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Studying the Effect of *Carica papaya* Leaf Extract on the Shelf Life of Platelets

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Abstract: ***Transfusion of blood is important in medical regimen, so for a smooth process a decent inventory should be maintained. Scientific community has given a huge attention towards storage of blood and its components but still there is scope for further research. Efforts have been made to improve the quality of platelets under storage conditions. Goal is to maintain the quality of platelets from the point of donation to the point of transfusion - to suspend the aging process. Recent works have proved that platelets can be maintained in storage condition for 5-7 days maintaining their quality, as measured by post transfusion recovery and survival. However, additional measures are needed to validate the development of technologies that may further reduce the aging of stored platelets, or enhance their hemostatic properties. Studies have suggested that Carica papaya leaf extract have the potential to increase platelet count. Knowing the medicinal value of Carica papaya and its association with diseases like dengue made it the best neutraceutical for the purpose. Promising components were obtained in the GC-MS analysis of Carica papaya leaf extracted in different solvent system namely, Hexane, Acetone, Ethanol and Water, which were further used in different concentrations in blood and platelet to access the quality of cells under storage condition. A few components such as vitamins, tannins, flavonoids, alkaloids, antifungal agents, anti-bacterial, oleic acid etc were identified, which could help in blood and platelet for quality improvement. The purpose of this work is to establish a relationship among the in-vitro damages in platelet during storage, the biochemistry of the cell heading to such damage and effect of Carica papaya leaf extract in controlling these damages. Blood Sample was collected from donors and stored at 4 degree C under aseptic condition for a few hours. Platelets were isolated and Papaya leaves extracts were added in different concentrations (Hexane, Acetone, 60% Ethanol, 40% Ethanol, Water in 3%, 6%, 9% concentration). Further various parameters such as Morphology, Platelet functional assay, pH, cell viability, Bacterial contamination, Glucose concentration were studied on Day 0, Day 4 and Day 7. The sterility test was carried out on the fifth day of storage. In stored Platelet, platelet storage lesions such as pH, glucose, platelet functional assay i.e. platelet factor 3 test determining the clotting factor time, cell viability along with bacterial contamination were overcome in papaya leaf extract with 9% Hexane solvent. Since the biggest problem faced by blood banks in storage of platelets is microbial growth because of the storage temperature (which is 22 – 24° C) we can say Hexane at higher concentration (9% in comparison to 3% and 6%) has maximum antimicrobial activity. It was observed that when 9% hexane extract was added to media plates no contamination was seen, thus the metabolites present in this extract can be considered for further analysis. The activity shown by the extract could be possibly due to tannis for its antimicrobial activity along with alkaloids and flavonoids. Leaf extract in solvent system hexane showed better results. Platelet storage lesions in hexane were controlled as compared to control and other extracts. Microbial contamination was absolutely zero. Discs prepared from these stored platelets showed no microbial growth. No growth in liquid broth was confirmed by spectrophotometer.***

Keywords: Platelets, Carica Papaya, Blood Shelf-Life, Hexane Extraction, Glucose Concentration Methods

1. Introduction

Platelets are the smallest cells in blood with size 1-2 µm which are enucleated, discoid in shape and play an important role in maintaining homeostasis, healing wounds, stopping bleeding. The concentration of platelets varies from 1.5 x 10^6 to 4 x 10^6 cells/µl in blood. Platelets are generated from megakaryocytes, their precursor cells in bone marrow with the help of various growth factors. The main role that platelets play is in wound healing; these cells stick with each other and recruit other factors which play important role in thrombosis and hemostasis and hence, transfusing platelets to control massive bleeding for treatment in accidental injuries and thrombocytopenic patients has flourished in clinical practices. In 1950s the idea of storing platelets in plasma free media came into existence with artificial preservatives such as salt, acetate, phosphate buffer solution, glucose that can be used along with plasma. Platelets have a shorter life span, in human body, after every 10 days platelet replenishes which creates a problem in platelet availability and inventory. The biggest problem which is faced in storing platelet is controlling the bacterial growth since platelet cannot be administered at either too high or too low temperature. The ideal temperature for storing platelets is 22 °C in a closed system (Ringwald et al., 2006). Research suggests three fundamental quality parameters for assessing the platelet shelf life namely platelet count, pH value, and absence of bacteria. All the deleterious changes taking place in platelets during storage are referred as 'platelet storage lesion' and it can be defined as the progressive detrimental changes in platelet structure and function that appear starting from the moment blood is withdrawn from a donor to the time they are transfused to a recipient (Figure 1). Since platelet has small life span, increasing the inventory by a day or two could be a great achievement. Currently, in blood banks platelets are stored at 22 - 24 °C, with gentle agitation. It has been reported that glycolysis at this temperature results in an increased lactate production and subsequent fall in pH.

