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Erythrocyte Morphologichal Characters on Wedang Empon Herbal Drink Extract: Anticoagulan in Vitro Study

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Abstract
The use of medicinal plants as a form of traditional medicine has a long history in Indonesia. The country's diverse population, comprising numerous tribes, cultures, and belief systems, has resulted in a rich tapestry of practices and traditions surrounding the use of medicinal plants. Several evaluations have shown that anticoagulant drugs have weaknesses, one of which is the use of heparin, which has the side effect of causing bleeding. The above side effects and problems of anticoagulant treatment cause the importance of finding alternative anticoagulant treatments made from natural ingredients that can safely prolong blood clotting time. Researchers employ reverse pharmacology and observational therapeutics to identify novel compounds in herbal sources, thereby facilitating the discovery of new drugs. This study aims to test the anticoagulant activity of wedang empon extract to provide scientific evidence regarding its utilization by the community. The anticoagulant activity test was performed in vitro using the blood smear method on blood smears treated with Wedang Empon extract 300µl, 400 µl, and 500 µl. The results showed that anticoagulant activity of extract wedang empon was suggested at volume 500 µL, which was characterized by the morphology of blood cells, the shape intact and contact separated from one to another.
Keywords: Erythrocyte, Anticoagulan, in Vitro, Wedang Empon

INTRODUCTION

The use of medicinal plants as a form of traditional medicine has a long history in Indonesia. The country's diverse population, comprising numerous tribes, cultures, and belief systems, has resulted in a rich tapestry of practices and traditions surrounding the use of medicinal plants. These practices and traditions vary considerably, reflecting the diverse processing methods and purposes associated with these plants(Silalahi, 2016). Herbs are an invaluable resource in the discovery of natural products, which are essential in addressing the evolving healthcare needs of populations worldwide. Researchers employ reverse pharmacology and observational therapeutics to identify novel compounds in herbal sources, thereby facilitating the discovery of new drugs(Subramani & Sathiyarajeswaran, 2022). The Coronavirus Disease (Covid-19) pandemic has resulted in the necessity for individuals to prioritize their health and wellbeing. This encompasses not only physical health but also the collective responsibility of communities to safeguard the health of their members. This can be achieved through a multifaceted approach, encompassing the treatment, prevention, and management of illness. Ancestral beliefs, passed down through generations, have shaped the utilization of natural ingredients in traditional medicine. Despite the advent of modern medicine, traditional medicine persists as a prominent aspect of healthcare in many communities(Romadhonsyah, 2023; Supriani; et al, 2022). One example is wedang empon, a herbal drink originating from the Special Region of Yogyakarta. It comprises a variety of medicinal plants, such as ginger, temulawak, turmeric, lemongrass, secang, and cinnamon. As a functional drink, this type of drink is known for its compounds that are beneficial to the body

because the rhizomes are known as natural ingredients that contain bioactive compounds with antioxidant activity such as phenolic groups(Fitriarni, 2021).

The current pandemic, caused by the novel coronavirus disease 2019 (Covid-19), is associated with an elevated risk of thromboembolic complications due to a hypercoagulability state of the blood, clinically referred to as "Covid-19 associated coagulopathy" (CAC)(Subramani & Sathiyarajeswaran, 2022). Coagulopathy is defined as an impairment in the blood's ability to form clots. Coagulopathy associated with the SARS-CoV-2 infection has emerged as a significant contributor to morbidity among those infected with the illness(Birkeland et al., 2020). Available evidence suggests that severe COVID-19 disease can develop coagulopathic complications in the form of disseminated intravascular coagulation (DIC), which is prothrombotic with a high risk of venous thromboembolism, where D-dimer markers can be used as parameters for blood coagulation disorders. Based on this, experts recommend the use of anticoagulants, reflecting the recognition of coagulation dysregulation in these circumstances(Rusdiana & Akbar, 2020).

