Clotting And Gel Separator Property Of Extract From The Roots Of Damong Maria (Artemisia Vulgaris) With Oryza Sativa

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Abstract: The root extract of Artemisia vulgaris with Oryza sativa promotes the blood clotting. Due to its protein-binding property, it could participate in thickening effects of the blood. With the emerging need for low cost-effective blood coagulant and gel separator, the researchers realized the urge in developing an alternative clot activator and gel separator from the root extract of Artemisia vulgaris with Oryza sativa. The study determined the effectiveness of clotting and gel separator property of the root extract Artemisia vulgaris with Oryza sativa using the normal human blood samples. An experimental approach to research method was used. The researchers were able to isolate the Oryza sativa starch and the root extract of Artemisia vulgaris and were subjected to different physical and phytochemical tests to confirm the presence of tannin. The participants were undergoing 10 to 12 hours of fasting before blood collection. The blood collected using the commercially prepared Serum Separator Tube and the alternative clot activator and gel separator made from the root extract Artemisia vulgaris with Oryza sativa. The data gathered from the participants were analyzed using mean and ANOVA. The study revealed that on the different concentrations of the Artemisia vulgaris root Extract the 25%, 50%, 75% and 100% has significant effect on the blood clotting time, and there are substantial variations on the blood clotting time using different concentrations of the Artemisia vulgaris root extracts and the commercially prepared Serum Separator Tube. It was concluded that varying levels of the root extract of Artemisia vulgaris do not affect the analysis of the Blood analytes. The researchers recommend to chemically isolating the specific component, pure tannin, from the roots of Artemisia vulgaris using the other methods for the extraction of tannin and other components to determine other components of the Artemisia vulgaris that may improve the clotting time and the gel separator.

Keywords: Clotting separator, gel separator, medical technology, Philippines

1. Introduction

In a fast-paced world, innovations are further explored through science and flow of modernity. Newer ideas are contributed in the field of science. New inventions that agree with the need for humanity and discoveries in health and medicine are undesirability observed from time to time. And with all the bane of this ever-changing world gives, there is also a boon that it brings. Evacuated blood collection system provides many advantages in blood collection procedures (Elliott & Peakman, 2008). It is safe, easy to use, shortens the time of collection, and allows multiple samples to be drawn in just a single needle entry (Miller, 2003). This method employs two-way needles, an adaptor, and evacuated tubes (Bell, 2004). Evacuated tubes facilitate the draw of a predetermined volume of blood into the tube. It enhances the precision and accuracy of the test results by reducing errors in the collection (e.g., blood-to-additive ratios and contamination). Evacuated tubes were initially produced using pop lime or borosilicate glass, yet pop lime tubes were found to discharge calcium and magnesium into blood examples (McCann, 1998). Glass evacuated tubes that are manufactured today are designed to be airtight, waterproof, and thermally resistant, which allows for vacuum preservation and long shelf lives (Bowen & Adcock-Funk, 2015). Additives are also employed in these tubes; it can range from those that promote faster clotting of the blood, to those that enable anticoagulation, and to those that preserve or stabilize certain analytes or cells. The inclusion of additives at the proper concentration in evacuated tubes greatly enhances the accuracy and consistency of test results and facilitates faster turnaround times in the laboratory (Pagana & Pagana, 2012). One of the additives used in the laboratory is the Serum separator gel. Serum blood gathering tubes with separator gel are utilized by numerous research centers for science examinations because of the upside of the obstruction gel that encourages snappy and finishes partition of serum from platelets as expressed by Lippi et al. (2013). Subsequently, Bowen and Adcock-Funk (2015), the tubes contain an inert, thixotropic polymer gel as the separator gel. Due to differences in density of these components, the gel is displaced and moves upward and forms a barrier between serum and blood cells upon centrifugation and prevents the crossing of molecules and proteins released from the cells to the serum. Tubes with separator gel improve serum stability for analysis, diminish sample manipulations, reduce aerosolization of hazardous substances, provide higher serum or plasma level and decrease the probability of contamination or sample switching. The use of serum separator gel tubes as primary tube also helps in eliminating the requirement of

