IN-VITRO ANTICOAGULANT PROPERTY OF SARGASSUM CONFUSUM F. VALIDUM

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ABSTRACT

The study sought to determine the in-vitro anticoagulant property of Sargassum confusum f. validum extract compared to EDTA and Heparin. The study used experimental laboratory based research design. The algae were air dried, milled and treated with 85% ethanol. A 100% extract was used. A blood to anticoagulant ratio of 1:1 until 1:9 was utilized. EDTA was used as a positive control for hematologic studies and Heparin was also used as positive control for chemistry analyses. Test for hematologic studies was limited to Complete Blood Count (CBC) only. For chemistry analyses, it was limited only to Blood Uric Acid, Creatinine, Cholesterol, AST, and ALT. For CBC results, all ratios gave increased values for White blood cell count, and a decreased amount for hematocrit, hemoglobin and platelet parameters. For chemistry analyses only the creatinine values have no significant difference compared to the positive control, for Blood Uric Acid, Creatinine, AST, and ALT all ratios gave an increased value, and for cholesterol all ratios gave normal values but still incomparable to the positive control.

Keywords*: anticoagulant, algae, hematology, chemistry*

INTRODUCTION

Tests that require blood as a specimen are very common and widely used for establishing prognosis and accurate diagnosis of the patient. These tests usually require high volumes of blood usually leading to multiple blood extraction, for example, the tube for hematological studies usually require a minimum of 2ml of blood in an EDTA containing tube and for chemistry analyses minimum of 5ml per tube without any anticoagulant, and 3 ml if with an anticoagulant. After collection of blood samples, these tubes are usually fed into different automated machines that measure specific test, and most of these machines require the entire sample to run. This common scene results in patients' refusing frequent blood collection because they cannot tolerate the pain or either they had experienced traumatic collection before due to multiple blood draws. To lower the cases of specimen wastage, a universal anticoagulant must be sought for clinical laboratory use (Kamura et al., 2000).

Anticoagulants are additives that inhibit blood and/or plasma from clotting, ensuring that the constituent to be measured is not significantly changed prior to the analytical process. Anticoagulation occurs by binding calcium ions (EDTA, citrate) or by inhibiting thrombin activity (heparinates, hirudin) (World Health Organization [WHO], 2002). There are different anticoagulants that are considered as additives for blood studies; these are Ethylenediaminetetraacetic acid (EDTA), Sodium Citrate and Heparin. EDTA is the anticoagulant of choice for hematologic studies and immunohematology. It is available as a liquid and sprays dried in a dipotassium or tripotassium salt form. Dipotassium for plastic, spray dried and tripotassim form in liquid form glass lavander-top tubes. EDTA must not be used for coagulation studies for it increases the result values for Prothrombin time and activated partial thromboplastin time. The anticoagulant is also limited to chemistry assays because it inhibits enzymes and may affect the levels of calcium for electrolyte determination. It is also used for coagulation studies. A light blue top that contains sodium citrate 3.2% or 0.105 M is commonly used to preserve clotting factors. Sodium citrate has also interference for some other tests, as it inhibits specific enzymes like AST, ALP, and ALT. It also stimulates acid phosphatase

and decreases amylase. This anticoagulant also affects electrolyte values.

Heparin, a mucositis polysurfuric acid, is effective even in small quantities without causing any interference on many assays. It has an advantage over EDTA because it does not affect ion levels, but it does interfere with the serologic test. It is restricted to hematology because it will cause a spurious increase in WBC, haemoglobin; it also distorts cell shape and causes interferences in some stains. It is available as Lithium and Sodium heparin, with Lithium heparin being the most commonly used form of chemistry test and Sodium heparin for trace elements assay and toxicology. Anticoagulants may affect the transport of water between plasma, thereby altering cell size and constituent plasma concentration. Excessive use of anticoagulants may induce cell shrinkage and may cause a false decrease in hemoglobin and hematocrit (Henry, McPherson, & Pincus, 2012).