Thus, platelet morphology begins to change around pH 6.8 and loss of viability at pH 6 (Shrivastava et al., 2009). Storage/preservation time is limited to 5 days due to platelet storage lesions along with the risk of bacterial contamination. (Chandra et al., 2006)

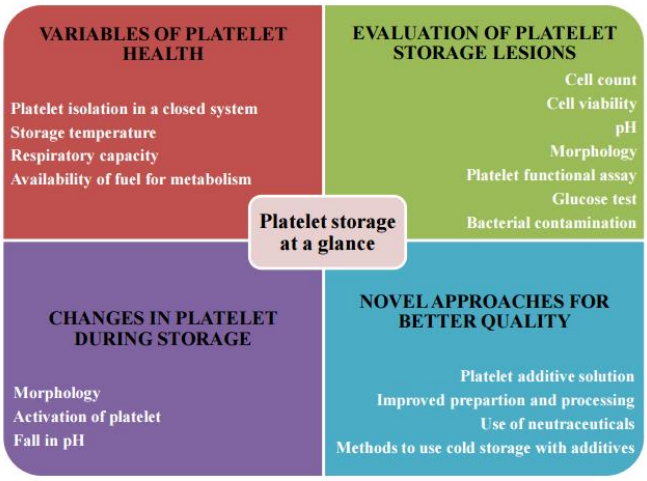

Figure 1: Platelet storage at a glance (Mittal K. et al, 2015)

Carica papaya, a neutraceutical plant, belongs to the family of Caricaceae, have a wide range of pharmacological properties. Every part of the plant has its own medicinal property. Papaya is an evergreen plant and is said to be the powerhouse of nutrients. Papaya is reported to be a rich source of antioxidants, minerals, vitamins, fibre and other inorganic compounds. Remedial use of the plant involves apportioning of roots, seeds, leaves, stems, and barks. Papaya leaves in particular are used for the treatment of various diseases like dengue, malaria. Papaya leaf extracts have phenolic compounds, Antimicrobial compounds (Anjum et al, 2013). The leaves of the papaya plant contain chemical compounds of karpain; it's a substance which kills microorganism. Fresh papayas leaves have antiseptic properties, whereas brown, stored dried leaves are used as blood purifier. (Amazu et al., 2010).

The idea of storing platelets started in 1950s. Various attempts to store platelets have been made ever since then including the use of various compounds such as Tullis' salt solution including acetate and gelatin, Phosphate buffered salt solution containing glucose, PRP in a modified Tyrode's medium at room temperature, Citrate and dextrose in combination with Plasma-Lyte A, some plasma was also used.

Table 1: Remedial properties of *Carica papaya* (Anjum, Vet al, 2013)

Properties	Component of Papaya involved
Abortifacient activity, hypoglycaemic, fever, asthma	Roots and immature fruits
Post testicular anti fertility drug activity	Seeds
Anthelmintic activity	Latex of plant
Wound healing properties	Extracts of seeds and fruit pulp
Treatment of dengue, jaundice, malaria, immunomodulatory and antiviral activity, gastric problems	Leaves

Similar to other living cells, survival of platelets depends on maintenance of biochemical balance amongst different substances; glucose and hydrogen ions in particular. To study platelet storage lesions various laboratory tests have been suggested, ranging from simple pH to the complicated tests of platelet function. (Akhoon, B. a., Gupta et al 2010).

2. Methodology

A brief methodology has been shown in figure 2.

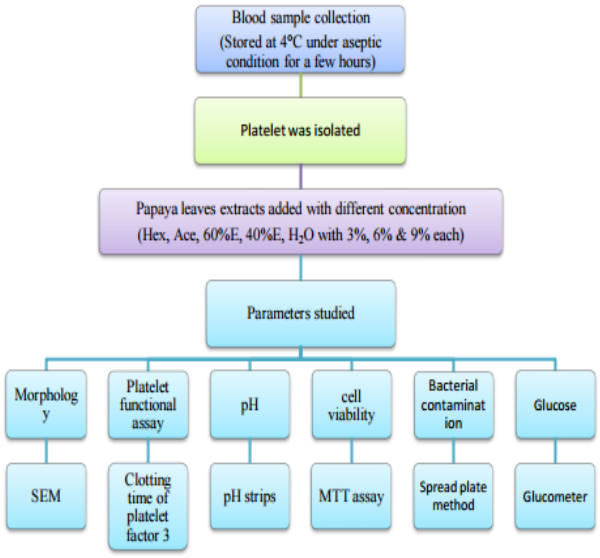

Figure 2: Platelet quality assessment methodology at a glance Platelet quality assessment methodology at a glance

2.1 Preparation of extracts

The *Carica papaya* leaves were washed and sun dried for 2 weeks to remove the residual moisture. The dried plant material was then ground into fine powder by removing the stalk and woody part using mortar and pestle and stored in an air tight container away from moisture to use for further study. Extraction of compounds from papaya leaves was carried out by sequential Soxhlet extraction with different solvents of increasing polarity. Fifty grams of powdered leaves were sequentially extracted in a Soxhlet extractor using 250 ml of hexane, acetone, 60% ethanol, 40% ethanol and water. The extraction time was about 5-8 hrs for each solvent. At the end of extraction the ethanol and water extracts were concentrated by using vacuum evaporator for GC-MS. The resulting extract was then filtered and the filtrate was stored at 4°C. GC-MS analysis of the *C. Papaya* leaf extracts were performed using a Shimadzu GC-MS QP2010 plus system and GC-MS equipped with Omega wax capillary column (30 meter) and a Flame Ionization Detector (FID) was used for detection. Helium was used as carrier gas at a constant flow of 1.21 ml/min and an injection volume was 2-5 micro litres. The column temperature was 60º C, with an increase of 5 °C /min, to 250 °C then 10 °C /min to 280 °C. Mass spectra were taken at a scan interval of 0.2s and fragments from 50 to 1000 Da.

