

Effect Of Binahong Leaf Extract ((Anredera Cordifolia (Tenore) Steenis) On Malondialdehyd, Hydroperoxide, Lipid, And Total Protein Levels In Cataract

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Abstract

Objective: A cataract is a condition where the lens loses its transparency or becomes cloudy; cataracts can occur due to oxidative stress due to the presence of free radicals. The free radical attack resulted in increased lipid hydroperoxide, MDA, and total insoluble protein. In this study, to overcome oxidative stress in diabetic cataract lenses, ethanol extract of binahong leaves was used, which has been reported to contain flavonoid compounds that can be used as antioxidants

Methods: This research was conducted at the Research Laboratory of the Faculty of Mathematics and Natural Sciences Unsyiah, The test materials used in this study were leaves of binahong *Anredera cordifolia* (Ten) steenis, liquid medium Nutrient broth (NB) distilled water, tween 80, 70% ethanol, HCl, Methanol, Mg, FeCl₃, NaCl, H₂SO₄, EDTA, NaCl, KCl, MgCl₂, NaHCO₃, NaH(PO₄)₂, CaCl₂, Glucose, Tris Buffer, Penicillin, Streptomycin, phenol, bovine serum albumin, butylated hydroxytoluene, sulfuric acid. Fresh goat eyeballs are collected from the slaughterhouse. Lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, MgCl₂ 2 mM, NaHCO₃ 0.5 mM, NaH (PO₄) 2 0.5 mM, CaCl₂ 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 hours. Principal Component Analysis (PCA) is a classical statistical technique that can be used to analyze a data set with many variables; in this study, the PCA technique was used to analyze MDA, LH, and total protein levels in regular lenses, diabetic lenses (cataracts), lenses after being treated with binahong leaf extract and lenses after being treated with quercetin (standard). PCA analysis in this study was carried out using the PYTHON application, using the Scikit-Learn Library, and visualized with the matplotlib and seaborn libraries.

Results: Based on the measurement results for each parameter, on the lipid hydroperoxide parameter, the lipid hydroperoxide level in normal lenses was 1.6611±0.01 g/mL, in diabetic cataract lenses 1.8508±0.01 g/mL, in diabetic cataract lenses after being given binahong extract at a dose of 100 mg. /mL lipid hydroperoxide levels became 1.5881±0.009 g/mL, while in binahong extract 200 mg/mL lipid hydroperoxide levels became 1.4390±0.01 g/mL. Measuring MDA levels in normal lenses showed results of 0.0252 ± 0.0015 g/mL, in diabetic cataract lenses 0.0562 ± 0.033 g/mL, after being given Binahong extract at a dose of 100 mg/mL the MDA levels became 0.0340 ± 0.0011 g/mL and at a dose of 200 mg/mL of MDA to 0.0300±0.0010 g/mL The same decreasing trend also occurred in total protein levels wherein normal lenses; the total protein level was 103.82±1.51 g/mL, in diabetic lenses it increased to 130.84±0.84 g/mL, total protein levels in diabetic cataract eyes were given 100 mg binahong extract. /mL dropped to 86.27±0.44 g/mL. and at binahong 200 mg/mL decreased to 73.92±0.62 g/mL

Conclusion: Binahong leaf extract gives good enough results to overcome the effects of free-radical attacks. The results of the PCA analysis showed that the levels of lipid hydroperoxide, MDA, and total protein tended to be different in both normal lenses, diabetic cataract lenses, and cataract lenses treated with binahong extract also with Quercetin.