re-barcoding. Improper design or use of blood collection devices can adversely affect the accuracy of laboratory test results. Components from blood collection tubes, such as stoppers, lubricants, surfactants, and separator gels, can lead into specimens and adsorb analytes from a specimen; special tube additives may also alter analyte stability. The serum separator gels accessible in the market today are comprised of manufactured polymers gel weighs 0.6g - 1.6g and has a relative outward power of 1200-1500 for 10 minutes. After blood collection and transfer, the tube is immediately inverted 6-8 times. The complete clotting time is more or less, 30 minutes if there is no dysfunction of the platelet. Numerous mechanisms have involved maintaining blood in a state of delicate balance. One of the essential actions of the blood is the clotting process. Several reports of components from blood collection tubes were altered the serum stability. Because of these interactions with blood specimens, blood collection devices are a potential source of pre-analytical error in laboratory testing (Lim, 2014). For centuries, people made use of different natural and synthetically available resources. It is the fact that people have long depended on the plant in their daily existence. Plants served as their clothing and food. Moreover, with the increasing economic expenses, the search for different alternatives emerged, especially in the field of medicine. In the Philippines, a country blessed with almost a hundred of plant species used these as an alternative. Artemisia vulgaris, as one of the commonly used herbal medicine, was being studied for research. Artemisia vulgaris was also known as Wormwood, Motherwort, Maidenwort, Mugwort in foreign countries; Arbaaka, Erbaka, Kamaria, Damong Maria in local areas. A plant present abundantly in areas at low and medium altitudes according to Ragragio et al. (2013), it was used by Herbalist or "Herbolaryos" to stop bleeding, inflammation, menstruation, cramps, menorrhagia, relieves the pain of indigestion, diarrhea, and dysentery, hematuria in the Philippine countryside. This plant was also used in other countries like Persia, Afghanistan, India, China, and Uruguay to induce coagulation in bleeding disorders (e.g., Von Willebrand Disease). Damong Maria contains tannins, alkaloids, volatile oil, saponins, calcium oxalate, iodine, vitamins A, B and C. Among the constituents being stated, tannin extract from Damong Maria can promote blood clotting. These compounds are naturally occurring plant phenols, which combine with proteins and other polymers to form stable complexes. Due to its protein-binding property, it can participate in clotting of blood. With the emerging need for low cost-effective coagulant, the researchers realized the urge in developing an alternative from the tannin extract from Damong Maria roots. The significance of this plant doesn't end with its medicinal plant properties, but it can also contribute to the medical field. This study aimed to employ the effectiveness of Artemesia Vulgaris and the rice starch Oryza sativa as a clot activator and gel separator that can be used in a clinical laboratory. Oryza sativa also was known, as is Rice; the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize, (Vareeket & Soytong, 2015). Rice starch granules are smaller than other cereal starches with amylose contents varying from virtually amylose-free in waxy to about 35% in nonwaxy and long-grain rice starches. Amylose content appears to be the major factor that controlled in almost all physicochemical properties of rice starch due to its influence on pasting, gelatinization, retrogradation, syneresis, and other functional properties.

2. Framework

The data derived from the assessment of clotting time of Artemisia vulgaris root extract with Oryza sativa that were tested in the typical blood sample. This was utilized to describe and analyze the differences between the clotting time of the control group and the experimental group. Due to the extreme increase of the synthetically prepared medicines available in the country, the researchers decided to make a study on Artemisia vulgaris. The researchers were based on the study of Riaz and Khan (2016) that there is significant coagulation in vivo effect of the tannins that introduced in rabbits. The Artemisia vulgaris contained tannins, which have a protein-binding property that could participate in clotting effect. This study is supported by Davie and Hanahan (2012), the presence of thrombokinase, prothrombin, Calcium ions, fibrinogen is essential for coagulation. However, this is different in a way that in the research conducted; it used the tannin content obtained from the root extract of Artemisia vulgaris. Many laboratories widely use serum blood collection tubes with separator gel for chemical analyses. SSTs are sometimes called "marble-top tubes" or "gold-topped tubes," referring to the stoppers, which are either gold or red-gray. SPS tubes have a paler color, sometimes leading confusion, these are known as "Yellow tops" not "Gold." Trademarked versions include Covidien "Corvac" tubes. It is used in medical clinical chemistry tests requiring blood serum. They contain an extraordinary gel that isolates platelets from serum, and also particles to make blood clump rapidly. The blood test may then be centrifuged, enabling the reasonable serum to be expelled for testing. Serum-separating tubes have a thixotropic gel that separates the whole blood into a serum portion and clotted portion. This thixotropic gel is composed of liquid polybutene polymer. The gel frames a physical hindrance between serum or plasma and platelets amid centrifugation. Serum Gel separator tubes were centrifuged for 15 minutes at 3500 rpm in a swing bucket centrifuge. The gravity of this gel is 1.04 g/cm3 to permit its proper positioning between serum and cellular constituents of blood upon centrifugation. The density of the liquid and cellular components of the blood typically ranges from 1.026 to 1.031 g/cm3 and 1.092 to 1.095 g/cm3, respectively. Rice also used in the study. It has been known as the staple food of Filipino people. Rice starch is primarily composed of amylopectin and amylose. Amylose is a large polysaccharide molecule made of D – glucose units and comprises about 20 to 30 percent of the total rice structure. The remaining amount consists of amylopectin, which is also a polysaccharide. This study aims to identify the potential of rice starch as an alternative to the artificially manufactured serum separator tubes. Rice starch was extracted by homogenizing rice with distilled water using a blender resulting in a mixture that was being filtered using cheesecloth. The filtrate obtained was refrigerated for 24 hours for the starch to settle at the bottom. Once the standing time is up, the