Each of the five extracts was checked by Thin Layer Chromatography (TLC) on silica gel plates. For each extract, three different solvent mixtures were used. These were chloroform: methanol::9:1, n-hexane:acetone::8.5:1.5 and benzene:ethyl acetate::1:1. After saturation with mobile

phase for 30 min, iodine vapours were used to detect the band spots on the TLC plates. The movement of the analytes expressed by its retention factor (Rf) were calculated in each solvent systems for different samples.

The phytocomponents in the hexane, acetone, 60% ethanol, 40% ethanol and H_2O extracts of the *C. Papaya* leaves were identified based on the retention time. Mass spectrums were interpreted using WILEY and NIST library having large number of patterns. The name, structure and molecular weight of the components of the extracts were identified. After obtaining the plant extract, phytochemical screening was performed with different tests to get an idea about the type of phytochemicals present in the plant extracts including NaOH test for flavonoids (Onwukaeme, D. N. et al, 2007), Kellar-Kiliani test for cardiac glycosides (Parekh et al, 2007), $FeCl_3$ test for tannins (Parekh et al, 2007), Foam test for saponins (Parekh et al, 2007), and lead-acetate test for phenolic compounds.

2.2 Blood Sample Preparation

Blood samples from the donors were contained into vacutainer containing ACD anticoagulant maintaining aseptic conditions throughout the process and transfer of sample was done only in laminar hood using autoclaved tips, pipettes and eppendorffs. Whole blood was centrifuged at room temperature at 200 X g for 20 min. After spin three distinct layers were observed, namely, bottom layer (Red blood cells), Middle layer (buffy coat) and top layer [platelet rich plasma (PRP)] (Figure 3). 2/3rd of the PRP was transferred in another tube. HEP buffer was added in 1:1 ratio (v/v). PGE 1 was added (1 µl final concentration) to prevent platelet activation. After a gentle mixing, spinning was done for 15 – 20 min at room temperature at 100 X g without any brake. Pellet platelet was centrifuged at room temperature at 800 X g for 15 – 20 min. Platelet wash buffer was used to rinse the pellet platelet by gently adding wash buffer and slowly removing it with a pipette. The pellet was slowly suspended in tyrode's buffer and freshly prepared BSA (3mg/ml). To prevent platelet activation, PGE 1 (1 µl) was added. Furthermore, hemoglobin estimation using AHD method, cell counting and analysis of morphology of blood cells using SEM was carried out.

2.3 Addition of extracts

The extracts obtained after extraction contained organic solvents hexane, acetone and ethanol which affects the blood cells if we directly add the extracts into platelet. So to remove these solvents the extracts were converted to crude sample by evaporating the solvent of sample. These crude samples were then dissolved in phosphate buffered saline (PBS) to add into platelet sample. The resulting extracts were than filtered using 0.2µ syringe filter and the filtrate were added with different concentration (Hex, Ace, 60%E, 40%E, H2O with 3%, 6% & 9% each) into blood under aseptic condition.

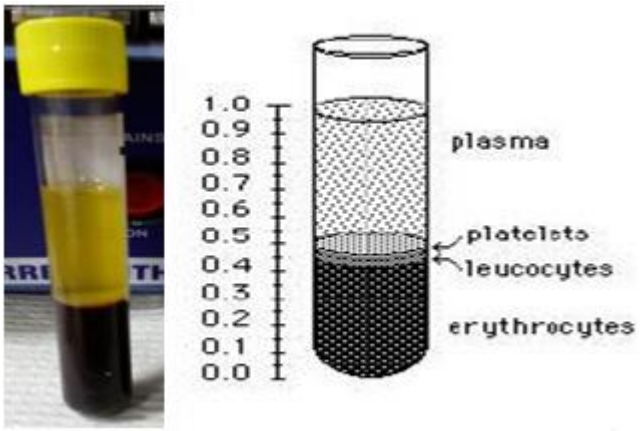

Figure 3: Platelet rich plasma separation after centrifugation

2.4 Effects of papaya leaf extracts

For studying the effects that the *C. papaya* leaves extract laid on the blood cells, various assays including glucose determination, platelet functional assay, MTT assay for cell viability, bacterial contaminations were conducted. Platelet factor 3 tests which determines the clotting time of blood, using kaolin/ silica for surface activation and $CaCl_2$ for clotting blood. 100 µl of platelet rich plasma with 100 µl of platelet poor normal plasma was added in a test tube held at 37° C in a water bath. 200 µl silica was added for an incubation period of 20 min with occasional shaking and 200 µl of $CaCl_2$ was added and clotting time was recorded. During storage, sample contains low glucose and high lactate concentration because of continued uptake and metabolism of glucose by blood cells. Changes in concentration can occur after blood sample collection, depending on glycolytic rate, temperature and pH. A simple glucometer test can be done to measure the amount of glucose present. As Platelets cannot be stored at cold temperature due to the activity of macrophages, they are stored at 22-24 °C, which when not stored extra cautiously can cause bacterial contamination thus, making them unfit for transfusion. Studies have suggested that *Carica papaya* leaf extract stabilizes erythrocytes membrane and have the potential to increase RBC and platelet count. 20 grams of premix LB agar powder was added. dd H_2O was added to make up the volume of 500 ml. (pH 7.5) and was autoclaved. Thin layer of agar was poured into each plate. Each plate was allowed to cool until it is solid. Plates can be stored at 4 °C. 35 µl sample was spread on nutrient agar plate and incubated at 37 °C for 48 hours. It was checked for bacterial growth. For MTT cell viability assay, 10^5–10^6 cells/ ml were maintained per sample. Incubation was done for 6 to 24 hours. 20 µL MTT Reagent (MTT stock solution) was added. Incubation was done for 4 hours at 37 °C. 200 µL detergent reagent (DMSO) was added. Left at room temperature in the dark for 1 hours. Absorbance was recorded at 570 nm, after diluting the sample with PBS. A graph was plotted between days and absorbance. Furthermore, the morphology of stored platelets was studied with SEM. It was observed that most blood cells can be identified by their unique topographic characters using SEM. The cells were fixed with 2.5% glutaraldehyde solution then dehydrated with 35-70% ethanol, dried and metal coated.