Blood is a vital fluid for sustaining life. It is opaque and reddish in color, serves as connective tissue, and contains a pale yellow-colored fluid matrix known as plasma. The plasma contains suspended blood cells, including erythrocytes or red blood cells (RBCs), leukocytes or white blood cells (WBCs), and platelets or thrombocytes (Plts), as well as other biochemical substances (Arshad, 2024). Erythrocytes represent the most prevalent element in the composition of blood. Their number is expressed in units of $106/mm^3$, exhibiting variations contingent on the species. For instance, the human erythrocyte count is approximately $5\times106/mm^3$(Cocan et al., 2022). A hematological test is a test that is often requested by clinicians. This hematology examination is used by clinicians as a basis for patient management. Therefore, this hematology examination must be performed properly and correctly to provide thorough and accurate results with good validation (Astina, 2020).

Anticoagulants function by interfering with the proteins responsible for blood clotting or clotting factors, thereby preventing the formation of blood clots(Subramani & Sathiyarajeswaran, 2022). Until recently, vitamin K antagonists were the only oral anticoagulant agents available. Warfarin remains the most commonly prescribed oral anticoagulant worldwide(Abebe, 2019). The most commonly utilized and recommended in vitro anticoagulant is ethylenediamine tetraacetic acid (EDTA). Calcium is a necessary cofactor for enzymes of the coagulation cascade. EDTA chelates this calcium, thus preventing the clotting of blood in tubes. Some anticoagulants are used for long-term preservation of blood. Additionally, anticoagulants have pharmacological effects that are employed to prevent venous thromboembolism.

The effects of different anticoagulants on blood cells and their storage times depend on their chemical composition(Subramani & Sathiyarajeswaran, 2022). However, these anticoagulants also have some disadvantages, which render blood transfusion unsafe and even life-threatening. Anticoagulants like EDTA also have disadvantages in the diagnostic field because they chelate metal ions and are used as sodium (Na+) and potassium but dilution of the sample could have significant effects (K+) salts; therefore, metal ions (calcium, iron, magnesium). They may cause changes in platelet count and alterations in blood cell morphology and biochemical processes. When the blood is stored in anticoagulants such as EDTA, constituents of blood are affected(Abebe, 2019; Arshad, 2024; Samuels, 2005). A number of evaluations have demonstrated that anticoagulant drugs possess inherent limitations. One such drawback is the use of heparin, which has the adverse effect of increasing the risk of bleeding. This is due to the inhibition of proteases involved in the clotting process, including factors IIa (thrombin), Xa, and IXa. Prolonged treatment is employed for the prevention of

stroke in at-risk groups, although this may result in the development of osteoporosis and an increased susceptibility to spontaneous fractures(Rusdiana & Akbar, 2020). This study was conducted to identify potential anticoagulant agents derived from natural sources. Wedang empon is a traditional Indonesian drink, comprising a mixture of spices. It is known to have various health benefits, such as boosting the immune system and reducing inflammation. However, its effect on red blood cell (erythrocyte) morphology has not been widely studied.

RESEARCH METHODS

This study used a completely randomized design Complete Randomized Design (RAL), comprising two control groups and three treatment groups. Each control and treatment group consisted of three replicates. The control groups were designated as (-) blood without treatment and (+) blood given 250 µl EDTA (10% concentration), while the treatment groups were designated as blood was given 300 µl, 400 µl, and 500 µl wedang empon extract, respectively.

Extraction and Preparation

The wedang empon decoction extract was prepared via boiling a mixture of spices in water to a specific temperature for an appropriate duration. After boiling, the extract underwent filtration and concentration for subsequent utilization in the study.

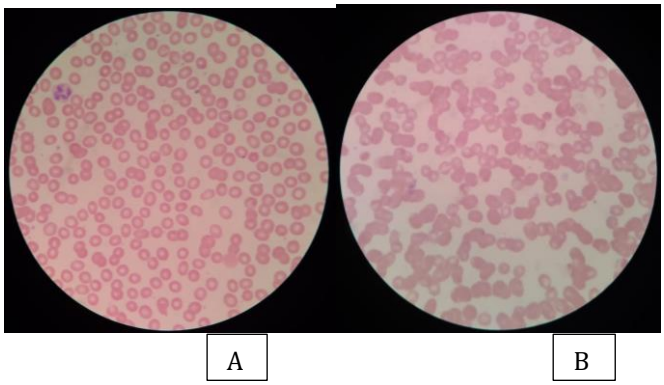
Morphological Analysis of Red Blood Cells