Keywords: Binahong Leaf Extract, Malondialdehyd, Hydroperoxide, Lipid, And Total Protein Levels

INTRODUCTION

Visual impairment is a severe problem worldwide. Blindness is one of the problems of visual impairment, a global problem where the incidence is more than 1% of the total world population. The World Health Organization (WHO) reports that the blindness experienced by the world's

population is as many as 45 million people whose distribution of problems is in several developing countries, including Indonesia 1,2,3,4. A survey of 39 countries globally reported that of the 285 million people who experience visual impairment, around 50% are age-related cataracts, and 13.7% are blind 5,6,7

The most common causes of visual impairment worldwide are uncorrected refractive errors (48.99%), followed by cataracts (25.81%) and Age-related Macular Degeneration (AMD, 4.1%) 8. Visual impairment, in general, is significantly associated with Age, cataracts being one of the most common eye diseases found in the older population 9. 10. Cataracts are generally a disease in the elderly, but many other factors influence cataracts, such as prolonged use of steroids, congenital metabolic disorders, chronic exposure to ultraviolet (UV) light, and even diabetes complications. The primary abnormality of various forms of cataracts lies in the retinal capillaries.11 The process of cataracts is quite complicated and not known with certainty; many reasons contribute to lens opacities, one of which is thought to play a role in accumulating free radicals 12,13,14. The effect of free radicals is the emergence of pathological reactions in the lens tissue resulting in an oxidative reaction that will disrupt the lens protein structure, causing cross-links between and intra-proteins that will increase the amount of high molecular weight protein, resulting in protein aggregation and will further cause cloudiness of the lens (cataract). Some derivatives of free radicals are malondialdehyde (MDA) and lipid hydroperoxides (LH). Currently, cataract surgery is the only treatment for cataracts and is very effective in restoring vision. Pharmacological therapy can efficiently reduce disease burden and medical costs and benefit patients15. Over the past few decades, much research has focused on pharmacological strategies to prevent and slow cataract progression using antioxidants, AR inhibitors, and combinations of sulfhydryl quinoa inhibitors16. Pharmacologically some herbal plants can be an alternative treatment for cataracts, such as the binahong plant. The binahong plant is believed to have various benefits, including the treatment of cataracts. To overcome cataracts caused by free radicals (oxidants), researchers used ethanol extract of binahong leaves as an alternative antioxidant in this study. The binahong plant (*Anredera cordifolia* (tenore) steenis) is known to contain a class of flavonoid compounds, as it has been known that flavonoid compounds have antioxidant activity 17. Based on the description above, this study aims to determine the effect of ethanol extract of binahong leaves on cataract lenses assessed based on several parameters, including lipid hydroperoxides (LH), malondialdehyde (MDA), and total protein content.

METHODS

This research was conducted at the Research Laboratory of the Faculty of Mathematics and Natural Sciences Unsyiah, Banda Aceh, from May to November 2020 after obtaining information on passing the ethical review published by the Research Ethics Commission of the Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

Tools and materials

Glass utensils, alcohol meter, vacuum rotary evaporator, evaporating dish, needle loop, cotton, simlicia grinding machine, spatula, micropipette, Bunsen, tweezers, aluminum foil, water bath, analytical balance, autoclave, oven, anaerobic chamber, incubator, caliper. Spectrophotometer, Centrifugal.

Ingredients

The test materials used in this study were leaves of binahong *Anredera cordifolia* (Ten) steenis, liquid medium Nutrient broth (NB) distilled water, tween 80, 70% ethanol, HCl, Methanol, Mg, FeCl₃, NaCl, H₂SO₄, EDTA, NaCl, KCl, MgCl₂, NaHCO₃, NaH(PO₄)₂, CaCl₂, Glucose, Tris Buffer, Penicillin, Streptomycin, phenol, bovine serum albumin, butylated hydroxytoluene, sulfuric acid.

Extract Making

Binahong leaves as much as 2 kg were washed and drained, then air-dried and not exposed to direct sunlight, after drying and then cut into small pieces, then macerated with 70% ethanol for three days with occasional stirring, after three days filtered to To get the filtrate, the obtained filtrate is then concentrated using a vacuum rotary evaporator so that it becomes a gel.

