In vitro, antioxidant activity and cream formulation of alkaloid extracts *Perna viridis*

Y D Franyoto¹, L Kusmita¹, Mutmainah¹, Y P Pertiwi¹

¹Department of Pharmacy, STIFAR “Yayasan Pharmasi Semarang” Jl. Letjend Sarwo dye Wibowo KM 1, Plamongansari, Pucanggading, Semarang 50193, Indonesia
E-mail: Yuvianti.franyoto@gmail.com

Abstract. Photoaging is a common problem that occurs in our community due to ongoing exposure to ultraviolet rays. The use of antioxidants is an effective approach to prevent symptoms related to photo-induced aging of the skin. The objective of this study was to purification alkaloid and to formulate antioxidant cream extract *Perna viridis*. Thus, the present study was to prepare and evaluate the antioxidant cream extracts of *Perna viridis* for their radical scavenging activity. *Perna viridis* is one of the economically important biotas. This biota is diverse in Indonesia waters and occurred in coastal, mangrove area and estuarine. Initially, the material was macerated gradually with ethanol. The extract obtained was filtered and evaporated. The extracts were fractionated and analyzed by Thin-layer Chromatography. The extracts were examined for DPPH (2, 2 - diphenyl - 1- picrylhydrazyl) radical scavenging activity with reference standard ascorbic acid through in vitro models. The ethanolic extract *Perna viridis* was found to act in scavenging DPPH radicals with ascorbic acid used as the reference standard, and an IC$_{50}$ value was found to be 90.17 µg/mL. The results showed that these extracts of *Perna viridis* could be considered as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration.

1. Introduction
Ocean offers large biodiversity of fauna and flora which is estimated to be over 500,000 species more than double of the land species [1]. This rich diversity of marine organism assumes a great opportunity for the discovery of new bioactive substances — the marine natural products isolated from the Bivalvia been investigated predominantly for their antioxidant, antimicrobial, cytotoxic, antitumor and anti-inflammatory properties [2-4].

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals. Many alkaloids often have pharmacological effects and are used as medications. Many studies demonstrate that alkaloids had many kinds of biological activities, such as anti-microbial, antioxidants, anti-cancer, anti-inflammatory, and anti-virus activities [5,6].

Antioxidant are substances the neutralize free radical ant their actions. There are natural antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxide, glutathione reductase, thioredoxin thiols, and disulfides bonding which form the buffering system in every cell. Ascorbic acid is also a part of the normal protecting mechanism. Other non-enzymatic antioxidant includes carotenoid, alkaloid, flavonoid and related polyphenols [7].

Free radical and reactive oxygen species are well known as inducers of cellular and tissue pathogenesis which is causing some diseases like diabetes, cancer, inflammatory and also
cardiovascular. Free radical reactions take place in the human body and food systems can cause injury and death [8]. Free radicals are one of the main factors which necessary to cause DNA mutation, which is involved in the initiation stage of carcinogenesis [9].

The skin is the body’s first line of defense for external exposure. The signs of aging are most visible in the skin. Although aging skin is not a threat to a person, it can have a detrimental effect on the psychology of a person [10]. Much of the premature aging occurs as a direct or indirect result of skin’s interaction with the environment. Exposure to sunlight is recognized as a major factor in the etiology of the unwanted progressive changes in the skin appearance [11]. Photochemoprotective agents are capable of preventing the adverse effects of ultraviolet radiation on the skin, which are caused by excessive generation of reactive oxygen species [12].

2. Materials And Methods
2.1. Materials
Perna viridis were collected from Semarang, central of Java. 2, 2’-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. All other chemicals used were of analytical grade. Perna viridis was shown in Figure 1.