To evaluate the morphological characteristics of red blood cells, blood samples were examined using light microscopy following the completion of the designated treatment period. The blood preparations were stained using Wright-Giemsa dye, which facilitated the identification of erythrocyte morphology. Blood smears were performed to erythrocyte morphology. Blood smears were prepared according to the method described by Coles et al. (1986). The blood smear preparation was stained with Diff Quick Dye (MDT Indoreagen®), which consisted of three steps: fixation with methanol solution for 40-60 seconds, staining with eosin solution for 20-30 seconds, and then staining with methylene blue solution for 15-30 seconds. The specimens were rinsed with distilled water, allowed to dry, and stored in an object glass box until reading. Five clean and lean glass slides were prepared and fixed in ethanol PA solution. Each specimen was assigned a number from 1 to 5. The glass slides numbered 1 to 5 were identical to those used in the Lee-White method. A total of 2 µl of blood was extracted from each of the glass objects numbered 1 to 5. Subsequently, the blood was photographed and observed under a microscope (SZ2-ILST) with 40x magnification.

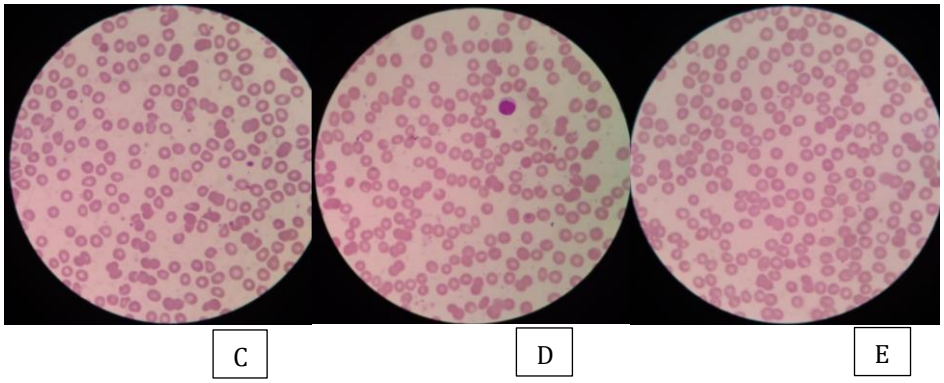
RESEARCH RESULTS AND DISCUSSION

Five 3-mL plain Vaculab tubes were prepared and filled with 1 mL of blood each. Each tube was supplemented with wedang uwuh extract, obtained through a decoction and steeping process with varying volumes: 300 µl, 400 µl, and 500 µl. The tubes were then incubated and observed at 15-second intervals to ascertain the occurrence of coagulation. A sample of blood was taken from each treatment, after which it was dripped onto a series of glass objects numbered from 1 to 6 in sequence. Subsequently, the slide method was employed on the blood sample until a thin blood smear was obtained. The preparations were then fixed with 95% alcohol for 15 minutes, after which they were aerated to dry. The preparations were then immersed in Giemsa solution for 30 minutes, after which they were rinsed with running water and aerated to dry and then observed under a microscope (SZ2-ILST) with 40x magnification. In blood smear preparations in which clotting occurs, erythrocytes are observed to be dense

and clustered, with transparent peripheral areas. In contrast, in blood smear preparations in which clotting does not occur, platelets are seen to be round and not clustered, with empty nuclei (Picture 1).



Description:
A = Positive control (+ EDTA)
B = Negative control (- EDTA)
C = Blood + 300 µl extract (1)
D = Blood + 400 µl extract (2)
E = Blood + 500 µl extract (3)



Discussion

Based on microscopic observations on blood smear preparations using Giemsa Staining (Figure 1), it can be seen that there is no clustering of blood cells in preparations that have 500 µl of wedang uwuh extract. This is in accordance with previous research which states that in blood smear preparations, the shape of non-frozen red blood cells will appear round and have empty nuclei and not grouped together(Nugroho et al., 2015; Rahmawati et al., 2018). Most common stains Giemsa staining is commonly used because Giemsa is more stable in tropical climates. Some hospitals and clinics, even Puskesmas, also use Giemsa staining to stain peripheral blood smears because Giemsa gives representative results with an orange-red granular color and peripheral blood smears can survive well (Astina, 2020).