supernatant formed above were discarded. Obtained 15g of the starch residue and diluted with 30ml of NSS, and added on the different concentrations of the root extract from Artemisia vulgaris. After the dilution, the solution will be heated at 195-240°C on a hot plate until it starts to gelatinize. The temperature of the gel maintained at a temperature of 63-69°C. The prepared gel concentrations were integrated into a tube and were used during blood collection. The potential of rice starch as a gel separator was determined by its ability to migrate between the serum and formed elements of blood after centrifugation. This study aimed to determine the clotting and gel separator property of Artemesia Vulgaris with the Oryza sativa as an alternative to the artificially manufactured serum clot activator and a gel separator. This vital information for both foreign and local literature and studies, regarding Artemisia vulgaris and Oryza sativa, which the researchers believed to have bearing to present study. The researchers used Damong Maria as the subject of the study, which is suspected, as the source of tannin extract.

3. Objectives of the Study

The study aimed to determine the clotting and gel separator property of extract from the roots of Artemisia vulgaris with Oryza sativa. In particular, the researchers would like to determine the: 1) Concentration of Artemisia Vulgaris Root Extract gives a significant effect on blood clotting and 2) Significant variations in the blood clotting time using different concentrations of Artemisia vulgaris root extracts.

4. Scope, Delimitations, and Limitations

The researchers used Artemisia vulgaris roots as the main experimental sample of the study. The study primarily deals with the clotting property of tannin extract from the roots of Artemisia vulgaris tested in human normal blood samples. The study included the experimental and procedural analysis of the plant. The experimental method was used to measure the physical property of Artemisia vulgaris Linn. While procedural methods involved the following: (1) identification test, extraction and purification of tannins and determination of percent yield; (2) Isolation of rice starch; and (3) the concentration at which tannins employed its clotting property. The study involved experimentation on human subjects specifically extraction and testing of their serum. The study focuses on the effectiveness of Artemisia vulgaris tannin and rice starch as serum gel separator to separate the solid components of the blood from the liquid part and clot activator, however it may vary with the factors such as components, procedures and chemical composition of Artemisia vulgaris and Oryza sativa that may affect the results of the test and to give find the proper dilution or quantity needed in order to have an alternative serum gel separator and clot activator through the use of Artemisia vulgaris root extract without interfering with the test results. This study aimed to look at the potential of Artemisia vulgaris root extract and the Oryza sativa as an alternative to the artificially manufactured thixotropic polymers as constituents for the commercial serum separator tubes. The experiment is only limited to healthy human subjects. The study was limited to specific chemistry test, and clotting time. The study does not cover other blood tests such as Blood Urea Nitrogen (BUN), Creatinine, Uric acid, other enzymes like Alkaline Phosphates (ALP), Amylase, and other blood electrolytes such as Phosphorous, Chloride, Magnesium, etc. This study also limited only to determine whether there is a significant variation in blood clotting and effect of the different concentrations of the Artemisia vulgaris root extract with Oryza sativa.