Chemistry analyses include assessment of Kidney Function Test, Liver Function Test, Electrolytes, Cardiac Panel and Lipid Profile, while the complete blood count is the calculation of the cellular (formed elements) of blood including White blood cells (WBC), Red blood cells (RBC), Platelet count (PlTct), Hemoglobin (Hgb) and hematocrit (Hct). Both the chemistry analysis and complete blood count rely on the use of in vitro anticoagulants to be able to provide the correct diagnosis of the symptoms being presented. Thus, if a client needs both tests, the blood would have to be placed in at least two containers and the client would have to wait longer while the blood is withdrawn. This provided the researchers the inspiration for the necessity to find an in vitro coagulant to make the blood collection become more efficient and cause less discomfort among clientele.

Ushakova et al. (2008) discussed that fucoidan's anticoagulant activity is closely the same as Anti thrombin III as it inhibits factor Xa and thrombin in the absence of antithrombin III. Also, Irhimeh (2009) discussed that Brown algae such as Sargassum confusum f. validum contain fucoidan. Moreover, Toshiko (2003) also discussed that in vitro effect of the fucoidan was prominent while its in-vivo effect on hemostasis was not obvious. According to Bureau of Fisheries and

Aquatic Resources (BFAR) Region II (Cagayan Chapter), Sargassum is found in many coastal areas in Cagayan Valley Philippines like Gonzaga, Sta. Ana, Claveria, Pamplona, Buguey, and Appari.

Thus, the researchers aimed to determine the potential anticoagulant activity of Sargassum confusum f. validum containing the component called fucoidan which has the same property as Heparin to be a potential universal anticoagulant.

Conceptual Framework

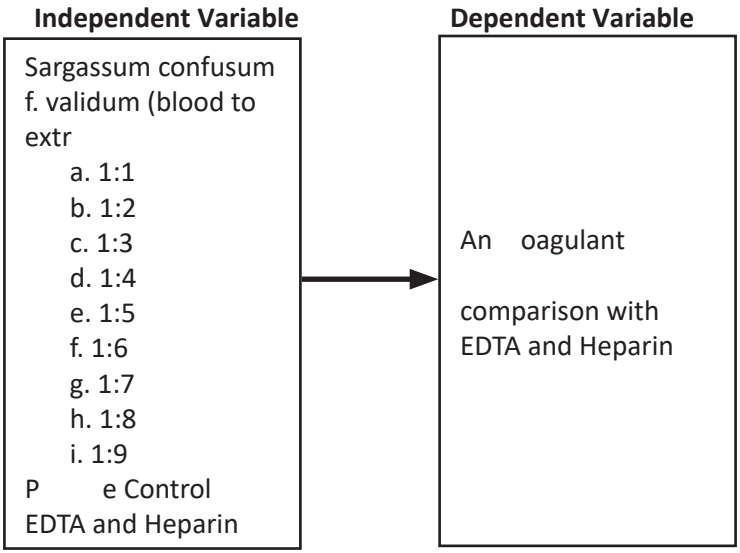

Figure 1. Paradigm of the study

The study aimed to determine if each of blood mixed to Sargassum confusum f. validum extract from 1:1 to 1:9 has an anticoagulant activity in comparison with EDTA and Heparin. EDTA was the anticoagulant of choice for blood hematologic studies with parameters used such as WBC, RBC, hemoglobin, and hematocrit, and platelet count and heparin was used for plasma chemistry tests such as BUA, cholesterol, creatinine, AST, AND ALT. Thus this study utilizes the independent variables, (the diff. ratio of blood to extract dilution and the positive control, to determine the dependent variable (anticoagulant activity

considering the different parameters of the study).

Statement of the Problem

Generally, this study determined if Sargassum confusum f. validum extract has the potential to become a universal anticoagulant for clinical laboratory analyses.