![Figure 1. Perna viridis](image)

2.2. Extraction of alkaloid extract Perna viridis
Extraction of the alkaloids from the species has been reported in the literature [13]. The Body tissue Perna viridis (500 g) were macerated with absolute ethanol at room temperature for 24 h. The solution was filtered and concentrated under reduced pressure at 50°C. The ethanolic residue was taken up in 10 mL of 2.5% hydrochloric acid and filtered. The aqueous acid solution was adjusted to pH=8 with concentrated ammonium hydroxide and extracted with dichloromethane (3 x 10 mL). The solvent evaporated to afford a crude extract of alkaloids. After evaporation, the yield of each fraction was calculated.

2.3. Qualitative Phytochemical Analysis
The stock solution was prepared from the crude extract and was dissolved in 10 ml of its mother solvent. The extract ethanolic Perna viridis was screened for tannin, saponins, flavonoids, alkaloids, and terpenoids as described by Trease and Evans [14].

2.4. Antioxidant assay
Alkaloids extract of Perna viridis dissolved in ethanol were plated out in triplicate. The ethanolic DPPH (50 µM) solution was added to alternating columns of the test samples, and ethanol was used for control of test samples. The percentage of decolorisation was obtained spectrophotometrically. The percentage of decolorisation was plotted against the concentration of the sample, and the IC50 values were determined. The DPPH absorbance decreases with an increase in DPPH radical scavenging activity. Results were expressed as IC50 concentration where 50% inhibition of the DPPH radical is obtained. This activity is given as the percent of DPPH radical scavenged, which is calculated with the equation:

\[
\text{DPPH radical scavenging activity (\%)} = \left(\frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}\right) \times 100\%
\]

2.5. Preparation of Formulation
Alkaloids extract of *Perna viridis* was used to prepare the antioxidant cream. The cream was formulated as per Table 1.

**Table 1.** The composition of antioxidant cream of Alkaloids extract *Perna viridis*

| Components                  | Amount (g) |
|-----------------------------|------------|
| Alkaloids Extract *Perna viridis* | 5          |
| Vaseline Album              | 100        |
| Stearic acid                | 150        |
| Na. Tetraborate             | 2.5        |
| TEA                         | 15         |
| Propylene glycol            | 80         |
| Methylparaben               | 0.1        |
| Distilled water             | 655        |

The aqueous phase and oil phase components were heated separately up to 70°C and mixed uniformly using homogenizer by addition of methylparaben and extract. Care was taken for even mixing; the remaining deionized water is added with continuous stirring until the mixture cooled and formed as cream.

2.6. **Evaluation of Antioxidant Cream**

2.6.1. Organoleptic Characteristics.

The cream was observed for color, odor, and appearance. These characteristics were evaluated by visual observation.

2.6.2. pH Values

About 0.5g of the cream was weighed and dissolved in 50 ml of distilled water, and its pH was measured. The pH meter was calibrated with standard buffer solutions (pH 4, 7, and 10) before each use.

2.6.3. Homogeneity

Homogeneity and texture were tested by pressing a small quantity of the formulated cream between the thumb and index finger. The consistency of the formulations and presence of coarse particles were used to evaluate the texture and homogeneity of the formulations.

2.6.4. Determination of Emulsion Type (Dye test)

The emulsion type was determined by using a dye test. The test was done by mixing the cream with red dye then place the drop of cream was placed on a slide and covered with a coverslip, observed under a microscope. If the dispersion phase appears in red colored globules, the cream was O/W type. If the continuous phase appears red color the cream was W/O type.

2.6.5. Viscosity

The viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm at 25 °C, using spindle no 7.

2.6.6. After Feel Effect

Emolliency, slipperiness and amount of residue left after the application of the fixed amount of cream were checked.

3. **Result and Discussion**
The body tissue *Perna viridis* (500 g) were macerated with absolute ethanol, yielding 76.10 g crude ethanolic extract of *Perna viridis*. Table 2 shows the summarized phytochemical screening of chemical constituents of ethanolic extracts of *Perna viridis* understudy on qualitative.