5. Methodology

The method used in the study is the Experimental method. The method was identified the clotting effect of tannin that is extracted from the roots of Artemisia vulgaris in the blood. Experiments were done to show the capability of the root extract from a particular plant such as Artemisia vulgaris in blood clotting. The participants of the study have composed of twelve healthy participants, six normal females and six normal males with the age of 18 years old and above. Artemisia vulgaris and Oryza sativa were collected from the province of Quirino, Isabela. The researchers conducted the study, Artemisia vulgaris, and Oryza sativa as Serum Gel Separator and Clot Activator at Centro Escolar University-Makati. The Centro Escolar University-Makati is located in Makati, Philippines.

Figure 1: *Roots of Atremisia vulgaris*

Figure 2: *Grinding of the roots using electric blender*

Figure 3: *Powdered Roots of Artemisia vulgaris*

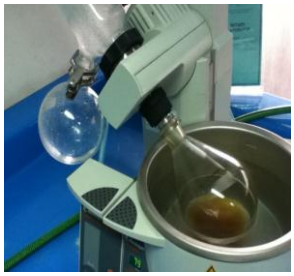

Figure 4: *Rotary Evaporator*

Figure 5: *Powdered Root Extract*

Figure 6: *Rice starch*

Figure 7: *Root extract with Starch*

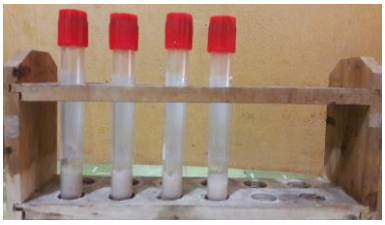

Figure 8: *Prepared Clot Activator & Gel Separator*

Figure 9: *H-19 α Kokusa Centrifuge*

5.1 Authentication/Verification and Collection of Sample

The roots of Artemisia vulgaris are collected from the Province of Isabela and processed for authentication. The plant sample was submitted to the Bureau of Plants and Industry located in Manila where the verification of the sample was done. The roots of Artemisia vulgaris were used as the primary sample of the study.

5.2 Preparation of the Plant Sample

The roots were washed with water to expel the soil holding fast to it. Then it was air dried at room temperature. After air-drying, the roots were ground using an electric blender and placed in a covered container.

5.3 Selection of Subjects

This study used a purposive sampling upon obtaining four different set-ups consists of five tubes: control (commercial serum separator gel) and four different concentrations of the alternative Artemesia Vulgaris root extract (25%, 50%, 75%, 100%) concentration. Determinations were come from the venous blood extraction using the syringe method. The subjects of the study were composed of twelve healthy participants, six normal females and six normal males with the18 years of age and above. The normal human as the subject of the study was undergo fasting for 10 to 12 hours before blood collection.

6. Procedures for Gathering of Data

6.1 Extraction, Isolation, Confirmation and Percentage Yield Determination of Tannins

Extraction and Isolation of the Extract from Artemisia vulgaris Roots. About 500 grams of ground-powdered roots were placed in a 500 mL Erlenmeyer flask with ethanol-water solvent and were macerated for 48 hours. After 48 hours, it was filtered using a cheesecloth; the residue was discarded while the filtrate was placed in a steam bath using a rotary evaporator until the extract became viscous, and put on evaporating dish over a steam bath at 80^{o}C until the extract was dried. When the final drying was completed, and the percentage yield of the extract obtained was computed.

Percent Yield Determination. Five hundred grams (500g) of the dried roots sample was used in the study. The ground roots were added to ethanol-water solvent using the Erlenmeyer flask at 500 mL capacity. The dehydrated root sample was soaked for 48 hours. The portion of the collected filtrate was weighed and evaporated over a steam bath using a rotary evaporator. The crystals are collected and weighed to determine the percentage yield.

Phytochemical Test for the Extract. This was done to confirm if the root extract from Artemisia vulgaris is the presence of tannins. About 0.2 grams of a gel is dissolved in 15 mL of water and divided equally into 5 test tubes. Tests were repeated in three trials – trial 1, 2, 3. The solution was tested with the following reagents. Add three drops of ferric chloride solution to 3 ml filtrate. The formation of blue-black or green precipitate was taken as

evidence for the presence of tannins. Into the filtrate of 3 ml, a few drops of lead acetate solution were added. The expected result yielded a yellowish brown precipitate for the presence of tannin. The filtrate is tested by adding three drops of 1 % gelatin solution containing 10% sodium Chloride to 3 ml of filtrate. A white precipitate appeared as evidence for the presence of tannins. Into 3-mL of the filtrate, three drops of bromine water were added. Brownish orange precipitate indicates the presence of tannin. Add three drops of copper sulfate solution into 3-mL of the filtrate. A grayish green precipitate indicates the presence of tannin.