In treatments 1 and 2, it was observed that some blood cells were in close proximity to one another, exhibiting gaps in between. Additionally, some cells were tightly adhered to neighboring cells, and cells were found to be aggregated in clusters. In treatment 3, it was observed that some blood cells were in close proximity to one another, exhibiting gaps between them. Some cells were tightly adhered to other cells, and no cells were found to be piled up. The relationship between each cell is such that no interference occurs, resulting in a clear shape for each cell. Anticoagulants are substances added to the blood to into the blood to inhibit or prevent the clotting process by binding or precipitating calcium ions and inhibiting the formation of thrombin from prothrombin. However, not all types of anticogulants can be used because some anticogulants may affect the shape of the erythrocytes or leukocytes being tested morphologically. Mixing the blood sample with the anticoagulant should be done very gently as it may cause hemolysis(Azzahrah et al., 2024; Nugroho et al., 2015). There are many types of

anticoagulants, depending on the type of examination or treatment to be performed. Each type of anticoagulant works differently to prevent blood clots from forming. A good anticoagulant is one that does not damage the components of the blood and is appropriate for the type of test being performed. Erythrocytes are the red blood cells most susceptible to this damage. Inappropriate concentrations of anticoagulants can also cause cell swelling, hemolysis, or crenation(Azzahrah et al., 2024). A crenation is a form of erythrocyte that is shrunken and has protrusions on its surface. Crenations usually form in blood that has been left for a long time, which also means that the longer the exposure to anticoagulants, the more likely it is that crenations will form(Astina, 2020).

Combining various rhizomes with other herbal plant to form functional drinks is becoming increasingly popular, especially in almost all cities in Indonesia at this present. It is consumed as traditional drinks and good sources for health promoting by its phenolic compounds. In addition, there are some people who believe that mixing rhizome drinks with other ingredients such as lemongrass, cinnamon, sappan wood will make this functional drink richer in efficacy and nutritional content as well as changes in taste, aroma and color attributes. Wedang empon is a traditional Indonesian beverage composed of a blend of spices, including ginger (*Zingiber officinale* Roscoe), secang (*Caesalpinia sappan* L.), turmeric (*Curcuma domestica*), temulawak (*Curcuma zanthorrhiza*), lemongrass (*Cymbopogon citratus*), and cinnamon (*Cinnamomum burmanni* Nees ex Bl.). The anticoagulant properties of gingerol-rich ginger have been well-documented. Cinnamon contains a coumarin chemical component that functions as an anticoagulant by inhibiting the action of vitamin K, which is essential for the activation of clotting factors (Abebe, 2019; Emilda, 2018; Subramani & Sathiyarajeswaran, 2022)..Turmeric contains an active substance in the form of curcumin and its derivatives, namely bisdemethoxycurcumin (BDMC) and demethoxycurcumin, which can act as anticoagulants. It has been demonstrated that curcumin and its derivatives are capable of inhibiting thrombin and factor Xa activation in human blood plasma without the involvement of thrombin III. The research conducted by Kim DC and colleagues demonstrated that the effects of curcumin and its derivatives can prolong the prothrombin time (PT) and activated partial thromboplastin time (aPTT) (Agung et al., 2021; Kim & Park, 2019).

The process of blood coagulation entails the transformation of blood components into a semisolid material, known as a blood clot. The composition of blood clots is primarily constituted by blood cells that are entrapped within fibrin networks. The process of blood clotting is initiated when a wound or injury occurs. This results in the severed blood vessel undergoing contraction and retraction, accompanied by a hemostatic reaction(Fabiana Meijon Fadul, 2019). Blood clotting occurs through the interaction of clotting factors with sequential or cascading proteolytic reactions (coagulation cascade). At each step, a clotting factor is proteolytically degraded and becomes protease active. The resulting protein activates the next clotting factor until a solidified fibrin clot is formed(Rizalallah, 2020). Initiation of the coagulation process can occur through one of two pathways, the extrinsic pathway and the intrinsic pathway. Regardless of which pathway is the initial process, the two pathways will converge into a common pathway which is the final pathway. The intrinsic pathway requires a cascade reaction in which the activation of one factor leads to the activation of the next successor form. This pathway involves the activation of factors Factor XII (Hageman Factor/Serine Protease), XI (Plasma Thromboplastin), IX (Chrismast Factor), Factor VIII, Platelet Phospholipid (PL), which then enter into a common pathway. These factors activate factor X with the help of factor VIII, phospholipids and Ca2+ ions. The extrinsic pathway requires the release of tissue factors, namely Factor III or thromboplastin and which simultaneously activates factor VII which then enters the pathway along with activating factor