Lens Preparation

Fresh goat eyeballs are collected from the slaughterhouse. Lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, MgCl₂ 2 mM, NaHCO₃ 0.5 mM, NaH (PO₄) 2 0.5 mM, CaCl₂ 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 hours. Penicillin 32 mg% and Streptomycin 250 mg% were added to the culture medium to prevent bacterial contamination (Langade, 2006). After incubation for 72 h, homogenized lenses (10% w/v) were prepared in Tris buffer (0.23 mM, pH 7.8) containing 0.25x10⁻³M EDTA. The homogenization was centrifuged at 10,000 g for 1 hour, and the supernatant was used to estimate biochemical parameters, including the determination of lipid peroxidation end products, namely malondialdehyde (MDA) and lipid hydroperoxides (LH).

Table 1. Goat Lens Preparation

No	Lens	Code	Total	Information
1	Normal	N	10	Solvent kontrol Artificial aqueous humor & Glukosa 5.5mM
2	Diabetes	D	10	Negatif kontrol Hanya Glukosa 55mM
3	Binahong	T1	10	Ekstrak daun binahong

	(100)			(100 µg/ml) + Glukosa 55 mM
4	Binahong (200)	T2	10	Ekstrak daun binahong (200 µg/ml) + Glukosa 55 mM
5	Quercetin	T3	10	Standard obat Vitamin E (100 µg/ml) + glucose 55 mM

Inspection Parameters

Lipid Hydroperoxides (LH).

About 0.1 ml of the tissue homogenate was reacted with 0.9 ml of Fox's reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg of xylenol orange, and 9.8 mg of ammonium sulfate ion added to 90 ml of methanol and 10 ml of 250 mM sulfuric acid.) and then incubated for 30 min. The absorbance was measured against a blank solution at 560 nm. Values are expressed as lens protein nmoles/mg.

Malondialdehyde (MDA)

As evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP), Lipid peroxidation was measured by the Nieshus and Samuelsson method. Approximately 0.1 ml of tissue homogenate (Tris HCl buffer, pH 7.4) was added with 2 ml (1:1:1 ratio) of TBA-TCA-HCl reagent (Thiobarbituric Acid 0.37%, 0.25N HCl, and 15 % TCA) and placed in a water bath for 15 min, cooled and centrifuged at 1000 g at room temperature for 10 min. The absorbance of the supernatant was measured against a blank solution at 535 nm. Values are expressed as MDA lens protein nmoles/min/mg.

Lens Difference Analysis Using PCA Technique

Principal Component Analysis (PCA) is a classical statistical technique that can be used to analyze a data set with many variables; in this study, the PCA technique was used to analyze MDA, LH, and total protein levels in regular lenses, diabetic lenses (cataracts), lenses after being treated with binahong leaf extract and lenses after being treated with quercetin (standard). PCA analysis in this study was carried out using the PYTHON application, using the Scikit-Learn Library, visualized with the matplotlib and seaborn libraries.

RESULTS

Estimation of lipid hydroperoxides (LH)

The process of cataracts caused by oxidative stress will occur when free radicals can exceed the ability of cells in the lens to eliminate these free radicals. Free radicals will react with unsaturated fatty acids in the lens epithelial cell wall; the reaction between free radicals and lens epithelial cells will produce lipid hydroperoxides (LH). Figure 1 shows lipid hydroperoxide levels in several groups of experimental lens samples.