6.2 Isolation of rice starch

The isolation of starch the researchers was based on the extraction methods of starch. The readiness of the separator gel was finished by extricating the starch from Oryza sativa (rice). Initial, five hundred grams (500 g) of rice was put in the blender with distilled water. The subsequent blend was separated utilizing the cheesecloth. The strong buildups were gripped until dried, and the filtrate was set in plastic holders and refrigerated for 24 hours for the starch to settle at the base. The supernatant of the filtrate was disposed of and could detach the rice starch.

6.3 Gelatinization and settling of Oryza sativa starch

The procedure used in this study was based on the modified procedure of Castro et al. (2015). The root extract of the Artemisia vulgaris was mixed in the Oryza sativa starch to form into a gel**.** Once the starch and the root extract have been extracted, the 15g of starch were weighed using the analytical balance and 30 mL of Normal Saline Solution (NSS). To make the concentrations, four different set ups of gelatinization were prepared that contains 15g of starch and 30 mL of NSS then add the root extract to different tubes: 1) 25g of root extract and 75mL of NSS to make it 25% concentrations of root extract, 2) 50g of root extract and 50mL of NSS to make it 50% concentrations of root extract, 3) 75g of root extract and 25 mL of NSS to make it 75% concentrations of root extract, 4) 100g of root extract to make it 100% concentrations. NSS was used since it does not lose the red blood cells when the gel comes in contact with the cells. The resulting mixture was heated at 195-240 ^{0}C on a hot plate until it starts to gelatinize. The temperature of the gel was maintained at a temperature of 63-69 C and a pH of 7.3-7.4. Subsequently, 1500 ul of the prepared gel was incorporated in the plain redtop evacuated tubes and cooled at 18 ^{0}C for 24 hours before blood collection.

6.4 Specimen Collections and Processing

Before blood collection, the patients undergo 10 to 12 hours fasting. Syringe method was used. Ten-milliliter of syringes were used to avoid multiple punctures on the antecubital fossa of the subject. The tubes of experimental and control groups were contained 3 mL of blood and observed for blood clotting.

Blood Clotting. Blood clotting tests were performed in the control group and experimental group in three-day trials, right after the blood extraction. The results of the clotting tests were noted and tabulated to see if there are any developments in the results. Tube method was used for this test. After the puncture starts the timer as soon as the blood appeared and dispensed the blood on the yellow top, which is the control tube, and on the clear tube that contains the different concentrations of Artemisia vulgaris root extract and Oryza sativa for clotting time analysis. After thirty seconds, check by slowly tilting the tube. Repeat in thirty-second intervals until the clotting was observed in the blood and the timer stopped. The results are recorded.

Centrifugation. After observing the clotting of the blood specimen, the different concentrations of Artemisia vulgarus root extract with Oryza sativa and the Controls were placed in the swing bucket assure that the tubes are arranged in balanced before closing the centrifuge and spin at 3500 rpm for 15 minutes. This was done to separate the serum from the clotted blood. The data gathered from the participants were analyzed using mean, and ANOVA