Specifically, it sought to answer the following questions:

1. How effective is the Sargassum confusum f. validum extract as an in vivo anticoagulant?
2. What is the effect of the different Sargassum confusum f. validum extract and blood ratio and the positive control on the Hematology, Chemistry, and enzyme parameters?
3. Is there a significant difference in the mean Hematology, Chemistry, and enzyme parameters values in the different Sargassum confusum f. validum extract and blood ratio when compared to the positive control - EDTA and Heparin?

METHODOLOGY

Research Design

Using the laboratory-based experimental design, the study was conducted to determine the potential of Sargassumconfussum f. validum as a universal anticoagulant. The researchers utilized 100% Sargassum confusum f. validum extract and used test samples with blood to the anticoagulant ratio of 1:1 up to 1:9. The test samples were run in triplicates for each analytic run for Hematology (CBC) and Chemistry tests (AST, ALT, BUA, Cholesterol, Crea). EDTA was used as positive control for CBC and Heparin for Chemistry analyses.

Subjects and Participant of the Study

Two (2) kilograms of Sargassum confusum f. validum was collected during low tide at Sta. Ana Cagayan Valley, Philippines (18.5604° N, 122.1405° E). Thirty (30) mL of Venous Blood sample was collected

from a normal healthy individual at St. Paul University Medical Technology Laboratory. Test samples for Chemistry were analyzed at St. Paul University Medical Technology Laboratory and test samples for Hematology (CBC) was sent to St. Paul Hospital Tuguegarao.

Data Collection Procedure

Collection of Plant Sample

The leafy parts of Sargassum confusum f. validum was carefully collected on February 2017 during low tide at Palaui Islands, Sta. Ana, Cagayan, Philippines. The seaweed was removed from selected mussel-harvesting lines by hand, making sure that nearby mussels were not damaged or removed during the process. Seaweed was rinsed with sea water and placed into resealable plastic bags, frozen, packaged in polystyrene containers and sent to St. Paul University Philippines the following morning.

Before the collection of the plant sample, the researchers asked permission from the Bureau of Fisheries and Aquatic Resources (BFAR) Region II to harvest Sargassum confusum f. validum at Palaui Islands, Sta. Ana Cagayan, Philippines.

Drying of Seaweeds

Frozen seaweed was thawed under running tap water. Visible debris was carefully removed with slight agitation, while the holdfast and any degraded areas of the seaweed were removed by hand. The algae were milled with a food blender (Krups 75 blender) and sieved using a metal sieve with a pore size of 600 microns (Endecott's Ltd) to obtain a fine powder. Milled seaweed was transferred into glass beakers, weighed and recorded. Beakers were transferred into the oven and dried at 60 degrees Celsius overnight and reweighed the next morning. A homogenous sample of dry seaweed powder was achieved when the weight of the powder remained constant.

Ethanolic Extraction

The extraction of Sargassum confusum f. validum was carried out by mixing 1.0g of ground Saragassum with 30.0 mL solvent in a flask. The suspensions were heated with constant stirring. After cooling to room temperature, the suspensions were centrifuged. To obtain the crude polysaccharide, ethanol was used for precipitation at an ethanol-to-filtrate ratio of 3:1. The sediments were washed twice with acetone and ether, sequentially. The depurated sediments were dried at 60°C (Zhang, 2013). The sample was stored in a sterile capped glass bottle and was kept at 4 degree Celsius.

Collection of Blood (Venipuncture)

Before performing venipuncture, the donor was allowed to rest for 30 minutes prior to arrival at St. Paul University Medical Technology Laboratory. After resting the phlebotomist palpated a suitable vein for extraction at the antecubital fossa of the elbow was identified with the aid of a tourniquet. After palpating and removing the tourniquet, the phlebotomists cleansed the site using isopropyl alcohol and allowed to air dry for 30 seconds then reapplied the tourniquet. The vein was then punctured using a sterile syringe (Terumo) at a 35-degree angle and 30 ml of venous blood was drawn. Two (2) ml of venous blood was then transferred to a 9.5 mL containing a plain top tube, 2 mL for EDTA tube and another 2 mL for heparin.

