Fractionation of secondary metabolites from *Serratia plymuthica* UBCF_13 based on polarity properties

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Abstract. *Serratia plymuthica* UBCF_13 bacteria produce compounds capable of suppressing the growth of various pathogenic fungi. There are several types of compounds produced by these bacteria. The compounds produced by *S. plymuthica* bacteria have different abilities and characteristics. So, to determine the capabilities and characteristics of each compound, a compound separation technique or method is needed. This study was conducted to determine the optimal mobile phase composition for the separation of compounds. Optimization of the mobile phase was carried out by spotting the extracellular extract of *S. plymuthica* on thin layer chromatography (TLC) using several comparisons of ethyl acetate with hexane and ethyl acetate with methanol. The separation technique in this research will be useful for further study of the antifungal compound *Serratia plymuthica* UBCF_13, such as the identification and characterization of each of the compounds it produces. In this study, the best mobile phase was not found in the separation of compounds. There are several mobile phases that separate the compounds, for further test the elution gradient method is used.

Keywords: Fractionation, TLC, Antifungal compounds.

1. Introduction

Bacteria are widely used as biocontrol agents for pathogenic fungi. One of the bacteria that has potential as a biocontrol agent is from the genus *Serratia* [1]. *Serratia plymuthica* can protect plants from various types of phytopathogenic infections [2,3,4,5]. [6] reported that *Serratia plymuthica* strain HRO-C48 has been commercialized as an agricultural fungicide. *S. plymuthica* UBCF_13 produces many compounds capable of suppressing the growth of various pathogenic fungi one of them is *Colletotrichum gloeosporioides*. [4] reported that *Serratia plymuthica* UBCF_13 was able to suppress the growth of *C. gloeosporioides* by 26%. The ability of *S. plymuthica* is regarded by compounds that its produces, such as chitinase enzymes, pyrrolnitrin, siderophores, proteases, and indole acetic acid (IAA) which function as antifungal compounds [2,7].

The compounds produced by *S. plymuthica* have different abilities and characteristics. To find out the characteristics of each compound, it is necessary to separate the compounds. The method often used for the separation of compounds is chromatography. The principle of chromatography is the separation of compounds based on the movement between two phases, namely the stationary phase and the mobile phase. The stationary phase and the mobile phase have different polarity properties [8]. Compounds that have a polarity similar to the mobile phase will be carried and separated into
individual components based on their polarity characteristics [9]. [14] tested bioactive compounds detected from Pseudomonas cepacia using thin layer chromatography, [15] purified the active compounds from Pencillium sp. using column chromatography, and [10] also using chromatography techniques to identify secondary metabolites produced from Dysidea fragilis. In this research, the method used is the thin layer chromatography (TLC).

2. Materials and methods

2.1. Preparation of Isolates and Culture Media

Serratia plymuthica UBCF_13 was obtained from Biotechnology Laboratory, Faculty of Agriculture, Andalas University and was cultured on 20ml of LB medium and incubated for 16 hours (150rpm, 27°C) and adjust OD=1.

2.2. Production of Antifungal Compounds

S. plymuthica UBCF_13 which has OD=1 was taken 2ml and put into 200ml of PDB + 1% glucose medium. The culture was incubated for 48 hours in the shaker (150rpm, 27°C). The culture was harvested to obtain extracellular compound (supernatant) using a centrifuge (ThermoFisher Scientific SL-16R) at a speed 12,000 rpm/17.709 xg at 4°C for 10 minutes.

2.3. Extraction of Antifungal Compounds

The supernatant was extracted using ethyl acetate in a ratio of 1:1. Mix 200ml of supernatant with 200ml of ethyl acetate in a 500ml Erlenmeyer flask then incubated for 6 hours. The mixture is put into a separating funnel until two phases are formed then separated. The lower phase was re-extracted with ethyl acetate. This extraction was carried out three times on the same culture.

Ethyl acetate containing extracts of bacterial extracellular compounds was separated using a rotary evaporator (45°C) to evaporate ethyl acetate. The extract was resuspended in a methanol solution.