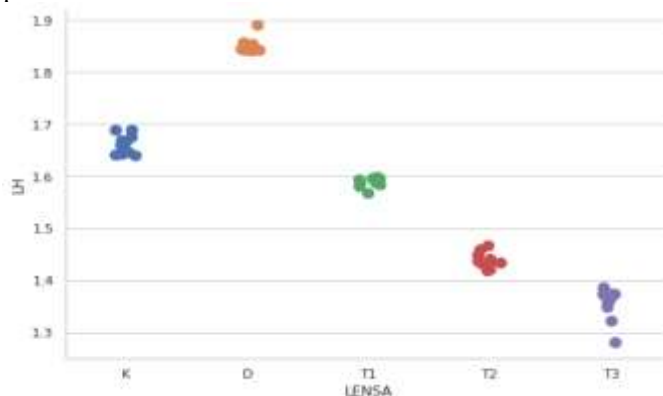


Fig -1. LH levels in 5 lens groups

Figure 1. Shows LH levels in the experimental lens, based on the measurement results obtained where the LH levels in the N lens are 1.6611 ± 0.01 , the D lens is 1.8508 ± 0.01 , the T1 lens is 1.5881 ± 0.009 , the T2 lens is 1.4390 ± 0.01 , and the T3 lens is 1.3555 ± 0.03 . The Anova test on the mean of all lens groups showed a p-value of 0.000 ($p < 0.05$); this indicates that the levels of LH in the five lens groups have significant differences. The picture shows that the LH level in the D lens (diabetic cataract lens) is higher than the N lens (standard lens); this indicates that the cataract lens has higher LH levels. However, there was a decrease in LH levels in the

cataract lens in diabetes after being treated with binahong extract at a dose of 100 mg/mL. The data obtained show that the higher the semiannual dose of the binahong extract, the more significant the decrease in LH levels in the diabetic cataract lens.

Estimation of malondialdehyde (MDA)

Malondialdehyde (MDA) is a natural compound that can be a marker of oxidative stress, high levels of MDA in the lens can affect the occurrence of cataracts. Fig. 2 shows the MDA content in several lenses consisting of a control group and a treatment group.

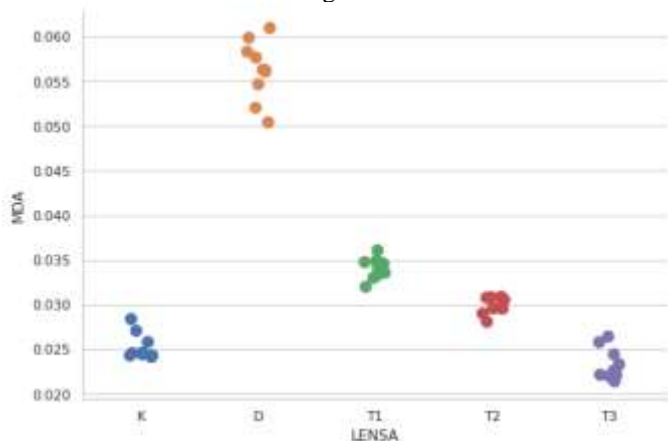


Fig -2. MDA levels in 5 lens groups

Figure 1. Shows the results of measuring MDA levels, the average MDA content of the N lens is 0.0252 ± 0.0015 g/mL, the D lens is 0.0562 ± 0.033 g, the T1 lens (100) is 0.0340 ± 0.0011 g, the T2 lens is 0.0300 ± 0.0010 g, and on the T3 lens 0.0233 ± 0.0017 g. Based on Figure 2, it can be seen that the diabetic cataract lens sample (D lens) has a very high MDA level compared to the MDA level in the standard lens sample (N lens). However, after being treated with binahong extract, there was a decrease in MDA levels in diabetic cataract lenses; this can be seen from the data on T1 and T2 lenses, where the higher the dose of binahong extract given, the MDA levels in the lens will decrease until the levels are almost the same as a standard lens. The T3 lens treated with quercetin showed the most significant reduction, even lower than the Normal lens.

Total Protein Content

One of the causes of visual disturbances is due to oxidation of the lens structure, one of which is cumulative oxidation. The previously reported protein is quite dominant in the pathogenesis of cataracts in the lens. Based on previous research, it is known that antioxidants can prevent or minimize oxidative damage to the lens (Zhang et al., 2012, Selin et al., 2013). Figure 3. Shows the comparison of total protein content in regular lenses, diabetic lenses, and diabetic lenses after being treated with binahong extract and quercetin.