Preparation of Test Sample

The researchers used samples of blood to an anticoagulant ratio of 1:1 up to 1:9. For 1:1 ratio, 2 mL of blood was placed in a 5 mL plain tube with 2 mL Sargassum confusum f. validum extract and was mixed by the figure of eight manner of inversion. For 1:2 ratio, 2 mL of blood was added to 1 mL Sargassum confusum f. validum extract in a 5 mL plain test tube mixed in the same manner. For 1:3 ratio, 2 mL of blood was added to 666 uL of Sargassum confusum f. validum extract in a 5 mL plain test tube mixed in the same manner. For 1:4 ratio, 2 mL of blood was added to 500 uL of Sargassum

confusum f. validum extract in a 5 mL plain test tube mixed in the same manner. For 1:5 ratio, 2 mL of blood was added to 400 uL Sargassum confusum f. validum extract in a 5 mL plain test tube mixed in the same manner. For 1:6 ratio, 2 mL of blood was added to 333 uL Sargassum confusum f. validum extract in a 5 mL plain test tube mixed in the same manner. For 1:7 ratio, 2 mL of blood was added to 285 ul Sargassum confusum f. validum extract in a 5 mL plain test tube mixed in the same manner. For 1:8 ratio, 2 mL of blood was added to 250 uL Sargassum confusum f. Validum extract in a 5 mL plain test tube mixed in the same manner. For 1:9 ratio, 2 mL of blood was added to 222 uL Sargassum confusum f. validum extract in a 5 mL plain test tube mixed in the same manner.

Tubes containing EDTA were used as controls for hematology studies and heparin for chemical analyses.

Test for Anticoagulant Activity

Before running any test, all test samples were left to stand at room temperature for 15 minutes and were checked for any clot formation by rimming the blood sample.

Complete Blood Count (CBC) was performed with a hematology analyzer (Sysmex XS 1000i) for quantitative analysis. Parameters evaluated were white blood cell count (WBC), red blood cell count (RBC count), hemoglobin (Hgb), hematocrit (Hct), and platelets (Plts). Collected samples were transported 30 minutes after time collection from SPUP Laboratory to SPH Laboratory. All tests were done in 3 analytical runs, and all tests were finished after 15 minutes.

Chemistry Test

Chemistry test was performed using Biosystems machine analyzer for determination of Aspartate aminotransferase (ALT), Alanine aminotransferase (ALT), Blood uric acid (BUA), Creatinine, and Cholesterol.

For ALT and AST procedure, the working reagent and instrument was in room temperature. Pipetted 1.0 mL of reagent and 50 uL of the sample in a cuvette. Then mixed and inserted in the machine. After 1 minute, initial absorbance was recorded one-minute intervals and 3 minutes after.Calculated the difference between consecutive absorbance, and the average absorbance difference per minute.

For BUA procedure, researchers brought reagent into room temperature. Then Pipetted 25 uL of the sample and 1.0 mL of reagent.25 uL of distilled water and 25 uL BUA for the Standard. Mixed and incubated the tubes for 10 minutes at room temperature. Measured the absorbance of the standard and the sample at 520 nm.

For the creatinine procedure, the reagent was brought into room temperature. Pipetted 1.0 mL of reagent and 0.1 mL of sample. Mix and inserted in the machine. Recorded the absorbance at 500 nm after 30 seconds and 90 seconds.

For Cholesterol procedure, the reagent was brought into room temperature. Pipetted 10 uL of standard in a cuvette. Put 10 uL of the sample and 1.0 mL of reagent A and 1.0 mL of reagent B in another cuvette. Mixed and incubated for 10 minutes at room temperature. Measured the absorbance of standard and sample at 500 nm.

