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**Research Article**

# Potential of Ribosome-inactivating proteins (RIPs) of *Mirabilis jalapa L.* as an antiacne: Effect on Proliferation of Cultured Sebocyte Cells and its Antibacterial Activities against *Propionibacterium acnes* and *Staphylococcus epidermidis*

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**Abstract**

Previous researches showed that Ribosome-inactivating proteins (RIPs) isolated from *Mirabilis jalapa L.* demonstrated some bioactivities such as *in vitro* cytotoxic on cancer cell lines, antiinflamation and inhibition of nodule growth in animal model. Therefore, this protein has a potential to be developed as antiacne. Pathogenesis of acne is caused by some factors such as growth of sebocyte cells that produce sebum and infection caused by bacteria of *Propionibacterium acnes* and *Staphylococcus epidermidis*. This research was aimed to determine whether the extract has an effect on proliferation of primary cultured sebocyte cells and whether the extract possess antibacterial activities against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Result of this study could have a benefit in the further development of use of protein of *M. jalapa* as antiacne.

RIPs of *M. jalapa* leaves were extracted using phosphate buffer. This protein fraction was then determined in its activity on cleaving of supercoiled double stranded plasmid DNA in order to identify presence of RIPs in the extracts. The protein fraction was then tested on cultured sebocyte cells and on its antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Results demonstrated that the protein extract at concentration of 0.3-2.5 mg/mL has inhibition activity on proliferation of cultured sebocyte cells. The protein extract has bacteriostatic effect on growth of *Staphylococcus epidermidis* (MIC 10 mg/mL).

**Keywords:** *Mirabilis jalapa*, Ribosome-inactivating protein (RIP), antibacterial, proliferation, sebocyte cells

## 1. Introduction

Ribosome-inactivating proteins (RIPs) are protein toxins that are widely distributed in plant kingdom. The toxic activity is due to the ability of the protein to inhibit protein synthesis by inactivating ribosomes through cleaving the N-glycosidic bond at the $A_{324}$ position of 28S RNA[1]. There are two classes of RIPs which are type I consists of a single N-glycosidase domain and type II of RIPs composed by an A-chain that is attached to a sugar-binding B-chain lectin domain[2]. The presence of RIPs in an extract of plant scan is determined by their activity to cleave super coiled double stranded DNA into the nicked circular and linear form[3]. Many reports have also showed that RIPs from plants possess activities as anticancer, antivirus and antibacterial[4].

Previous studies reported that *Mirabilis jalapa* L contains RIPs that has cytotoxicity activity in some cell lines such as T47D and HeLa. One of mechanism of the cytotoxicity activity has been known by mechanism of inducing apoptosis[6]. Furthermore, another study showed that RIPs of *M. jalapa* have some bioactivities such as anti-inflammatory, anti-allergic and inhibition of nodule growth in animal model[7]. Two types of RIPs of *Mirabilis expansa* (ME1 and ME2) have been purified and were known to have antibacterial activity on some bacteria such as *Pseudomonas syringae*, *Agrobacterium radiobacter* and *Agrobacterium tumefacien.* In addition, the ME1 and ME2 have antifungal activity against *Pythium irregulare* and *Fusarium oxysporum solani*[8]. ME1 has been known to have antibacterial activity by inhibition of bacterial protein synthesis[9].

Antibacterial activity of crude protein extracts of seeds of some medical plants against bacterial strains has been reported[10]. For example protein extract of *Allium ascolinicum* extracted using sodium phosphate citrate buffer was active to inhibit growth of *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus*. The other protein extracts of seeds of *Rumex vesicarus*, *Ammi majus*, *Cichorium intybus*, *and Curcuma sativus* have also been reported to have antibacterial activity against some bacterial strains[10]. The researches showed that protein from plants is considerable potential to be developed as antimicrobial proteins.

There is limited studies investigating potency of the RIPs from *M. jalapa* as antiacne. This research was therefore aimed to determine whether protein extracts of *M. jalapa* have effects on proliferation of sebocyte cells *in vitro* and on growth of bacteria involved in acne pathogenesis.

## 2. Material and Methods

*Mirabilis jalapa L.* leaves were obtained from local garden in Universitas Gadjah Mada University (UGM), Yogyakarta, Indonesia. pUC18 was obtained from laboratory stock of Laboratorium Penelitian Dan Pengujian Terpadu (LPPT), UGM, Yogyakarta, Indonesia**.** Bacteria of *S. epidermis* was stock of Microbiology Laboratory, Faculty of Medicine, UGM and bacteria of *P. acne* was obtained from Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia.