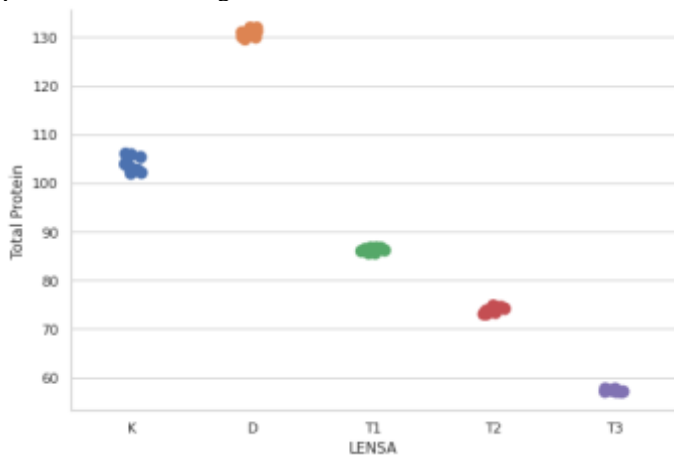


Fig-3. Total Protein (TP) levels in 5 lens groups

Figure 3. Shows the total protein content in several groups of lenses; from the measurement results obtained, the average total protein content in the N lens is 103.82 ± 1.51 , in the D lens, the average total protein content is 130.84 ± 0.84 , the T1 lens is 86.27 ± 0.44 , the T2 lens is 73.92 ± 0.62 , and the T3 lens is 57.26 ± 0.24 . From the mean data of the measurement of total protein content, the p-value was 0.000 ($p < 0.05$). The results of the ANOVA test showed that there was a significant difference. Based on Figure 3, it can be seen that the D (diabetic) lens shows a higher total protein content than the N (Normal) lens, the high total protein content is an indication that there has been protein clumping in the eye lens, which causes cloudiness in the eye lens. Treatment using binahong extract (T1

and T2) showed a significant decrease in Total Protein content at an extract concentration of 100 g/ml (T1). Then the diabetic lens, which was treated with 200 g/ml (T2) binahong extract, showed the lower total protein content.

Analysis of the Effect of Giving Binahong Extract on Cataract Lenses

Based on the PCA technique analysis, the effect of binahong extract treatment on diabetic lens samples can be seen. PCA technique can analyze the effect of several treatments by analyzing the three parameters and visualizing them in a plot score. Figure 4. shows a PCA plot illustrating the effect of treatment on a diabetic lens sample.