Data analysis

During the experimentation of the anticoagulant activity of the fucoidan, the researchers adopted the normal values of the Complete Blood Count parameters from Saint Paul Hospital Laboratory Department. For white blood cells, the researchers used 5.0-10.0x10^9/L. For the hemoglobin, 12.0-16.0 g/dL was used. Lastly, for the hematocrit values, 37.0-48.0% was used and the platelet values of 150,000 to 450,000 uL.

For the chemistry tests, the researchers used the normal values

from Bishop (2010). For creatinine, 0.9-1.3 mg/dL is used. For the BUA (blood uric acid), the researchers adopted the normal values used in Uricase method. For male patients, the normal value is 3.5-7.2 mg/dL. For total cholesterol, 140-200 mg/dL is used as the normal value.

For enzyme determinations, the researchers used the normal values from Biosystems test kit. For AST (aspartate aminotransferase) values, the researchers used 40 U/L and for ALT (alanine aminotransferase) values, 41 U/L.

The researchers used Mean values to determine the results of all the analyses which were run in Triplicates. And to determine the significant differences, the researchers used One-way Analysis of Variance (ANOVA) and Scheffe Post Hoc analysis for multiple comparisons.

RESULTS AND DISCUSSION

Sargassum confusum f. validum extract as Anticoagulant

Initially, the Sargassum confusum f. validum extract was tested for its anticoagulant activity. McGill Physiology Virtual Lab (n.d.) presents that the expected clotting time range is 4-10 minutes. Likewise, Legacy Health (n.d.) discusses that the blood in the tube should be allowed to clot for 20-30 minutes in a vertical position before centrifugation. Also, the tube will be centrifuged for 10-15 minutes at about 1300-1800xg. Thus, the results show that from 15 minutes, the blood with Sargassum confusum f. validum extract did not have fibrin formation, thus did not clot. Adding 25 minutes waiting time in the test tube in a horizontal position and 15 minutes centrifugation, the blood in the tube with the extract had not yet clotted. This therefore means that the Sargassum confusum f. validum extract is effective as an anticoagulant, since the blood with the Sargassum confusum f. validum extract did not clot after 15 minutes and even after centrifugation.

Hematology Parameters

Table 1
Mean Results for White Blood Cell Count (WBC) in the Different Extract and Blood Ratio

Dilution	WBC x10^9/L	Remarks
Positive control	7.92	Normal
1:1 ratio	416.31	Increased
1:2 ratio	118.71	Increased
1:3 ratio	114.45	Increased
1:4 ratio	118.52	Increased
1:5 ratio	130.29	Increased
1:6 ratio	176.93	Increased
1:7 ratio	203.45	Increased
1:8 ratio	221.67	Increased
1:9 ratio	242.29	Increased

Table 1 shows the increase in the number in WBC parameter in all ratios; this shows an increased result since the normal values used were 5.0-10.0x10^9/L. The result can be associated with the machine used, which uses the principle of Fluorescence Cytometry or Flow Cytometry which fluorescently labels cell components or particles and then excited by a laser to emit light at varying wavelengths. The fluorescence can be measured to determine various properties of single particles which are usually cells. Fluorescent molecules that emit fluorescence are detected by lenses. According to Sea (n.d), some particles such as marine algae are naturally fluorescent. The researchers used extract based on a marine alga; the machine read some of the algal particles as WBC, thus an increase in WBC count.

Table 2
Mean Results for Hematocrit (Hct) and Hemoglobin (Hgb) in the Different Extract and Blood Ratio

Dilution	Hct Values (%)	Remarks	Hgb Values (g/dL)	Remarks
Positive control	46.20	Normal	15.23	Normal
1:1 ratio	23.76	Decreased	8.56	Decreased
1:2 ratio	32.40	Decreased	11.30	Decreased
1:3 ratio	36.60	Decreased	12.63	Normal
1:4 ratio	38.01	Decreased	12.73	Normal
1:5 ratio	38.33	Decreased	12.80	Normal
1:6 ratio	40.16	Decreased	13.53	Normal
1:7 ratio	40.26	Decreased	13.60	Normal
1:8 ratio	41.43	Decreased	13.53	Normal
1:9 ratio	42.70	Decreased	14.53	Normal

Table 2 represents hematocrit and haemoglobin values across the dilutions of the Sargassum confusum f. validum extract and blood. The two parameters are joined in one table because the two of them represent the Red blood cell (RBC).