3. Results

3.1 Phytochemical screening of extracts

Firstly, a papaya leaf powder was prepared by sun drying the washed papaya leaves and then grinding them into a fine powder by using mortar pestle to remove the woody parts. Then, the powder was used to prepare the extracts using different solvents namely hexane, acetone, 60% ethanol, 40% ethanol and water. These extracts hence obtained were tested using various tests. Different classes of phytocompounds were present in the papaya leaf extract as determined by the various tests conducted in phytochemical screening assay. As mentioned in Table 2, flavonoids were tested positive in the extracts from Hexane, acetone, 60% ethanol, and 40% ethanol by using NaOH test.

Table 2: Phytochemical screening assay of each extracts

Phyto-constituents	Tests	Hexane	Acetone	60% Ethanol	40% Ethanol	Water
Flavonoids	NaOH test	+	+	+	+	-
Cardiac glycosides	Kellar-Kiliani test	+	+	-	-	-
Tannins	$FeCl_3$ test	-	-	+	+	+
Phenolic compounds	Lead acetate test	+	+	+	+	+
Saponins	Froth forming test	-	+	+	+	+

For cardiac glycosides, Kellar-Keliani test was conducted which showed the presence of these glycosides in the extracts from hexane and acetone only. $FeCl_3$ test showed that tannins were present in the extracts from 60% ethanol, 40% ethanol and water. Lead-acetate test was conducted to check the presence of phenolic compounds and it showed that the extracts from all of the solvents (hexane, acetone, 60% ethanol, 40% ethanol and water) had these phenolic compounds. Saponins were found to be present in all solvents but hexane as determined by froth forming test.

3.2 pH and glucose estimation

The pH values of the control and blood with CPDA and extract from day 0 to 42 have been shown in the Table 3.

Table 3: Change in pH during storage period

Days	pH of Blood with	
	CPDA and Extracts	Control
Day 0	7.4	7.2
Day 7	7.2	7
Day 14	7.1	7
Day 21	7	6.9
Day 35	6.9	6.8
Day 42	6.9	6.8

The graph of pH shows change in pH from day 0 to day 42, pH change shows that the acidity of stored blood is increased day by day but the presence of CPDA + extracts in blood slows down the rate of acidity in comparison to CPDA alone in blood (Figure 4).

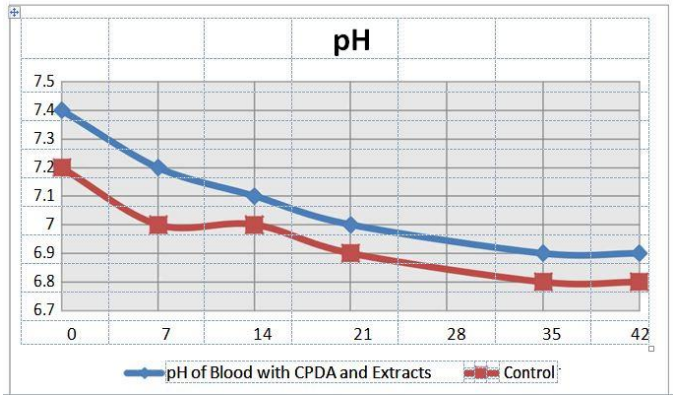

Figure 4: pH of blood with CPDA and extracts vs. Control

The graph shows change in pH from day 0 to day 42, pH change shows that the acidity of stored blood is increased day by day but the presence of CPDA + extracts in blood slows down the rate of acidity in comparison to CPDA alone in blood.

A comparative analysis of pH amongst platelet samples stored in papaya leaf extracts of different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H_2O), Fresh (F) and control was also done (Table 4).