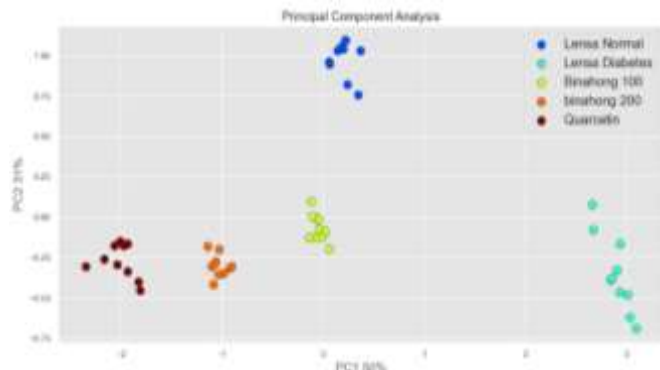


Fig- 4. PCA plot the effect of the type of treatment on the cataract eye lens

The score of the PCA plot in Figure 4 shows a fairly multiplier result, where each lens sample is grouped according to the type of lens; this indicates that the three parameters, namely MDA, LH, and Total Protein levels, have different contents in different types of lenses. The PCA scot plot obtained explained the total variance of 81% (PC1 50% + PC2 31%). The reaction between free radicals and unsaturated fatty acids will produce lipid hydroperoxides; lipid hydroperoxides are a type of fat that is unstable and can decompose into several forms of aldehydes, one of the decomposition products of lipid hydroperoxides is Malondialdehyde (MDA). The MDA formed reacts very quickly with lens proteins which can cause protein aggregation so that the weight of the protein model will increase and reduce the lens's crystallinity^{18,19,20}. The chain effect of oxidative stress caused by free radicals can be overcome with antioxidants; many plants have been reported to have antioxidant activity, one of which is the ethanol extract of binahong leaves. Binahong leaf extract is known to contain flavonoid compounds that can be an agent of antioxidant activity. Based on the results of the tests that have been carried out, it is known that the binahong extract shows quite good results in overcoming the effects of oxidative stress; this can be seen from the three measurement parameters carried out, namely the measurement of MDA, LH, and total protein levels.

In measuring MDA levels, the administration of binahong leaf extract on diabetic cataract lenses showed a significant decrease in MDA levels, where the MDA levels obtained after being given binahong extract were almost the same as the MDA levels in standard lenses. However, compared with the MDA levels in the eyes given quercetin as an antioxidant, the MDA levels were slightly better; this can be understood because quercetin is a pure type of flavonoid. Based on the test results, it was concluded that the decrease in MDA levels was directly proportional to the increase in the dose of the extract given. The same results were also shown in the measurement of lipid hydroperoxides. The administration of binahong leaf extract showed a significant decrease in lipid hydroperoxide levels in diabetic cataract lenses, and lipid hydroperoxide levels after being administered binahong extract were lower than lipid hydroperoxide levels in standard lenses.

The effect of giving binahong extract on total protein levels in diabetic cataract eyes also showed significant hunting; the larger the dose given, the higher the percentage of decrease in total protein levels. Based on the PCA analysis that has been carried out, it shows that the levels of MDA, LH, and total protein tend to be different in Norma lenses, diabetic cataract lenses, and lenses that have been treated. The PCA results show that the effect of binahong extract on the cataract lens as a whole was measured using the parameters of MDA, lipid hydroperoxide, and total protein. Oxidative stress is thought to have an essential role in initiating and developing cataracts^{21 22}. Several studies have reported higher levels of membrane lipid peroxidation and reactive oxygen species (ROS) in cataract tissue than in normal tissue^{23,24,25}. Hydrogen peroxide (H₂O₂), the primary oxidative substance in the lens and aqueous humor, was found at high levels in cataract samples—as many as 30 times that in standard samples²⁶. Such an increase in ROS induces apoptosis of lens epithelial cells and denaturation of crystallin proteins, promoting decreased crystallin solubility and aggregation formation^{27, 28 29}. It can be concluded that binahong leaf extract has good potential to overcome oxidative stress in cataract lenses in diabetes because it contains good antioxidant activity. Utilizing antioxidants as a defense on the lens from the radical attack has also been shown from several research reports, such as research conducted by showing that administration of bilberry extract with its active component, flavonoid anthocyanidin, for 14 days has an effect in reducing oxidative stress levels. As levels of malondialdehyde (MDA) measure it. Giving *Crataegus pinnatifida* leaf extract, which is rich in antioxidants, can also delay the occurrence of cataracts at concentrations of 0.1% and 0.2% which can increase levels of superoxide dismutase (SOD) and reduce levels of MDA in the serum of rat models of cataracts^{30 31}.

CONCLUSION

Binahone extract has the potential to overcome the effects of oxidative stress due to free radical attack by reducing the levels of

MDA, lipid hydroperoxide, and total protein in diabetic cataract lenses.