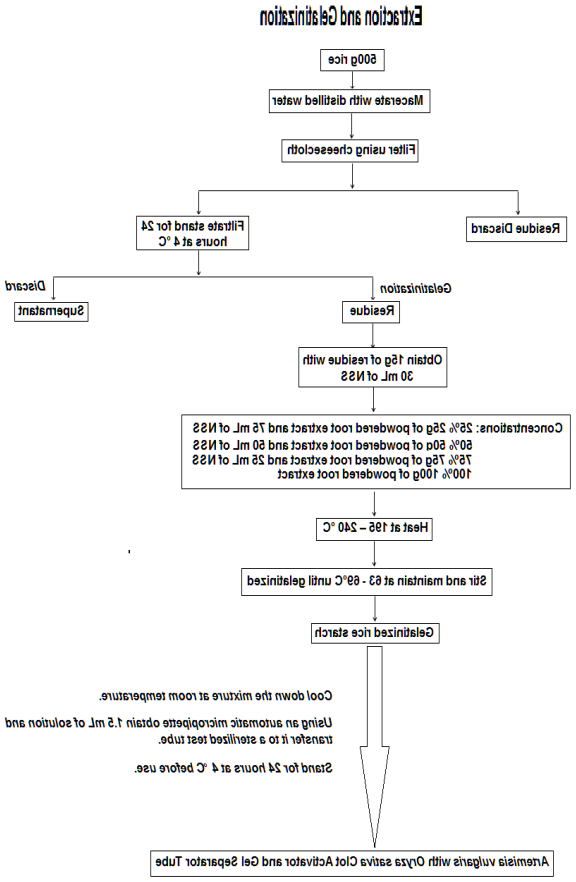

Figure 10. *Modified Procedure of the Starch Extraction and Gelatinization*

7. Results and Discussions

Effects of Different Concentrations of Artemisia vulgaris Root Extract to Blood Clotting Time

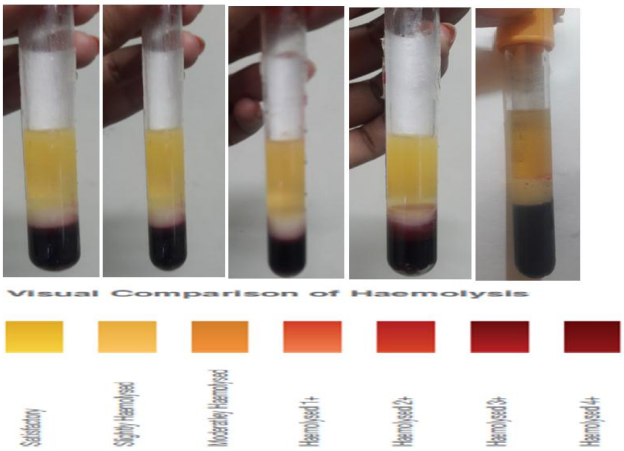

Figure 10: *Serum Index (1. 25%; 2.50%; 3. 75%; 4. 100%; 5.Control)*

After centrifugation of the different concentrations of the Artemisia vulgaris Root Extract and the control, the clotted blood and the serum were separated entirely. Based on the serum index the 25% on the first tube from the left, as compared to the control, they have successfully separated the serum from the clotted blood and have satisfactory serum index. The 50% concentration on the second tube, as compared to the control, the serum is completely separated from the blood, and it is satisfactory serum index. The 75% concentration on the third tube has satisfactory serum index, the same with the control. And the 100% concentration on the fourth tube has satisfactory and also the 100 % concentration. As compared to the control, they are all successfully separated the serum and the clotted blood and has satisfactory serum index since the application of centrifugal force would allow the different particles to migrate and settle base on the specific gravity.

Table 1: *Blood Clotting Using Artemisia vulgaris Root Extract in Different Concentrations*

Specimen	25%	50%	75%	100%	Control
	Clotting Time (in minutes)				
A	16.00	11.33	7.33	3.33	19.67
B	16.00	12.00	7.33	4.33	20.00
C	16.33	12.33	8.33	5.67	20.00
D	15.00	11.33	7.33	4.67	19.67
E	17.33	12.67	8.67	5.33	21.67
F	15.33	11.67	8.33	4.67	21.67
G	15.00	11.00	7.00	3.00	20.00
H	16.67	13.00	9.00	5.33	20.67
I	16.00	12.33	8.00	4.33	20.33
J	16.00	12.67	9.00	5.67	20.33
K	15.00	11.67	7.00	3.00	20.00
L	15.67	12.00	8.00	4.67	20.33
Mean	**15.86**	**12.00**	**7.94**	**4.50**	**20.36**