The Hematocrit parameter in the different ratios shows decreased values considering the 37.0-48.0% normal values. This means that there is an excess in the anticoagulant which may cause shrinkage in the cells. Or there was an in vitro hemolysis, resulting in a false decrease in hematocrit values. This is supported by Nemec, Drobnic-Kosorok, and Butinar (2005) who showed that a significant decrease in hematocrit was seen in samples with high K3EDTA concentration.

For Hemoglobin there is a markedly decreased in 1:1 and 1:2 ratios because the sample might be over diluted making the reading low or the presence of sulfhemoglobin, but the ratios 1:3 to 1:9 show normal results. Considering the 12.0-16.0 g/dL as normal values, the 1:9 ratio shows the nearest reading to the positive control.

This means that only the 1:3 to 1:9 ratios of the Sargassum confusum f. validum extract and blood provide relevant anticoagulant activity findings in terms of hemoglobin count.

Table 3
Mean Results for Platelet Count (Plt) in the Different Extract and Blood ratio

Dilution	Plt	Remarks
Positive control	360.00	Normal
1:1 ratio	48.00	Decreased
1:2 ratio	31.00	Decreased
1:3 ratio	29.00	Decreased
1:4 ratio	29.67	Decreased
1:5 ratio	29.00	Decreased
1:6 ratio	52.00	Decreased
1:7 ratio	55.67	Decreased
1:8 ratio	58.00	Decreased
1:9 ratio	64.00	Decreased

Table 3 shows that there is decreased count in Platelet in the different extracts and blood ratios considering the 150,000-450,000 mL normal values. This means that the extract caused platelet satellitism or a rare peripheral blood finding that is due to the clumping and adherence of platelets to neutrophils or rarely, to monocyte (College of American Pathologists, 2007). Another cause of decreased platelet values is that the patient has giant platelets; therefore, it will be read as a WBC instead of a platelet causing a false decreased values for platelet parameter and false elevated values for WBC parameter.

Generally, for the Complete Blood Count analysis, the values for WBC have a marked increased in all ratios. For haemoglobin, ratios 1:1 and 1:2 have a decreased value, and the remaining ratios have a normal value. For hematocrit, all ratios have decreased values while for haemoglobin, only 1:1 and 1:2 have decreased values. While for platelet, all ratios have decreased value.

Chemistry Parameters

Table 4
Mean results for Chemistry Parameters in the Different Extract and Blood Ratio

Dilution	Creatinine	Remarks	BUA	Remarks	Cholesterol	Remarks
Positive Control	.23	Normal	4.66	Normal	152.46	Normal
1:1 ratio	.28	Normal	2.55	Decreased	21.89	Normal
1:2 ratio	.31	Normal	2.60	Decreased	23.06	Normal
1:3 ratio	.31	Normal	3.29	Decreased	46.43	Normal
1:4 ratio	.56	Normal	3.08	Decreased	59.91	Normal
1:5 ratio	.60	Normal	3.95	Decreased	132.48	Normal
1:6 ratio	.86	Normal	3.97	Normal	138.60	Normal
1:7 ratio	.63	Normal	3.13	Decreased	153.59	Normal
1:8 ratio	.56	Normal	4.75	Normal	155.71	Normal
1:9 ratio	.60	Normal	4.66	Normal	145.59	Normal

Table 4 shows that the creatinine results are slightly higher than the positive control but all the different ratios fall under the normal value which is 0.9-1.3 mg/dL. For Blood uric acid, only ratios 1:6, 1:8, and 1:9 show that it is within the normal values and the nearest value to the positive control, are ratios 1:8 and 1:9. Ratios 1:1 to 1:5 and 1:7 have decreased results; a probable cause of this is the sample which was over diluted or the plasma was not separated on time from the red cell from ratios 1:1 to 1:5 and 1:7, which, according to Bishop (2013), it could have been diluted by intracellular components. For cholesterol, across the different ratios, all values fall within normal and ratios 1:7 and 1:8 show the nearest value to the positive control.