CONFLICT OF INTEREST

The author declares no conflict of interest

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REFERENCES

1. Shanmugam PM, Barigali A, Kadaskar J, et al. Effect of lanosterol on human cataract nucleus. *Indian J Ophthalmol*. 2015;63(12):888-890. doi:10.4103/0301-4738.176040
2. Shih KC, Wong JKW, Lai JSM, Chan JCH. The case for continuing elective cataract surgery during the COVID-19 pandemic. *J Cataract Refract Surg*. 2020;46(6):921. doi:10.1097/j.jcrs.000000000000225
3. Singh G, Grzybowski A. Evolution of and developments in simultaneous bilateral cataract surgery. Update 2020. *Ann Transl Med*. 2020;8(22):1554. doi:10.21037/atm-20-3490
4. Lin D, Chen J, Lin Z, et al. A practical model for the identification of congenital cataracts using machine learning. *EBioMedicine*. 2020;51:102621. doi:10.1016/j.ebiom.2019.102621
5. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. *Br J Ophthalmol*. 2012;96(5):614-618. doi:10.1136/bjophthalmol-2011-300539
6. World Health Organization. Global Estimates of Visual Impairment 2010.
7. Chew FLM, Salowi MA, Mustari Z, et al. Estimates of visual impairment and its causes from the National Eye Survey in Malaysia (NESII). *PLoS One*. 2018;13(6):e0198799. Published 2018 Jun 26. doi:10.1371/journal.pone.0198799
8. Liu Y, Fu M, Zhou Q, Tian M, Zhang X, Wang Z. The application of patient-centered care bundle significantly reduces incidence of perioperative respiratory complications in hip fracture patients aged 80 and over. *Geriatr Nurs (Minneapolis)*. 2022;43:213-218. doi:10.1016/j.gerinurse.2021.11.014
9. Ekman I, Wolf A, Olsson LE, et al. Effects of person-centred care in patients with chronic heart failure: The PCC-HF study. *Eur Heart J*. 2012;33(9):1112-1119. doi:10.1093/eurheartj/ehr306
10. Rathert C, Wyrwich MD, Boren SA. file:///C:/Users/YASIN-01/Documents/BU FRIDA/JURNAL IBU/6.pdf. *Med Care Res Rev*. 2013;70(4):351-379. doi:10.1177/1077558712465774
11. Australian Commission on Safety and Quality in Health Care (ACSQHC). Patient-Centred Care: Improving Quality and Safety by Focusing on Care on Patients and Consumers. Discussion paper: Draft for public consultation, September 2010. 2010;(September). [http://www.health.gov.au/internet/safety/publishing.nsf/Content/36A B9E5379378EBECA2577B3001D3C2B/\\$File/PCCC-DiscussPaper.pdf](http://www.health.gov.au/internet/safety/publishing.nsf/Content/36A B9E5379378EBECA2577B3001D3C2B/$File/PCCC-DiscussPaper.pdf)
12. Jo Delaney L. Patient-centred care as an approach to improving health care in Australia. *Collegian*. 2018;25(1):119-123. doi:10.1016/j.collegn.2017.02.005
13. Larson E, Sharma J, Bohren MA, Tunçalp Ö. When the patient is the expert: Measuring patient experience and satisfaction with care. *Bull World Health Organ*. 2019;97(8):563-569. doi:10.2471/BLT.18.225201
14. Morgan S, Yoder LH. A Concept Analysis of Person-Centered Care. *J Holist Nurs*. 2012;30(1):6-15. doi:10.1177/0898010111412189
15. Nelson KM, Helfrich C, Sun H, et al. Implementation of the patient-centered medical home in the veterans health administration: Associations with patient satisfaction, quality of care, staff burnout, and hospital and emergency department use. *JAMA Intern Med*. 2014;174(8):1350-1358. doi:10.1001/jamainternmed.2014.2488