Table 1 shows the total mean value in minutes on every concentration made in three-day trials. In 25% concentration, the specimen D, G, and K have the mean value of 15.00 minutes. Specimen F has the mean value of 15.33 minutes, and 15.67 mean the Specimen L. The Specimen A, B, I, and J has 16.00 mean value. And Specimen C has 16.33 mean value and 16.67 minutes is the Specimen H. The Specimen E has 17.33 minutes. The overall mean value of the 25% concentration has 15.86 minutes. In the 50% concentration which has 12.00 minutes, grand mean, the Specimen A, D, F, G, and K the mean values were the range in 11.00 minutes to 11.67 minutes. The mean value of Specimen B, C, E, I, J, and L are a range of 12.00 minutes to 12.67minutes. And the Specimen H has 13.00 mean value. The 75% concentration has 7.94 overall mean values for the three-day trials. It composes of Specimen A, B, D that has 7.33 mean values and 7.00 is the specimen G and K. The mean value of Specimen C, E, F, I and L were a range of 8.00 minutes to 8.67 minutes. And the mean value of Specimen H and J is 9.00 minutes. The 100% concentration has 4.50 mean values as compared to the control which has the mean value of 20.36 minutes. Based on the result it has the big difference on the clotting time of the 100% concentration of the root extract Artemisia vulgaris compared to the Control. Based on the mean of the different concentrations it signifies that the Artemisia vulgaris root extracts with Oryza sativa provide acceleration of the blood clotting. If the root extract of the Artemisia vulgaris has higher concentration the faster to clot, when it is diluted it also has the slightly low affinity to clot but when it is compared to the Control, the different concentrations of Artemisia vulgaris root extracts with Oryza sativa are fastest to clot. The different levels of Artemisia vulgaris root extracts with Oryza sativa can be used in the Clinical Laboratory to accelerate blood clotting. All of the concentrations Artemisia vulgaris root extracts are lower than the mean value of the control, and thus, they are more preferable concentrations than the control because of its highest mean value. This shows that the higher the concentration of Artemisia vulgaris root extracts, the lower the mean, therefore, the shorter the clotting time. On the other hand, the data show that the lower the concentration of Artemisia vulgaris root extracts, the longer the clotting time occurs. The findings were similar to the study made by Dhananjaya et al. (2006) on the effects of tannins on in vivo clotting time, at high concentrations the clotting time was shortened. This is achieved through the protein binding property of tannins that can participate in coagulating effects to the blood. According to Synder and Keegan (2016), the tannins are considered to be the same with the factor VIIa that accelerates in the conversion of the factor Xa.In which the factor VIIa, calcium, and phospholipids also helps in the activation of factor Xa. It means that also of tannins, and the action of the cofactors with the factor VIIa are more accelerated in the formation of blood clot. Also, Melzig et al. (2005) say that the effect of tannins shows the catalytic activity in thrombin. In coagulation pathway, after the conversion of Factor Xa the prothrombin is converted to thrombin by the help of the cofactors such as calcium, Factor V, and the phospholipids, the Factor Xa will also participate. Also of the tannins the conversion of prothrombin into thrombin are accelerated (Dahlbäck, 2000).

Variations of the Blood-Clotting Time Using Different Concentrations of Artemisia vulgaris Root Extracts and the Control

Table 2: *Comparison between Blood Clotting Time of Each Concentration of Artemisia vulgaris Root Extracts and the Control (df = 22; critical value = 1.717 at α = .05)*

Groups	Computed t	Decision
A. 25 % and Control (Means -15.86 and 20.36)	-15.84	Ho accepted
B. 50 % and Control (Means -12.00 and 20.36)	-31.62	Ho accepted
C. 75 % and Control	-43.06	Ho accepted
D. (Means - 7.94 and 20.36)		
E. 100 % and Control (Means - 4.50 and 20.36)	-46.85	Ho accepted

