

Analysis of protein profile of neem leaves juice (*azadirachta indica l. Juss*)

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Abstract—Introduction. The current trend of the management of diseases has shifted toward modulating the immune response of the patients. This is achieved by the administration of immunomodulatory substance obtained from the immunogenic medicinal plant, such as neem. The immunogenic properties of neem has been associated with its protein content. Aim. This study aimed to characterize the proteins and immunogenic components of neem leaves juice using sodium dodecyl Sulphate method Polyacrilamide gel electrophoresis (SDS-PAGE). Materials and Methods. Neem leaf juice was extracted for proteins with freeze dry method. Samples that were to be run were added with RSB with the ratio of 1:1, then heated in boiled water for \pm 5 minutes. An amount of 15 μ l from each sample was then put into the wells. Samples were run in 120 Volt for 60-80 minutes. Gel was then taken, stained with silver nitrate for 1 hour, and was then stained every 1.5 hours. Protein bands formed were then observed. Results. The protein fraction of neem leaves juice consisted of proteins with molecular weights (MW) as follows: 11 kDa, 13 kDa, 30 kDa, 62 kDa, 70 kDa, 81 kDa. Conclusion. All protein fractions of neem leaves juice are potentially immunogenic components.

Keywords—neem leaves juice; immunogenic components; SDS-PAGE; protein fractions

INTRODUCTION

The current trend of the management of diseases has shifted toward modulating the immune response of the patients. This is achieved by the administration of immunomodulatory substance obtained from the immunogenic medicinal plant, such as neem. The immunomodulatory capacity of a substance can be determined from its protein content. Neem (*Azadirachta Indica L Juss*) has been well known as a plant containing immunomodulatory substances. Therefore, certain communities has regarded this plant as a traditional medicine to overcome various diseases, including: worm infestation, scabies, malaria, fungal infection, and it might also serve as anti-bacterial and anti-allergy. The fraction of water-acetone extract of neem leaves known as IRAB has been showing properties to cure diseases. This phenomena has strengthened the notion that neem contains immunomodulatory components that are able to modulate immune response. Some research has demonstrated that neem modulates the innate and adaptive immunity[1][2][3][4][5][6][7]. While prevalent in Indonesia, the use of neem leaves as a natural medicine is still highly limited, due to the dearth of information and research supporting its use. Studies directed toward demonstrating the potentials of neem as a medicinal plant are therefore warranted.

Taxonomy included neem in the division of *Spermatophyte*, Subdivision *Angiospermae*, Class *Dicotyleclonae*, ordo *Rutales*, suku *Meliaceae*, marga *Azadirachta*, species *Azadirachta Indica Juss.*[7][8] All parts of neem leaves contain active compounds including azadirachtin, salanine, meliantriolo, nimbin, nimbolide, gedunine, mahmodine, gallic acid, catechin, epicatechin, margolone, margolonone, isomargo-lonone, cyclictrisulphide, cyclictetrasulphide and polisaccharide, and results of such studies have been inconclusive.[7][8][9] Neem leaves contain fiber (20.11 \pm 0.45%) protein (13.42 \pm 0.12%). Other research found that fresh neem leaves contain 7.1% protein, 1% fat, 6.2% fiber, 22.9% carbohydrate, 3.4% minerals and 59.4% moisturizer[9][10][11][12]

Neem has been demonstrated to be able to modulate PMN, macrophages, lymphocytes and in turn, it affects the phagocytic activity, TNF- α , IFN- γ , macrophage activity, and the immunoglobulin production. This supports the notion that neem modulates the innate, cellular, and humoral immunity.[1][2][4][7][8] Some studies demonstrated immunomodulatory effect involves modulation of cellular and humoral immunity response including the increase of IgG, IgM level[13]. The immunomodulatory potential of neem extract involves its effect on CD4, CD8, Th1 cells, TNF- α , IFN- γ and macrophage activities in mice and monkeys. Neem

leaves also modulate humoral and cellular immunity, including: phagocytosis, expression of MHC (*Major Histocompatibility Complex*) class I and II, production of IFN γ , CD4, CD8, Th 1, TNF- α , IFN γ , IL-1 β as has been studied since 1997 by Ray *et al*, Talwar *et al*. In addition, IRAB (fraction of water-acetone extract) has been considered as savior and has been demonstrated to increase the level of CD4+ cell in patients with HIV/AIDS [14].

