapment efficiency that are Soya Phosphatidyl Choline (SPC) and ethanol concentration. The amount of these materials influenced the entrapment of ketoprofen inside lipid vesicles in a positive way. It has been said the entrapment efficacy of 75-80% was provided by formula containing 30-40% of alcohol and 2,5-3% of SPC. While the lower efficacy was performed by formula containing 20-25% of alcohol and 1-2% of SPC (Chourasia,2011).

In addition, research about aceclofenac ethosome has shown that the highest and the lowest entrapment efficiency was given by formula containing 30% and 50% of ethanol respectively. There was increase in percent entrapment efficiency was observed with an increase of ethanol concentration, but when ethanol concentration exceeded 30%, a decrease in entrapment efficiency was observed. If ethanol concentration increased above 30% resulting into leakage of drug from fluidized bilayer of vesicles (Barupal, 2010). The obtained results of this research was in line with previous mentioned research which indicated that the higher the concentration of ethanol, the higher its entrapment efficiency. Besides, based on mechanism of drug entrapment in ethosome, hydrophilic drugs are entrapped in the aqueous core of lipid carrier while lipophilic drugs are retained in the nonpolar chain (Chourasia, 2011). So, earthworms extract which is hydrophilic was entrapped in the core of ethosomes vesicle.

Conclusion

The highest percentage of entrapment efficiency of earthworms extract ethosome was 72.58% presented by formula containing earthworms extract 5%, soya lecithin 2%, propyleneglycol 10%, and ethanol 50%. The entrapment efficiency of the formulations was observed to increase along with the increasing concentration of alcohol.

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