Table 4: The measurements of clotting factor time, change in pH, glucose concentration, cell viability and turbidity for extracts from different solvents namely Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H_2O), Fresh (F)

Sample	Clotting factor time			Change in pH			Glucose determination			Cell viability			Absorbance
Control + Antibiotic	Day 1 (sec)	Day 4 (sec)	Day 7 (sec)	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 4	Day 7	Relative viability %	0.13
Control	82	293	420	7.5	7.3	7.2	294	217	201	0.002	0.001	50	0.664
3% H	82	147	203	7.5	7.5	7.5	294	267	248	0.009	0.006	67	0.114
6% H	82	151	225	7.5	7.5	7.5	294	210	197	0.006	0.004	67	0.236
9% H	82	145	202	7.5	7.5	7.5	294	285	263	0.008	0.006	75	0.087
3% A	82	149	199	7.5	7.4	7.4	294	252	237	0.021	0.015	71	0.119
6% A	82	156	207	7.5	7.5	7.4	294	216	213	0.015	0.008	53	0.138
9% A	82	155	210	7.5	7.4	7.4	294	247	225	0.004	0.002	50	0.082
3% 60% E	82	165	225	7.5	7.3	7.2	294	275	252	0.004	0.002	50	0.352

6% 60% E	82	169	203	7.5	7.3	7.2	294	245	226	0.015	0.007	47	0.467
9% 60% E	82	161	220	7.5	7.4	7.2	294	252	239	0.021	0.011	52	0.513
3% 40% E	82	180	243	7.5	7.3	7.1	294	238	217	0.02	0.004	20	0.478
6% 40% E	82	164	236	7.5	7.3	7.1	294	215	198	0.003	0.001	33	0.224
9% 40% E	82	178	240	7.5	7.3	7.1	294	212	193	0.003	0.001	33	0.358
3% H2O	82	165	230	7.5	7.4	7.1	294	230	215	0.006	0.002	33	0.363
6% H2O	82	168	230	7.5	7.4	7.1	294	226	204	0.003	0.002	67	0.37
9% H2O	82	168	232	7.5	7.4	7.1	294	224	201	0.003	0.002	67	0.362
3% F	82	170	232	7.5	7.4	7.1	294	258	241	0.002	0.001	50	0.3
6% F	82	169	240	7.5	7.4	7.1	294	245	227	0.004	0.003	75	0.347
9% F	82	173	246	7.5	7.4	7.1	294	156	135	0.005	0.001	20	0.47

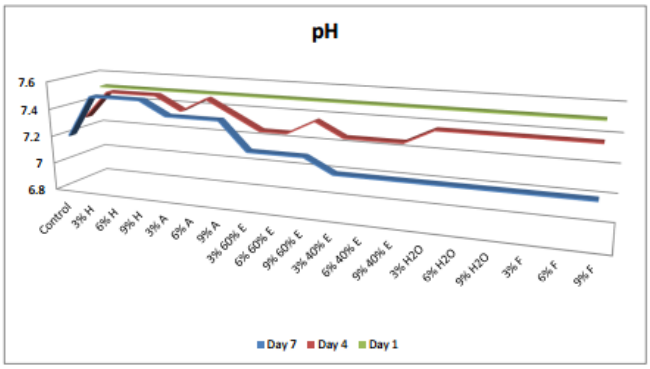

Figure 5: Comparative analysis of pH amongst platelet samples stored in papaya leaf extracts of different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H2O), Fresh (F) and Control. The graph is plotted against platelet stored in various extracts and pH on day 1, 4, and 7.

The graph was plotted for pH values of platelet stored in various extracts on day 1, 4, and 7 (Figure 5). The glucose concentration values for platelets stored in extracts from various solvents at day 1, 4 and 7 have been mentioned in Table 4 and a graph was plotted for the comparative analysis of glucose concentration of different extracts (Figure 6).

3.2 Platelet functional assay (clotting time of platelet factor 3)

A comparative analysis of Clotting time of platelets stored in papaya leaf extract in different solvent systems and control was carried out. The values of the clotting time in seconds have been summarized in Table 4. A graph was plotted for platelets stored in various extracts and time (sec) on day 1, 4, and 7 (Figure 7).

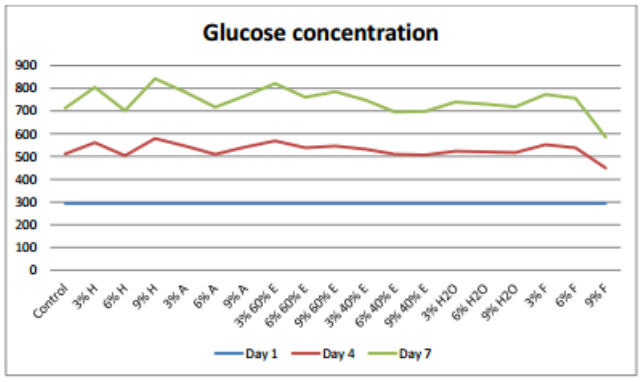

Figure 6: Comparative analysis of glucose concentration of Platelet stored in papaya leaf extract in different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H2O), Fresh (F) and Control. The graph is plotted against platelet stored in various extracts and glucose concentration on day 1, 4, and 7.

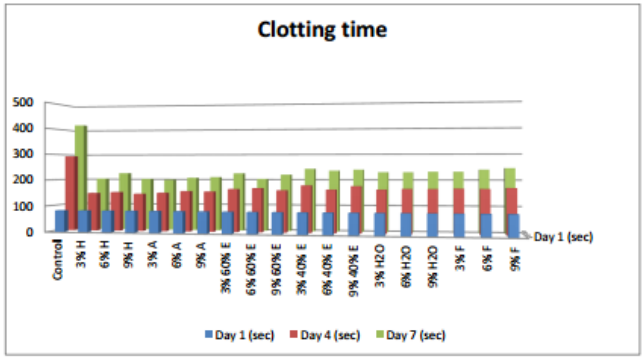

Figure 7: Comparative analysis of Clotting time of Platelet stored in papaya leaf extract in different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H2O), Fresh (F) and Control. The graph is plotted against platelet stored in various extracts and time (sec) on day 1, 4, and 7