The author's previous research has demonstrated that neem leaves juice inhibited the growth of *C. albicans in vitro*. Neem leaves juice decreased the count of inflammatory cells in mice inoculated with *C albicans* and inhibited the growth of *Candida albicans in vitro*. The effect of different concentrations of liquid extract of neem leaves on macrophages expressing CD14 was studied as well. Ethanol extract of neem leaves had demonstrated to increase macrophage activity in expressing TLR2, TLR4, TNF- α , and its phagocytic activity. Proteins from neem leaves liquid extract with molecular weights of 70 and 100 kDa did not differ significantly in affecting the activities of TLR2, TLR4, TNF α , phagocytic activity and the colony number of *C. albicans*. The author then developed a study by creating a murine model exposed to allergen of white egg (ovalbumin) [15][16][17][18][19].

This research was conducted to characterize the proteins of neem leaves juice using the method *Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis* (SDS-PAGE). Gel electrophoresis is a technique to separate charged molecules based on its physical characteristics such as mass or its charge through gel matrix provided with electrical current[20][21].

MATERIALS AND METHODS

Electrophoresis method

Neem leaf juice was extracted for proteins with freeze dry method made in the laboratory of Center for Development of Advanced Sciences and Technology (CDAST) Jember University. SDS-PAGE method in Biomedic laboratory of Brawijaya University. Fractionation was then conducted using SDS-PAGE method with electrophoresis set mini protein gel (Bio-Rad) with 12.5% gel, stained by silver stain SDS-PAGE Standards (Bio-Rad)[20][21]. Samples that were to be run were added with RSB with the ratio of 1:1, then heated in boiled water for \pm 5 minutes. An amount of 15 μ l from each sample was then put into the wells. Samples were run in 120 Volt for 60-80 minutes. Gel was then taken, stained with silver nitrate for 1 hour, and was then stained every 1.5 hours. Protein bands formed were then observed.

RESULTS AND DISCUSSION

The SDS-PAGE results of neem leaves showing the intensity of each band is presented in Figure 1. Lane 2 and 3 represent the protein profile of neem leaves containing proteins with high molecular weight of 11 kDa, 13 kDa, 30 kDa, 62 kDa, 70 kDa, 81 kDa.

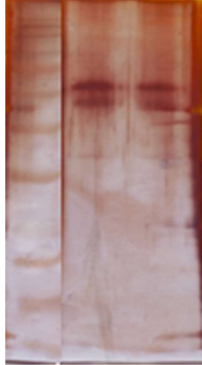


Figure 1. Protein electrophoregraph of neem leaves juice

Lane 1: marker marker
 Lane 2: neem leaves proteins
 Lane 3: neem leaves proteins

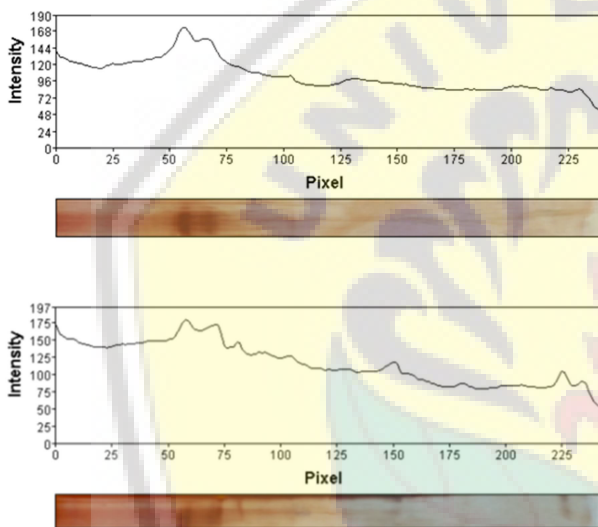


Figure 2. Graphical fit of the results of SDS-PAGE neem leaves juice protein profile

Table 1. Intensity of neem leaves juice proteins with SDS-PAGE method

Molecular weight (kDa)	Intensity (pixel)
11	993
13	1095
30	1532
62	1268
70	1958
81	2477

Figure 2 and table 1 show that the degree of fraction intensity is affected by the amount of distance value and highest intensity value in each fraction. The bigger the distance value and the higher the intensity in each fraction, the bigger the intensity of a particular protein fraction. The bigger the intensity of the fraction, the higher the protein content [23]. Highest intensity was found for protein with molecular weight of 81 kDa (2477). This means this type of protein has the highest level.