2.4. Mobile Phase Optimization

The solvents used as the mobile phase are methanol, ethyl acetate, and hexane. The solvent for the mobile phase used was obtained from a mixture of 2 solvents (methanol with ethyl acetate, hexane with ethyl acetate) with a ratio of 0:10,1:9,2:8,3:7,4:6,5:5, 6:4,7:3,8:2,9:1,10:0.

The mobile phase optimization was tested using TLC, the length of TLC is 10cm. The extract of bacteria extracellular was spot on the origin line (0.5cm from the bottom of the TLC) as much as 3µl then put into a chamber containing the mobile phase according to the comparison treatment. When the mobile phase has reached the upper limit of TLC (1cm from the top of the TLC) the separation is stopped.

Visualization of the results of TLC separation using a staining reagent (10% HCl in 100ml of methanol) then heat it on a hotplate until it forms a spot then mark it with a pencil.

3. Results and Discussion

3.1. Optimization of The Mobile Phase

The production of antifungal compounds from Serratia plymuthica UBCF_13 was obtained from the extraction using ethyl acetate. Ethyl acetate is used because it has a wide range of polarity. Ethyl acetate is semi-polar, it can bind polar and non-polar compounds. The results of compound extraction are evaporated using a rotary evaporator to obtain a crude extract of the compound. This crude extract which is assumed to be an antifungal compound will be fractionated into a pure fraction. Before fractionation, it is necessary to optimize the mobile phase.

The mobile phase optimization is carried out to determine the best mobile phase for the separation of compound extracts. Various types of organic solvents have been used as mobile phases, but not all
of them are efficient. It is necessary to know the strength of each solvent to increase the selectivity of separation, so it is necessary to optimize the mobile phase[11]. Optimization is carried out by a series of experiments by changing the ratio of the solvent ratio or changing the type of solvent[11]. [12] said that a mixture of two solvents is better as a mobile phase, for example, variations in the ratio of polar and non-polar solvents will get better separation. Using two solvent mixtures can also cover the entire polarity range [11].

The polarity of the *S. plymuthica* UBCF_13 extract has unknown, so an organic solvent is used which can cover overall range of polarity. The organic solvents used are methanol (polar), ethyl acetate (semi-polar), and hexane (non-polar).

![Image](image_url)

**Figure 1.** Optimization of the mobile phase using a solvent mixture of methanol and ethyl acetate at the ratio 0:10, 1:9, 2:8, 3:7 and 4:6

The solvent mixture of methanol and ethyl acetate in Figure 1 shows a good separation, namely at a ratio of 0:10, 1:9, 2:8, and 3:7. At a ratio of 4:6 there is the separation of compounds but there are spots that accumulate and there are tails at some points. At a ratio of 5:5 to 10:0 the separation that occurs is not good and has a tail [13]. Because several ratios have good separation, the elution method used is gradient elution. Elution gradient is a good method for separating compounds that have narrow polarity [12]. The ratios 1:9 and 2:8 have similar separation patterns so that only one of them is used, namely 1:9.
The results of the separation of compounds from the mixture of hexane and ethyl acetate solvents in Figure 2 show that good separation is in the ratios of 0:10, 3:7, and 4:6. The separation that occurs in the ratios 3:7 and 4:6 is similar so that only one ratio is chosen. At the ratio of 1:9 and 2:8 there is separation but there are many compounds still left on the origin line so it is not good for further test. At a ratio of 5:5 to 10:0 the separation that occurs is not optimal and the spots have tails.

Based on the results of TLC and the spots formed in the mobile phase which are semi-polar to polar compounds, tend to be carried upward. This shows that the compounds obtained are assumed to be polar. The compound will only be adsorbed if it is eluted with a polarity that matches it [12].

4. Conclusion
The optimal mobile phase for the separation of compounds in this study has not been found. To obtain a good separation of the compound several mobile phases are used. For further test, the elution method used is stepped gradient polarity.

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