This means that in terms of the chemistry parameters, only the 1:6, 1:8 and 1:9 ratio of the Sargassum confusum f. validum extract and blood provide relevant anticoagulant activity findings in terms of the chemistry parameters.

Enzyme Parameters

Table 5
Mean Results for AST and ALT in the Different Extract and Blood Ratio

Dilution	AST	Remarks	ALT	Remarks
Positive Control	36.53	Normal	37.90	Normal
1:1 ratio	17.40	Normal	22.40	Normal
1:2 ratio	23.36	Normal	28.06	Normal
1:3 ratio	45.60	Increased	45.30	Increased
1:4 ratio	44.66	Increased	57.76	Increased
1:5 ratio	108.90	Increased	78.70	Increased
1:6 ratio	87.43	Increased	73.33	Increased
1:7 ratio	86.80	Increased	70.46	Increased
1:8 ratio	95.20	Increased	73.23	Increased
1:9 ratio	91.40	Increased	71.70	Increased

The AST and ALT are separated from the rest of the Chemistry parameters because they represent liver function test and are the only enzymes in the parameters. Table 5 shows that AST and ALT results are higher than the positive control though ratios 1:1 and 1:2 are within normal values in both the AST and ALT. This means that there was an in vitro hemolysis of the sample because these enzymes are found inside the red blood cell and may leak out once the red blood cells are ruptured causing the false increase. Also, the levels of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may increase with strenuous exercise.

Table 6
Significance of Sargassum confusum f. validum Compared to EDTA in CBC

Parameter	F value	Sig.	Remarks
WBC	652500.47	.000	Significant
Hemoglobin	276.21	.000	Significant

Hematocrit	3432.94	.000	Significant
Platelet Count	35186.87	.000	Significant

Table 6 shows that there is a significant difference in all the Complete Blood count parameters. WBC gave markedly increased values compared to the positive control. Hgb and Hct gave a decreased value compared to the positive control. All values for platelet were markedly decreased compared to the positive control.

Table 7
Significant Difference of Fucoidan Compared to Heparin Using ANOVA

Parameter	F value	Sig.	Remarks
Creatinine	2.45	.045	Not Significant
BUA	53.37	.000	Significant
Cholesterol	54867.99	.000	Significant
AST	37678.45	.000	Significant
ALT	37897.30	.000	Significant

Table 7 shows that all chemistry test values gave significant difference except for creatinine. Values for BUA gave decreased amount compared to the positive control. Values for Cholesterol gave normal results but still incomparable to the positive control. AST and ALT have increased values compared to the positive control. This means that only creatinine has a value comparable with the positive control.

CONCLUSION

Taking consideration of the findings, the researchers conclude that the Sargassum confusum f. Validum extract exhibited anticoagulant activity in vitro. However, Sargassum confusum f. validum cannot be substituted to EDTA for CBC. Also, the extract can be used for creatinine only and not for Cholesterol, Blood uric acid, ALT and AST for chemical analyses.

RECOMMENDATIONS

Based on the findings and conclusion of the study, the researchers recommend that future researchers:

May do varying concentrations of the extract from 95% until 25%;

May include other test parameters for hematological test in vitro like Prothrombin time, Activated Partial Thromboplastin Time and Erythrocyte Sedimentation Rate, Platelet studies;

May try another extraction method for succeeding experiments like High-Performance Liquid Chromatography Enzyme assisted fucoidan extraction or ultrasonic extraction in removing impurities and to minimize interferences; and

May conduct experiment on this substance in vivo to act as a potential Heparin substitution or replacement since it has the same biological structure to heparin.

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