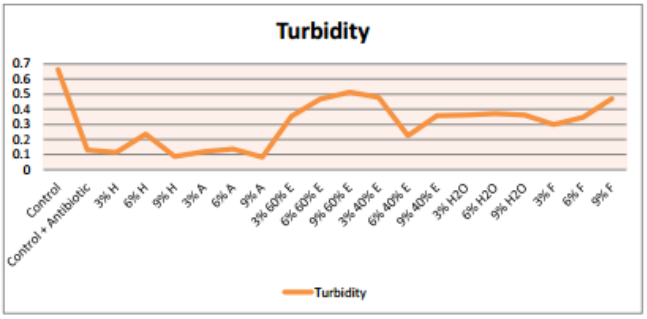

Figure 8: The graph is plotted against platelet stored in various extracts and absorbance on day 4 for bacterial contamination. Absorbance was taken at 560 nm. Turbidity was checked in nutrient broth after addition of the platelet sample stored in papaya leaf extract in different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H2O), Fresh (F), Control and control + antibiotic on day 4

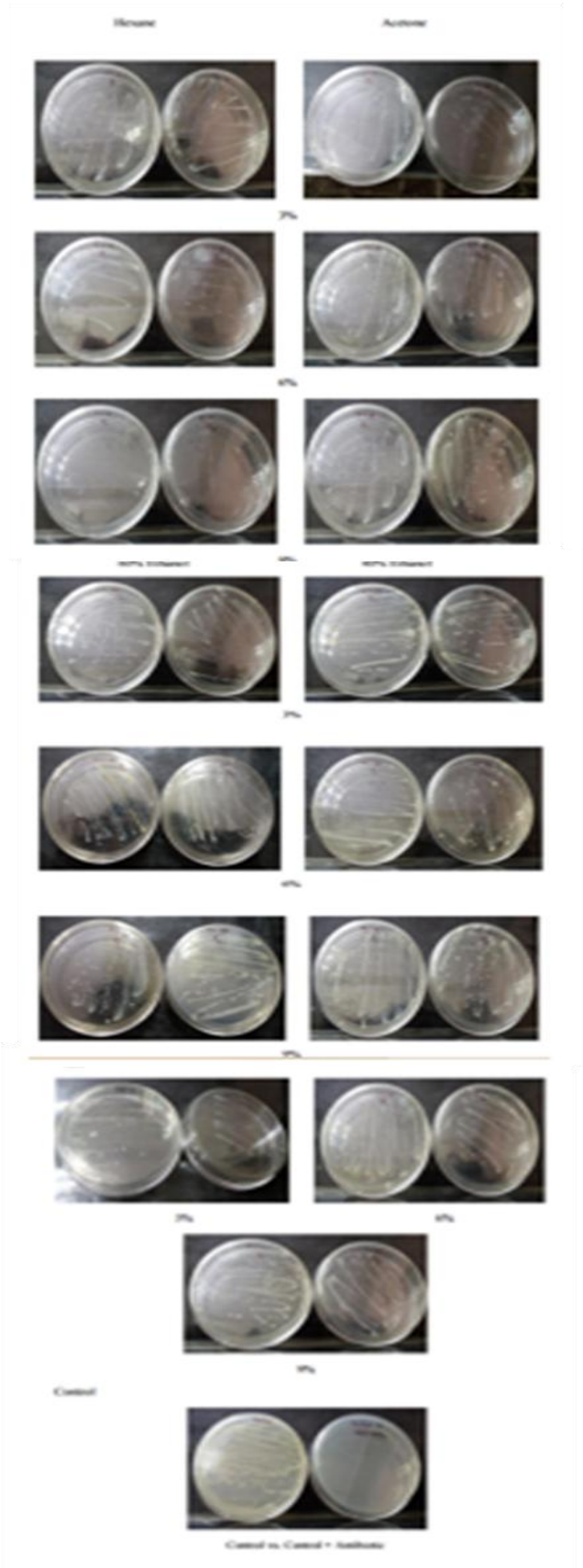

Figure 9: Confirmatory test for Bacterial contamination. These plates contain Luria Broth agar along with papaya leaf extracts in different solvent systems namely, Hexane, Acetone, 60% Ethanol, 40% Ethanol, Water in different concentrations i.e. 3%, 6%, 9% and the presence or absence of bacterial growth was checked by streaking the plates with E.coli DH5α strain. It's a confirmatory test for the experiment of bacterial contamination performed on stored platelets. 9% Hexane showed maximum anti-bacterial activity with no microbial growth.

3.3 Bacterial contamination

Turbidity was checked in nutrient broth after addition of the platelet sample stored in papaya leaf extract in different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H_2O), Fresh (F), Control and control + antibiotic on day 4. Absorbance was taken at 560 nm. The turbidity measurements of platelets with different extracts have been mentioned in Table 4 and a graph was plotted against platelet stored in various extracts and absorbance on day 4 (Figure 8). Further, a confirmatory test was also performed for bacterial contamination. The plates containing Luria Broth agar along with papaya leaf extracts in different solvent systems namely, Hexane, Acetone, 60% Ethanol, 40% Ethanol, Water in different concentrations i.e. 3%, 6%, 9% were checked for the presence or absence of bacterial growth by streaking the plates with *E.coli* DH5α strain (Figure 9). It was a confirmatory test for the experiment of bacterial contamination performed on stored platelets. 9% Hexane showed maximum anti-bacterial activity with no microbial growth.