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**2.5 Extraction of protein from *M. jalapa* leaves.**

About 5 gram of *M. jalapa* leaves was weighted and then grinded by using mortar and pestle, followed by addition of 10 mL of phosphate buffer pH 7.2 with 0.14M NaCl. The grinded solution was transferred to eppendorf and centrifuged at speed 7500 rpm for 30 minutes at 4°C. The supernatant was crude protein extract of *M. jalapa* and then was partial purified using acetone to get different protein fractions. All isolates were kept in the fridge for further analysis.

**2.6 Preparation of super coiled DNA and measurement of cleavage activity of super coiled DNA**

Plasmid DNA pUC18 was isolated from *Escherichia coli* DH5α and purified using modified method of alkaline lysis procedure. A portion of supercoiled double stranded plasmid DNA (pUC18) was incubated with various amounts of protein fraction containing RIPs to a final volume of 20 µl in 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, pH 8.0, at room temperature for 1 hour. At the end of the reaction, 10 µl of loading buffer (30% glycerol, 200 mM EDTA, 0.25% bromophenol blue and 0.25% xylene cyanol FF) were added. Electrophoresis was carried out using a 1% agarose gel in 0.5 x TBE (tris-borat) buffer. DNA bands were visualized by staining the gel with ethidium bromide.

**2.7 Preparation of primary culture of sebocyte cells for studying effect of protein fraction on proliferation of sebocyte cells.**

Preparation of primary culture of sebocytes cell was carried out based on method of Xia *et al*., (1989)[11]. Pieces of prupitium were washed using phosphate buffer and then incubated in 2.4 U/mL dispase for 20 h at 4ºC. Epidermis and dermis were separated, followed by isolation of sebaceous glands in epidermis. Sebaceous gland was seeded in DMEM containing 4.5 g/L glucose, Ham's F 12 medium (3:1) and was supplemented with 10% FCS, 10 ng/mL epidermal growth factor 0.4 µg/mL hydrocortisone, 3.4 mM L-glutamin and penicillin /streptomycin

**2.4 Antibacterial Activity Test**

Antibacterial activity was tested using disk diffusion method. Positive control used in this study was clindamycin (0.8µg) and as negative control was phosphate buffer at pH 6.5. Protein extracts loaded for antibacterial activity test against *Staphylococcus epidermidis* were series of concentration that was 31µg, 63µg, 125µg, 250µg and 500µg/disk and incubation at 37°C for 24 hour. The activity against bacteria of *Propionibacterium* acnes were carried out at concentration of 25µg, 50µg, 100µg, 200µg and 400µg/disk and incubation for 2 x 24 hours, 37°C under anaerobic conditions and blood MHA media. The bacterial suspension used in the treatment was equivalent to 0.5 McFarl and standard. Antibacterial activity was determined by measuring the diameter of the inhibitory zone (mm)

**2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

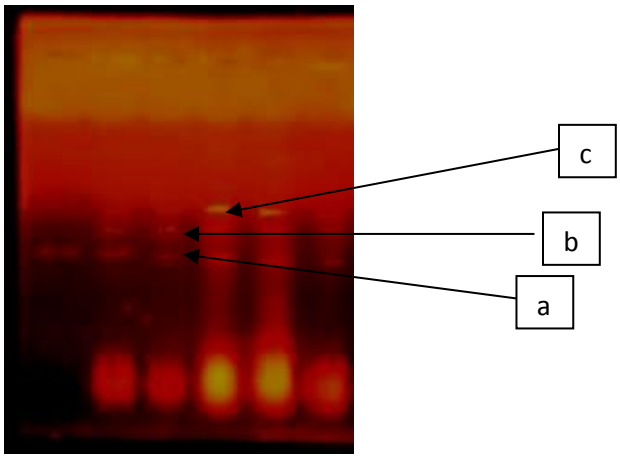
Determination of MIC and MBC of protein extract against *Staphylococcus epidermidis* was done at concentration series of 0.62, 1.25, 2.5, 5, 10 mg / mL using a liquid dilution method. The OD measurements were carried out using visible spectrophotometry at wavelength of 625 nm.