Table 2. Phytochemical constituents of ethanolic extract of *Perna viridis*.

| Sample                  | Tannin | Saponin | Flavonoid | Alkaloid | Terpenoid |
|-------------------------|--------|---------|-----------|----------|-----------|
| Ethanolic extract of *Perna viridis* | -      | -       | -         | +        | -         |

+: presence of constituent (positive); −: absence of constituent (negative)

Having alkaline alkaloids is present in a salt form in the animal, so choose water or acidic water to extract them. Usually, inorganic acidic extraction is used, so that the organic acid of alkaloids salt is replaced inorganic acid salt, and increasing its solubility. Use a small amount of NaOH and then extracting, so that makes the alkaloid-free. Chloroform can be used to extract free alkaloid. The percentage yield of the alkaloid-rich fraction from extract ethanolic *Perna viridis* reflected in Table 3.

Table 3. Nature, Percentage Yield of the Extract

| Extract Alkaloid of *Perna viridis* | Nature              | Percentage Yield (%) |
|------------------------------------|---------------------|---------------------|
| Extract                            | Brown, semisolid    | 9.71                |

TLC of alkaloid extracts from the *Perna viridis* used in this study revealed the presence of these compounds by using Dragendorff’s reagent to reveal characteristic orange bands of alkaloids.

3.1. Antioxidant activity.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the extract [15]. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation. DPPH is usually used as a substance to evaluate the antioxidant activity [16]. Antioxidants either transfer an electron or a hydrogen atom to DPPH•, thus neutralizing its free radical character. DPPH test, which is based on the ability of DPPH•, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action [17]. The DPPH• assay has been largely used as a quick, reliable and reproducible parameter to search the in vitro general antioxidant activity of pure compounds as well as plant extracts. The reducing capacity of compounds could serve as an indicator of potential antioxidant property.

*Perna viridis* is the potential source of antioxidant. Alkaloids in perna viridis having the capability to scavenge the free radicals. The effects in a biological system have drawn the attention of many experimental works. Antiradical activity of the alkaloids extracts from the *Perna viridis* was measured in term of hydrogen donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple color in methanol solution, antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character [18]. Antioxidant assay with DPPH is based on the ability of DPPH, the stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action [19] and has been largely used as a quick, reliable and reproducible at in vitro antioxidant activity assay [20]. The reducing capacity of compounds could serve as the marker of potential antioxidant activity 15-18. Alkaloids are compound which contains OH and NH functional group; there are could be donating their hydrogen to DPPH.

The antioxidant activity of extracts was measured regarding their efficient IC$_{50}$ concentration corresponding to the sample concentration that reduced the initial DPPH absorbance of 50%. The IC$_{50}$ value for *Perna viridis* extracts determined by linear regression. IC$_{50}$ value for the IC$_{50}$ value of ethanol extracts and ascorbic acid was used as positive control of antioxidant were given in Table 4.
Table 4. The IC$_{50}$ value of standard vs. sample extract

| Sample                  | IC$_{50}$ (µg/mL) |
|-------------------------|-------------------|
| Ascorbic acid           | 10.25             |
| Alkaloids extracts Perna viridis | 90.17             |

Herbal creams offer several advantages over other creams. The majority of existing creams which has prepared from drugs of synthetic origin extras gives fairness to face, but it has several side effects such as itching or several allergic reactions. Herbal creams do not have any of these side effects, without side effects it gives the fairness look to the skin. The formulated cream extract alkaloid of *Perna viridis* was shown in Figure 2.

Figure 2. Antioxidant Cream of alkaloid extract *Perna viridis*

The pH of the formulated antioxidant creams of alkaloid extract *Perna viridis* was found to be 6.1 which was matching to the skin pH. The dye test confirms that the formulated creams were o/w type of emulsion cream. The formulated antioxidant cream was evaluated for several physicochemical tests, and the results were shown in table 5.