3.4 MTT assay and cell morphology

The viability of cells was determined by MTT assay. The cell viability percentage of RBCs stored in papaya leaf extract in solvent systems and control with respect to fresh blood (Table 4). All concentrations of H, A, 60% E, along with 6% 40% E Water, 6% W, 3% F, 6% F were found to have a positive effect on the viability of cells, whereas 3% 40% E, 9% 40% E, 9% W, 9% F had lesser cell viability than control (Figures 10 and 11).

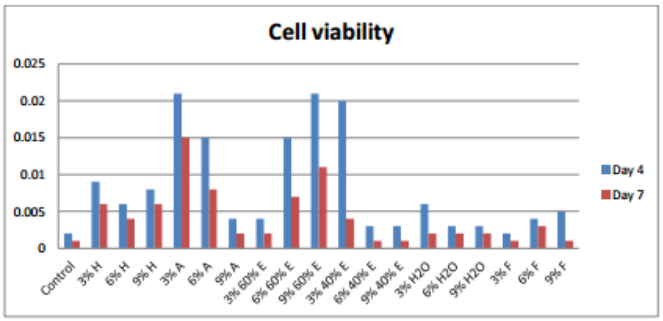

Figure 10: Comparative analysis of cell viability of Platelet stored in papaya leaf extract in different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H2O), Fresh (F) and Control on day 4 and 7. The graph is plotted against platelet stored in various extracts and absorbance on day 4, and 7.

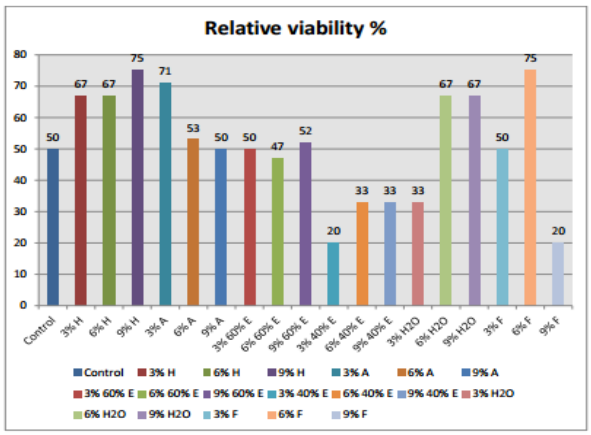

Figure 11: Relative viability of Platelet stored in papaya leaf extract in different solvent systems namely, Hexane (H), Acetone (A), 60% Ethanol (60% E), 40% Ethanol (40% E), Water (H2O), Fresh (F) and Control. The graph is plotted against platelet stored in various extracts and relative viability w.r.t day 4 and 7.

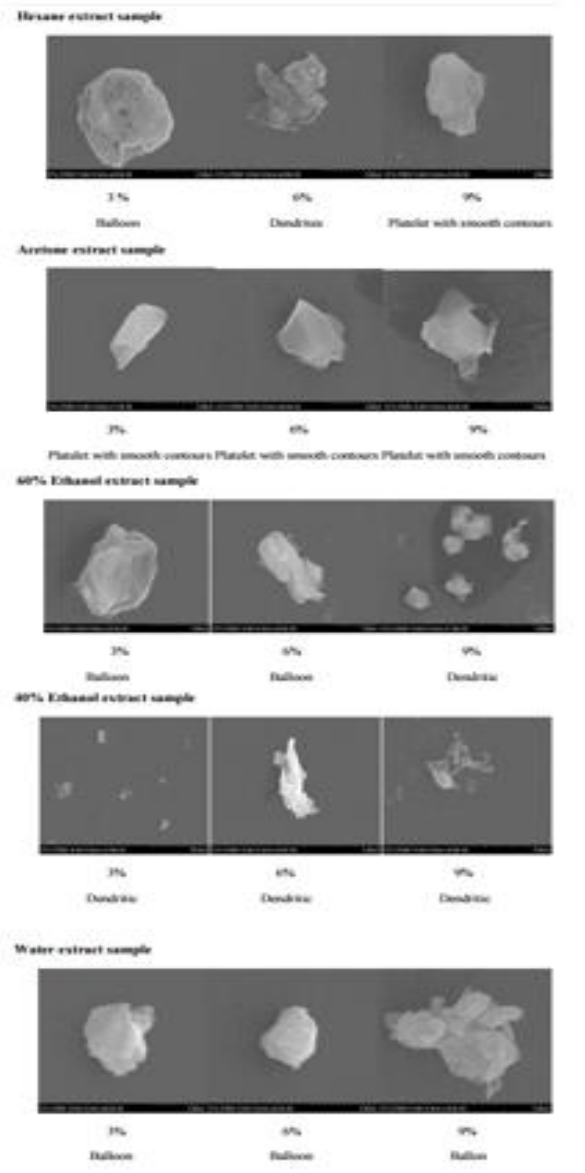

Figure 12: Morphology study of platelets at Day 5 using SEM Platelets with smooth contours — discs and spheres retaining normal size. Dendrites — Platelets that have developed pseudopodia or dendritic processes. Balloons — Platelets that have undergone swelling after losing the capacity to maintain an osmotic gradient across their membrane, clumps of multiple Platelets and could not be counted individually. (Jain A. et al, 2015)