## 3. Results and Discussion

**3.5 Cleavage activity of supercoiled double stranded DNA by protein fraction of *M. jalapa***

Activity on cleavage of supercoiled double stranded DNA was used to determine presence of RIPs in protein fraction isolated from *M. jalapa*. In this experiment, pUC18 was incubated with a concentration of protein fraction of the samples at 25°C. The protein extracts was positive to cleavage of supercoiled double stranded DNA activity when the supercoiled DNA band in the agarose gel become to disappear and new band was found (linier form). As shown in figure 1, protein fraction at different stages of protein isolate showed activity to cleave of supercoiled DNA. The supercoiled DNA band (line 2-5) in the agarose gel fainted and new bands were found (nick circular and linier). These results showed that protein fraction of *M. jalapa* has activity on cleavage of supercoiled double stranded DNA and it means that protein fractions that isolated from *M. jalapa* contains RIPs.

**Figure 1. Electrophoregram of agarose gel.**

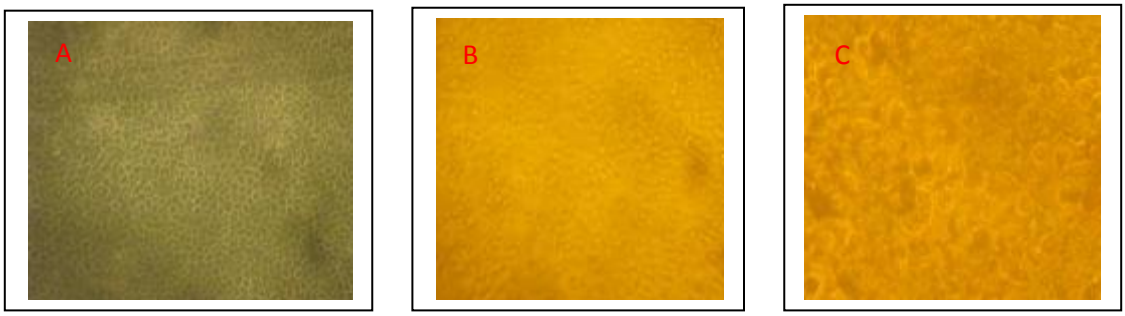



The activity was shown by disappearance of supercoiled DNA and appearance the new band of linier or nick circular form.(a). Supercoiled DNA (b). Linier DNA (c). Nick sircular DNA. The order from left to right is: 1. Control of supercoiled DNA; 2. Supernatant of crude extract; 3. Crude extract ; 4. Precipitat of aceton; 5. Supernatan of precipitat aceton; 6. Freezed drying extract.

**3.2 Treatment of protein extract on cultured sebocyte cells**

Cultured sebocyte *in vitro* is a useful tool for studying pathophysiology of acne and other disease related to sebaceous gland. Some studies have isolated sebaceous glands from human skin and developed them into primary and secondary cultured cell lines[11]. Due to there is no availability of collection of both primary or secondary cultured sebocyte cell lines in our laboratory for studying effect of protein extract on growth of sebocyte cell lines, it has been isolated sebaceous gland and prepared primary cultured cells based on method of Xia et al., (1989). The result of this study was shown in Figure 2.

**Figure 2. A: Cultured human sebocyte cells as control (morphology of the cells is diagonal) B: treatment at concentration 0.31 mg/ml and C: treatment at concentration 2.5 mg/ml)**




Cultured sebocyte cells were treated with various concentrations of protein extract of *M. Jalapa* (0.31 – 2.5 mg/ml) and at higher concentration (2.5 mg/ml) the protein fraction had a higher inhibition activity on proliferation of cultured sebocyte cell than that of at lower concentration (0.31) mg/ml. It was suggested that the protein at the range of concentration can inhibit proliferation of cultured sebocyte cells.

**3.3 Antibacterial Activity of protein fraction of *M. jalapa***

Result of antibacterial activity assay against *Staphylococcus epidermidis* showed that protein extracts of *M. jalapa* have bacteriostatic activity by mechanism of inhibition of bacterial growth and the activity started at loading concentration of 250µg. As shown in Table 1 diameter of inhibition zone around the disk of protein extracts at concentration of 250µgand 500µg was10.18mm and10.74mm, respectively. Whilst, protein extract with concentration of 31µg, 63µg, and 125µg did not affect growth of *Staphylococcus epidermidis* because no inhibition zone formed around the disk. Sodium phosphate buffer as negative control had no effect against the bacteria of *Staphylococcus epidermidis*. Positive control of clindamycin results in an inhibition zone with diameter average of 10.0mm.