Table 5. Evaluation of the formulated cream

| Parameter        | Formulation                      |
|------------------|----------------------------------|
| Appearance       | Yellowish semisolid cream         |
| Odor             | Good                             |
| Homogeneity      | Homogenous                       |
| pH               | 7.6                              |
| Spreadability    | Good                             |
| After feel       | Emollients and slipperiness       |
| Removal          | Easily removed with tap water    |
| Viscosity        | 740 cps                          |

4. Conclusion

According to data obtained from the present study, Alkaloid extracts from the *Perna viridis* was found effective antioxidant in vitro assay including reducing power DPPH radical. Based on the research, it can be concluded that study Alkaloid extracts from the *Perna viridis* have antioxidant activity with an IC$_{50}$ value of 90.17 µg/mL. The cream prepared from study Alkaloid extracts from the *Perna viridis* is an ideal oil-in-water cream with good consistency, smooth & shining texture and can be used for the antioxidant activity.

References

[1] Kamboj VP. 1999. *Bioactive agents from the Ocean bioat*. In: Ocean science Trenda and Future Directions. Indian national Science Academy, New Delhi. 197-227

[2] Tadesse, M., B. Gulliksen, M.B. Strim, O.B. Styrvold, & T. Haug. 2008. *J. Invertebrate Pathology*. 99 286-293.

[3] Zhou, D.Y., B.W. Zhu, L. Qiao, H.T. Wu, D.M. Li, J.F. Yang, & Y. Murata. 2011. In vitro antioxidant activity of enzymatic hydrolysates prepared from abalone (Haliotis discus hannaino) viscera. *Food and Bioproducts Processing*, in press.
4) Defer, D., N. Bourgougnon, & Y. Fleury. 2009. *J. Aquaculture*. **293** 1-7.
5) Yan D, Jin C, Xiao XH, et al. 2008. *J Biochem Biophys Methods*. **70 (6)** 845–849.
6) Zhou H, Tai YP, Sun CR, et al. 2005. *Phytochem Anal*. **16 (5)** 328–333.
7) Ansari A.Q., Ahmed S.A., Waheed M.A., and Juded A.. 2013. *J. Exp. Bio*. **3 (5)** 502-507.
8) Halliwell, B., 2008, *Arch. Biochem. Biophys.*. **476 (2)**, 107–112.
9) Johnson, I.T., 2007, *Proc. Nutr. Soc.*. **66 (2)**, 207–215.
10) Ugandar RE and Deivi KS. 2013. *International Journal of Pharmaceutical Science and Research* **4 (9)** 3375-3380.
11) Saraf S, Chhabra SK, Kaur CD, and Saraf. 2012. *Journal of cosmetic science*, **63** 119–131.
12) More BH, Sakharwade SN, Tembhurne SV, Sakarkar DM. 2013. *International Journal of Research in Cosmetic Science* **3 (1)** 1-6
13) Suau R, Cabezudo R, Rico R, F. Najera andLopez-Romero J.M. 2002. *Phytochem. Anal*. **13** 363 - 367
14) Trease GE, Evans WC. 2002, Pharmacognosy. 15th ed. B Saunders, London, 137- 440
15) Bhuiyan MAR., Hoque MZ., Hossain SJ. 2009. *World J. Agr. Sci*. **5** 318-322.
16) Tara Chand, Anil Bhandari, Bhupendra K. Kumawat, Pawank Basniwal, Sanjay Sharma, Rajesh Verma. 2012. *American Journal of Pharmtech Research*. **2 (3)** 2249-3387.
17) Raquibul Hasan SM., Mokarram Hossain MD., Raushanara A., Mariam J., Ehsanul Hoque Mazumder MD., Shaﬁqur Rahman. 2009. *Full length Research paper* **3 (11)** 875-879.
18) Jebitta, R., Allwin, J., 2016 *Asian J Pharm Clin Res*. **9** 361-363
19) Pan, Y., Wang, K., Huang, S., Wang, H., Mu, X., He, C., 2008. *Food Chem.* **106** 1264-1270.
20) Hasan, M.S., Ahmed, M.I., Mondal, S., Uddin, S.J., Masud, M.M., Sadhu, S.K., 2006. Antioxidant, antinociceptive activity and general toxicity study of Dendrophthoe falcata and isolation of quercitin as the major component. *Opem.*, **6** 355-60