X Active factor X together with active Factor V (activated thrombin), with the help of Ca2 + ions and phospholipids to catalyze and activate prothrombin into thrombin. Furthermore, thrombin together with Ca2+ converts fibrinogen into fibrin in the form of monomers. This monomeric fibrin is converted by active Factor XIII (activated by thrombin with Ca2+ ions) into solid fibrin in the form of a polymer. The intrinsic and extrinsic pathways meet at the so-called common pathway, i.e. Factor X activation occurs through the reaction of the extrinsic and intrinsic pathways. Most clotting factors are precursors of known proteolytic enzymes with zymogen and circulate in an inactive state. Most procoagulants and anticoagulants are produced by the liver, with the exception of factors III, IV and VIII(Birkeland et al., 2020; Kim & Park, 2019; Rizalallah, 2020; Rohmah & Fickri, 2020).

The observation parameters indicated that the anticoagulant activity of the wedang empon extract was effective in preventing blood clotting. These findings demonstrate the potential of wedang empon extract as a natural anticoagulant alternative. Consequently, wedang empon may be employed as a natural remedy for cardiovascular diseases, including myocardial infarction. The majority of myocardial infarctions are caused by the formation of blood clots. Anticoagulants are pharmaceutical agents that are used to thin the blood. In other words, anticoagulants are designed to prevent the formation of blood clots. The administration of these pharmaceutical agents is crucial for the prevention of complications associated with cardiac arrhythmias. Among the most commonly utilized pharmaceutical agents are warfarin and aspirin (Hastuti et al., 2021; Kim & Park, 2019).

CONCLUSION

Based on the observation of blood cell morphology on blood smear preparations, it can be concluded that 500µl wedang empon extract may have potential as an anticoagulant.

BIBLIOGRAPHY

Abebe, W. (2019). Review of herbal medications with the potential to cause bleeding: dental implications, and risk prediction and prevention avenues. In *EPMA Journal* (Vol. 10, Issue 1, pp. 51–64). Springer International Publishing. https://doi.org/10.1007/s13167-018-0158-2

Agung, R., Sakti, M., Roslaeni, R., & Harihardjaja, W. (2021). *Pengaruh Ekstrak Kunyit (Curcuma Longa) Terhadap Proses Prothrombin Time (PT) Effect Of Turmeric Extract (Curcuma Longa) On Blood Cloting Process Based On Prothrombin Time (PT) Examination*.

Arshad, et al. (2024). Biological and Clinical Sciences Research Journal. *Biological and Clinical Sciences Research Journal*, 1–10.

Astina, F. (2020). *Analisis Kualitatif Morfologi Eritrosit Pada Apusan Darah Edta (Ethylene Diamine Tetraacetic Acid) Untuk Pemeriksaan Segera (0 Jam) Dan Pemeriksaan Ditunda (2 JAM) Qualitative Analysis of Erythrocyte Morphology in EDTA (Ethylene Diamine Tetraacetic*. 326–334.

Azzahrah, P., Esfandiari, A., Mihardi, A. P., Widhyari, S. D., Wulansari, R., & Ningtias, P. I. (2024). *Erythrogram Profile of Blood Samples Anticoagulated with Tri- potassium Ethylene Diamine Tetraacetic Acid (K 3 EDTA) Stored for 48 Hours at 4 o C*. *7*(1), 76–87. https://doi.org/10.20473/jmv.vol7.iss1.2024.76

Birkeland, K., Zimmer, R., Kimchi, A., & Kedan, I. (2020). Venous Thromboembolism in Hospitalized COVID-19 Patients: Systematic Review. *Interactive Journal of Medical Research*, *9*(3), e22768. https://doi.org/10.2196/22768

Cocan, D., An, V. M. Ş., Ducu, C. R. Ă., Uiuiu, P., Giurgiu, A., & Puc, T. P. Ă. (2022). *The Impact Of Two Anticoagulants On Erythrocytes Morphology In Different Vertebrate Species*. https://doi.org/10.46909/journalalse-2021-023

Emilda. (2018). Efek Senyawa Bioaktif Kayu Manis (Cinnamommum burmanii) Terhadap Diabetes Melitus. *Jurnal Fitofarmaka Indonesia*, *5*(1), 246–252.