16. Epstein RM, Fiscella K, Lesser CS, Stange KC. Analysis & commentary: Why the nation needs a policy push on patient-centered health care. *Health Aff*. 2010;29(8):1489-1495. doi:10.1377/hlthaff.2009.0888
17. Otero C, Luna D, Marcelo A, et al. file:///C:/Users/YASIN-01/Downloads/1472-6963-13-251.pdf. *Yearb Med Inform*. 2015;10(1):30-33. doi:10.15265/IY-2015-013
18. Mirzaei M, Aspin C, Essue B, et al. file:///C:/Users/YASIN-01/Downloads/sidani2008.pdf. *BMC Health Serv Res*. 2013;13(1). doi:10.1186/1472-6963-13-251
19. Sidani S. Effects of Patient-Centered Care on Patient Outcomes: An Evaluation. *Res Theory Nurs Pract*. 2008;22(1):24-37. doi:10.1891/0889-7182.22.1.24
20. Juwariyah T, Joyo NBW, Santosa WRB. Hubungan Perilaku Caring Perawat dengan Tingkat Kepuasan Pasien di Poli VCT RSUD Gambiran Kota Kediri Berdasarkan Teori Watson. *J Ners dan Kebidanan (Journal Ners Midwifery)*. 2014;1(3):177-183. doi:10.26699/jnk.v1i3.art.p177-183
21. Stewart M, Brown JB, Donner A, et al. The impact of patient-centered care on outcomes. *J Fam Pract*. 2000;49(9):796-804.
22. Wolf DM, Lehman L, Quinlin R, Zullo T, Hoffman L. Effect of patient-centered care on patient satisfaction and quality of care. *J Nurs Care Qual*. 2008;23(4):316-321. doi:10.1097/01.NCQ.0000336672.02725.a5
23. Rivai F, Abubakar Z, Sapada NA. Effect of patient-centered care application on inpatient outcomes after emergency cesarean section surgery. *Enferm Clin*. 2020;30:130-133. doi:10.1016/j.enfcli.2020.06.030
24. Nowak M, Lee S, Karbach U, Pfaff H, Groß SE. Short length of stay and the discharge process: Preparing breast cancer patients appropriately. *Patient Educ Couns*. 2019;102(12):2318-2324. doi:10.1016/j.pec.2019.08.012
25. Sjarifudhin M, Maria Rosa E. Effectiveness of Patient Centered Care to Reduce Anxiety Level and Improve Satisfaction in Patients Undergoing Cataract Surgery. *J Medicoeticolegal dan Manaj Rumah Sakit*. 2018;7(3):188-195. doi:10.18196/jmmr.7372
26. Secretary CV, Dusik-sharpe J, South A, et al. In This Issue : Board of directors , committee chairpersons and associated organization representatives / Conseil d'administration , responsables des comités et représentants des groupes associés 2011 Executive. 2011;33(1):14-27.
27. Tomes AE, Peng Ng SC. Service quality in hospital care: The development of an in-patient questionnaire. *Int J Health Care Qual Assur*. 1995;8(3):25-33. doi:10.1108/09526869510089255
28. Cowie MR. Person-centred care: More than just improving patient satisfaction? *Eur Heart J*. 2012;33(9):1037-1039. doi:10.1093/eurheartj/ehr354
29. Amran, Stang, and Anwar Mallongi. 2017. Analysis of dengue fever risk using geostatistics model in bone regency. *AIP Conference Proceedings* 1825, 20002 <https://doi.org/10.1063/1.4978971>
30. Muhith A, Winarti E, Perdana SSI, Haryuni S, Rahayu KIN, Mallongi A. Internal Locus of Control as a Driving Factor of Early Detection Behavior of Cervical Cancer by Inspection Visual of Acetic Acid Method. *Open Access Maced J Med Sci [Internet]*. 2020 Apr. 20 [cited 2022 Nov. 10];8(E):113-6.
31. Masriadi, Rahmawati Azis, eha Sumantri, Anwar Mallongi. Effectiveness of non pharmacologi therapy through surveillance approach to blood pressure degradation in primary hypertension patients, Indonesia. *Indian Journal of Public Health Research & Development*, 2018; 9(2): 249-255