The comparison of the effects of blood clotting time between each of the concentrations of Artemisia vulgaris Root Extracts and the Control is presented in Table 2, on item 1, 25% has a t-value of -15.84 with a computed critical value of 1.717 which means there is a significant difference between the 25% concentration and the control. On item 2, the 50% concentration the mean response is 12.00; the test of significance of the mean has computed t of -31.62 with the computed critical t 1.717. This test shows the mean of responses is highly significant. On item 3, the 75% concentration the mean response is 7.94, and the control is 20.36. The test of significance of the mean has a computed t of -43.06. The test shows that the mean of responses is highly significant at alpha=0.05. On item 4, 100% concentration the mean response has 4.50, and the Control is 20.36. The test significance of the mean has a computed t of -46.85. Based on the results the 100% concentration of Artemisia vulgaris Root Extract is the fastest to clot compared to the control. Second is the 75% concentration of Artemisia vulgaris Root Extract. Third, the 50% concentration and last, the 25% concentration compared to the Control. According to Ondua (2015) that Artemisia vulgaris had the presence of tannin and proved its acceleration off in vivo clotting. He concluded that the higher concentration of the tannins is the faster for in-vivo clotting. The alternative clot activator and gel separator are made up of the different concentrations of Artemisia vulgaris Root Extract. Dahanukar et al. (2000) say that the higher the concentration of the Artemisia vulgaris root extracts the fastest to clot. Because of the tannin content of the Artemisia vulgaris that participates in promoting the blood clot formation. The diluted concentration of Artemisia vulgaris root extract also has the faster to clot compared to the Control. Table 2 shows that there is sufficient evidence to conclude that the mean of the control and the four concentrations (25%, 50%, 75%, 100%) are different. The difference in the mean of the control and the 25%, 50%, 75% and 100% concentrations are statistically significant. The more significant part of the figured t esteems are higher than the primary t-value, this infers to dismiss the wrong theory and reason that there are generous contrasts between the control and the four fixations. Therefore, the 25%, 50%, 75% and 100% concentrations are not only comparable to the control but can be used effectively as alternative clot activator for blood analytes having shorter clotting time as compared to the control. The phytochemical properties of Artemisia vulgaris, the findings are also supported by the previous study of Ivanescu and Corciova (2014) that presence of tannins can help to accelerate blood clotting. Hence, the presence of tannins in the phytochemical properties of Artemisia vulgaris possibly possesses procoagulant activity. Due to the presence of tannins exhibited shorter clotting time as compared to the control serum separator tube that is available in the market.

Variations of the Blood-Clotting Time Using Different Concentrations of Artemisia vulgaris Root Extracts

Table 3: *Variations of the Blood-Clotting Time Using Different Concentrations of Artemisia vulgaris Root Extracts (df = 3/44)*

Groups	Critical F (α = .05)	Computed F	Decision
Vulgaris Root extracts in 4 Different Concentrations of Blood Clotting Time	2.82	4.931	Ho rejected

Using the analysis of variance (ANOVA) are presented in Table 3, the researchers tried to determine if there are significant variations in the clotting time of the blood using the different concentrations of Artemisia vulgaris root extracts. Table 3 shows the variations in the blood clotting time in the different concentrations of Artemisia vulgaris Root Extracts. The computed F is 4.931, which is greater than the Critical F value. Therefore, there is significant effect using the four different concentrations; the decision is to reject the null hypothesis. Patterson et al. (2001) made detailed on sponges coated blood clotting agent that can accelerate on a clotting effect of the blood. The study made use of the root extract Artemisia vulgaris were mixed with the Oryza sativa starch to become a gel form, and tested in vitro on blood clotting time. Based on the results, the different concentrations can accelerate the blood clotting that can be used in the clinical laboratory compared to the commercially prepared Serum Separator Tube the clotting of the collected blood it takes 19 to 30 minutes. The made alternative clot activator, and gel separator can be used in the Laboratory for blood clotting. In the statistical analysis of the different concentrations of blood clotting time, it signifies that there are variations on the blood clotting time but differ in the Control, different levels have the fastest to clot. The table shows that there is enough evidence to conclude that the means of the clotting time of the blood with the different concentrations of the root extract Artemisia vulgaris are different. The rejection of the null hypothesis means that the differences in the clotting time of the blood among the different concentrations of Artemisia vulgaris root extract are statistically significant. When using different concentrations of Artemisia vulgaris root extract, it was noted that based on the one-way ANOVA it has a computed F value of 4.931, which is above the critical value of 2.82. Therefore, the null hypothesis is rejected. This establishes the fact that using different concentrations of Artemisia vulgaris root extract will give different clotting times when compared to one another.

8. Conclusions

On the different concentrations of the Artemisia vulgaris root extract the 25%, 50%, 75% and 100% has a significant effect on the blood clotting time. There are significant variations in the blood clotting time using different concentrations of the Artemisia vulgaris root extracts. As a result, all of the concentrations of the Artemisia vulgaris root extracts with Oryza sativa can be formulated as clot activator and a gel separator. The 100% is the best that could be formulated to accelerate the clotting of the blood and as a serum gel separator.

9. Recommendations

The researchers would like to suggest for future studies that enable to support and give more accurate results; the pathologic human samples should be tested. Also, they recommended determining other components of the Artemisia vulgaris roots, which have a coagulation property.

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