**Table 1: Antibacterial activity of RIPs on *Propionibacterium acnes* and *Staphylococcus epidermidis***

| ***Staphylococcusepidermidis*** | | | | | ***Propioni bacterium acnes*** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Protein concentration (µg/disk)** | | | | | | | | | |
| 31 | 63 | 125 | 250 | 500 | 25 | 50 | 100 | 200 | 400 |
| - | - | - | + | + | 0 | 0 | 0 | 0 | 0 |

+ : showed diameter of inhibition zone (10.00 – 11.00 mm); - : no activity or no inhibition zone; 0: bacteria did not grow

*Propionibacterium acnes* under anaerobic conditions and blood MHA media in this research was not able to grow and therefore antibacterial activity of the protein extract on *Propionibacterium acnes* in this study is still unknown. The less selectivity of the blood MHA media may affect growth of *Propioni bacterium acnes* in the media.

**3.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

Determination of MIC and MBC value of protein extracts against *Staphylococcus epidermidis* was done using a liquid dilution method at concentrations of 0.62, 1.25, 2.5, 5, 10 mg/mL. The optical density (OD) of protein treatment on the bacteria at concentrations of 5 and 10 mg/mL was - 0.177 and - 0.307, respectively. The negative value indicated that number of bacterial cells after incubation was declined and the activity was categorized as bacteriostatic. The lowest value of Δ OD was - 0.307 (at concentration of 10 mg/mL) suggested that the value of MIC of the protein extract was 10 mg/mL. MBC value was represented by Δ OD = 0 and this value did not appear on this results. It was suggested that the protein extracts were not able to kill bacteria and that means it has no bactericide activity.

## 4. Conclusions

Protein extract of *M jalapa* leave contains *Ribosome Inactivating Proteins*(RIPs) and the extract has bacteriostatic activity to *Staphylococcus epidermidis* with MIC value of 10 mg/mL and the extract at a range concentration of 0.31 – 2.5 mg/mL is able to inhibit proliferation of cultured *sebocyte* cells.

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## References

1. Park SW, Vepachedu R., Owens RA, Vivanco JM, The N-Glycosidase Activity of the Ribosome-inactivating Protein $ME_1$ Targets Single-stranded Regions of Nucleic Acids Independent of Sequence or Structural Motifs. *J. Biol. Chem*. 2006; 279: 34165-34174.
2. Peumans W, Hoa Q, Van Damme E, Ribosome-inactivating proteins from plants: more than RNA N-glycosidase? *J FASEB* 2001; 15:1493-1506.
3. Ling J, Lui W, Wang TP, Cleavage of supercoiled double stranded DNA by several ribosome inactivating proteins *in vitro*. *FEBS Letters* 2004; 345: 143-146.
4. Puri, Ribosome Inactivating Protein (RIPs) from *Momordica charantia* for antiviral therapy. *Current Molecular Medicine* 2009; 9:1080-1094.
5. Sudjadi, Sismindari, Herawati T, Prasetyowati AT, Purification of Ribosome-Inactivating Protein (RIP) from *Mirabilis jalapa* L leaves by CM-Sepharose CL-6B and Sephacryl S-300HR columns. *Indon. J. Pharm*. 2003; 14: 316 - 321.
6. Ikawati Z, Sudjadi, Widyaningsih E, Puspitasari D, Sismindari, Induction of apoptosis by protein fraction isolated from the leaves of *M.jalapa* L on HeLa and Raji cell-lines. *OPEM* 2003;3*:* 151-156 .

7. Sismindari and Husana, A., Preclinic assay and formulation development of Ribosome Inactivating Protein of *Mirabilis jalapa* as supplement for therapy and prevention of skin cancer. *Research Report of Hibah Bersaing* XIV, 2008.
8. Vepachedu R, Park SW, Sharma N, Vivanco J, Bacterial expression and enzymatic activity analysis of ME1, a Ribosome Inactivating Protein from *Mirabilis expansa*. *Protein Expr Purif* 2005; 40: 142-151.
9. Vivanco J, Savary BJ, Flores, HE, Characterization of two novel type I Ribosome Inactivating Protein from the storage roots of the Andean crop. *Mirabilis expansa*, *Plant Physiology* 1999; 119 : 1447-1456.
10. Al Akeel, R, Al-Shekh, Y, Mateen, A, Syed, R, Janardhan, K, Gupta, VC, Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains. *Saudi Journal of Biological Sciences*, 2014; 21, 147-151.
11. Xia L, Zouboulus CC, Ju, Q, Culture of human sebocyte *in vitro*. *Dermato-Endocrinology* 2009; 1: 92-95.