Fabiana Meijon Fadul. (2019). *Perbedaan Jumlah Trombosit Menggunakan Antikoagulan EDTA Dan Bawang Putih Sebagai Antikoagulan Alternatif*. 6–15.

Fitriarni, D. M. R. (2021). *Formulation of Indonesian traditional functional drink wedang empon based on Zingiberaceae rhizomes mixed with fruits Formulation of Indonesian traditional functional drink wedang empon based on Zingiberaceae rhizomes mixed with fruits*. https://doi.org/10.1088/1755-1315/913/1/012028

Hastuti, I., Nurrochmad, A., Puspitasari, I., & Fakhrudin, N. (2021). Studi Aktivitas Antiplatelet Dan Antitrombosis Ekstrak Air Daun Sukun (Artocarpus altilis (Park.) Fosberg). *Jurnal Tumbuhan Obat Indonesia*, *14*(1), 85–94. https://doi.org/10.22435/jtoi.v14i1.4227

Kim, K., & Park, K. Il. (2019). A Review of Antiplatelet Activity of Traditional Medicinal Herbs on Integrative Medicine Studies. In *Evidence-based Complementary and Alternative Medicine* (Vol. 2019). Hindawi Limited. https://doi.org/10.1155/2019/7125162

Nugroho, R. A., Hewan, M., & Biologi, J. (2015). *Uji Aktivitas Antikoagulan Ekstrak Propolis Trigona Laeviceps Terhadap Darah Mencit (Mus musculus L .)*. *September*, 1–10.

Rahmawati, R., Fawwas, M., Razak, R., & Islamiati, U. (2018). Potensi Antikoagulan Sari Bawang Putih (Allium sativum) Menggunakan Metode Lee-White dan Apusan Darah. *Majalah Farmaseutik*, *14*(1), 42. https://doi.org/10.22146/farmaseutik.v14i1.41927

Rizalallah, A. A. (2020). Uji Aktivitas Antikoagulan Ekstrak Etanol 96 % Daun Sirih Merah (Piper Crocatum Ruiz & Pav) Secara In Vitro. *Skripsi, STIKES Rumah Sakit Anwarfile:///C:/Users/Advan/Downloads/BAB II.Pdf Medika*, 1–114.

Rohmah, M. K., & Fickri, D. Z. (2020). Uji Aktivitas Antiplatelet, Antikoagulan, dan Trombolitik Alkaloid Total Daun Pepaya (Carica papaya L.) secara in Vitro. *Jurnal Sains Farmasi & Klinis*, *7*(2), 115. https://doi.org/10.25077/jsfk.7.2.115-125.2020

Romadhonsyah, F. (2023). Studi Etnomedisin Pada Masyarakat Di Kampung Demi Kabupaten Bantul, Yogyakarta. *Jurnal Ilmiah Ibnu Sina*, *8*(2), 286–296.

Rusdiana, T., & Akbar, R. (2020). Perkembangan Terkini Terapi Antikoagulan Pada Pasien Covid-19 Bergejala Berat. *Jurnal Sains Farmasi & Klinis*, *7*(3), 244. https://doi.org/10.25077/jsfk.7.3.244-250.2020

Samuels, N. (2005). Herbal remedies and anticoagulant therapy. *Thrombosis and Haemostasis*, *93*(1), 3–7. https://doi.org/10.1160/TH04-05-0285

Silalahi, M. (2016). *Studi Etnomedisin Di Indonesia Dan Pendekatan Penelitiannya*. 117–124.

Subramani, B., & Sathiyarajeswaran, P. (2022). Current update on herbal sources of antithrombotic activity—a comprehensive review. *The Egyptian Journal of Internal Medicine*, *34*(1). https://doi.org/10.1186/s43162-021-00090-9

Supriani; et al. (2022). *Studi Etnomedisin Tumbuhan Berkhasiat Obat Pada Masyarakat Desa Karangjengkol Di Masa Pandemi Covid-19*. *11*(3), 189–194.