Currently there are many emerging methods of drying used in the making of pharmaceutical preparations. One method that is considered to be the best one is the method using freeze drying. This method stands out in its ability to preserve the quality of drying results, especially for products that are sensitive to heat. This method has been demonstrated to be able to preserve the stability of the product (avoiding changes in odor, color, and other organoleptic elements). It's also able to preserve the

stability of material structure (shrinking and shape change after drying is minimal)[22].

SDS-PAGE profile of neem leaves juice revealed proteins with molecular weight of 11 kDa, 13 kDa, 30 kDa, 62 kDa, 70 kDa, 81 kDa. The order of intensity of molecular weight from the smallest was as follows: 11 kDa, 13 kDa, 30 kDa, 62 kDa, 70 kDa, 81 kDa. This intensity was shown by the thickness of the bands and the height and the width of the graphics. The thicker the band, the higher its intensity. It was suspected that this protein was polar protein since the aquades solvent in this research has polar characteristic. In addition, it was also suspected that protein fraction of neem leaves juice has the potential as an immunomodulator. Plant and human protein has been shown to be similar in essence, but there were differences in term of the proteins related to photosynthesis. This makes the plant protein unfamiliar to human, so when one's exposed to plant protein, immune system response will be activated[23][24]. The protein fraction of neem bark has high molecular weight so it is highly immunogenic. Proteins with molecular weight of more than 10 kDa are strong immunogens. Proteins with molecular weight of less than 10 kDa might have an antigenic or non-antigenic properties. The bigger the molecular weight is the more immunogenic protein. [25][26] Proteins with low molecular weight is not excludable as an imunogen, although proteins with high molecular weight is much likely to be one.

The differences in intensity of each protein fraction of neem leaves juice shows the different level of protein in each protein fraction. ajor proteins is thicker and has more intense color as compared to other protein fractions, Thick bands shows high concentration while thin band shows low protein level. The difference of the thickness of the bands is caused by the difference in the migrating molecules, thick bands are fractionation of some bands. Bands with higher ionic charge would migrate further than the bands with lower ionic strength. The fractions of m so the major protein fractions are the ones with higher level as compared to other fractions. The thickness of protein fraction of the SDS-PAGE result was affected by sample concentration. The low level of protein fraction from neem bark extract is affected by the low sample concentration. The higher the protein concentration of a sample to be done for electrophoresis, the resulting bands will be cleared and thicker.[26] Sample concentration can be increased by adding the sample volume in its ratio with the solvent or adding both the amount of samples and the solvent.

Neem is the plant with different protein components from human, which leads to its potential immunogenicity. The neem leafs juice contain proteins as shown by SDS PAGE results, including the ones with the molecular weight of 11 kDa, 13 kDa, 30 kDa, 62 kDa, 70 kDa, 81 kDa and is strongly suspected to be highly immunogenic since proteins with high immunogenicity usually have high molecular weight, but it is still possible that proteins with low molecular weight also have immunogenic nature. Further research should be conducted to test their immunogenicity. The discovery of these proteins could suggest for its use as an alternative therapy for many types of diseases. In addition, two dimensional electrophoresis should also be conducted to determine the proteins within certain bands since each band might consist of many proteins. Furthermore, to determine the name of the protein, sequencing should be done.

CONCLUSION

The SDS-PAGE profiles of proteins from neem leaves juice demonstrated proteins with molecular weight of 11 kDa, 30 kDa, 70 kDa and 81 kDa. All protein fractions of neem leaves juice are potentially immunogenic components.

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