The morphology of the cells was studied by inverted microscope and SEM. As observed with inverted microscope, the morphology changes from discoid or biconcave shape to Echinocytes to spherocytes and finally to Elliptocytes but changes in shape are different in all extract. Morphological studies explained about the cell death. Observation with a SEM shows that platelets have three major shape changes namely, platelet with smooth contours, dendritic and balloon (Figure 12). In platelets with smooth contours, the platelets are shaped as discs and spheres retaining normal size. In dendrites form, the platelets developed pseudopodia or dendritic processes; and long tubular forms. In balloons form, the platelets underwent swelling after losing the capacity to maintain an osmotic gradient across their membrane, clumps of multiple platelets formed and could not be counted individually. (Jain et al, 2015)

4. Discussion

Platelets are small enucleated cells in blood which have an important role in maintenance of homeostasis, wound healing and ceasing of bleeding. Platelets have a short life span of 10 days in human body, after which they are replenished. This creates a problem in the availability of platelets and its inventory. Bacterial contamination also poses to be a major problem in storage of platelets as they cannot be administered at neither too high nor low temperatures. The three fundamental quality parameters for assessing the platelet shelf life are platelet counts, pH and absence of bacteria. Many agents have been found to affect the shelf life of platelets. Our work aims to study the various effects that *Carica papaya* extract lays on the half life of the platelets. *Carica papaya* has been found to have many medicinal properties such as antimicrobial, antiseptic properties and its extract has also been found to be useful for the treatment of dengue and malaria.

Firstly, the *Carica papaya* leaves were washed and sundried for 2 weeks to remove the moisture and then ground into fine powder by mortar and pestle and the powder was then stored in an air tight container for further use. The extracts from papaya leaves were extracted by Soxhlet extraction technique using 250 ml of hexane, acetone, 60% ethanol, 40% ethanol and water and an extraction time of about 5-8 hrs for each solvent. The extracts were filtered and the filtrates were stored at 4°C. GC-MS analyses of extracts were performed. Each of the five extracts was checked by Thin Layer Chromatography (TLC) on silica gel plates. The movement of the analytes expressed by its retention factor (Rf) were calculated in each solvent systems for different samples. The phytocomponents in the hexane, acetone, 60% ethanol, 40% ethanol and H_2O extracts of the *C. Papaya* leaves were identified based on the retention time. Mass spectrums were interpreted using WILEY and NIST library having large number of patterns. After obtaining the plant extract, phytochemical screening was performed with

different tests including NaOH test for flavonoids, Kellar-Kiliani test for cardiac glycosides, FeCl3 test for tannins, Foam test for saponins, and lead-acetate test for phenolic compounds. Different classes of phytocompounds were found to be present in the papaya leaf extract as determined by phytochemical screening assay. Flavonoids tested positive in the extracts from Hexane, acetone, 60% ethanol, and 40% ethanol. Only the extracts from hexane and acetone tested positive for cardiac glycosides. $FeCl_3$ test showed that tannins were present in the extracts from 60% ethanol, 40% ethanol and water. The extracts from all of the solvents (hexane, acetone, 60% ethanol, 40% ethanol and water) had phenolic compounds. Saponins were found to be present in all solvents but hexane.

Blood samples from donors were prepared by adding ACD anticoagulant. The blood was centrifuged to finally separate the platelets in the form of a pellet and hemoglobin estimation using AHD method, cell counting and analysis of morphology of blood cells using SEM was carried out. After removal of organic solvents (hexane, acetone etc) from the extracts by evaporating the solvent, the extracts were dissolved in PBS to be added to platelet sample in different concentrations. For studying the effects that the *C. papaya* leaves extract laid on the blood cells, various assays including pH estimation, glucose determination, platelet functional assay, MTT assay for cell viability, bacterial contaminations were conducted. The pH changes showed that the acidity of stored blood is increased day by day but the presence of CPDA + extracts in blood slows down the rate of acidity in comparison to CPDA alone in blood.

Hexane showed maximum activity in overcoming platelet storage lesions such as pH, Morphology, cell viability, clotting factor time, Glucose and controlling bacterial contamination. The results of phytochemical analysis and GC-MS of the extracts confirmed the presence of glycosides, saponins, tannins, flavonoids, phenolic etc. Literature survey confirmed that the metabolites present in the extracts possess medicinal value such as antimicrobial, antioxidant, antiinflammatory, analgesic, anti-atherogenic, antithrombotic, anticoagulant, neuroprotective, antiviral, immune-modulatory, cell membrane-stabilizing and antiproliferative activities. Metabolites helped in overcoming the storage lesions both in blood and platelet.

In stored platelets, platelet storage lesions such as pH, glucose, platelet functional assay i.e. platelet factor 3 test determining the clotting factor time, cell viability along with bacterial contamination were overcome in papaya leaf extract with 9% Hexane solvent. Since the biggest problem faced by blood banks in storage of platelets is microbial growth because of the storage temperature (which is 22 – 24° C) we can say Hexane at higher concentration (9% in comparison to 3% and 6%) has maximum antimicrobial activity. It was observed that when 9% hexane extract was added to media plates no contamination was seen, thus the metabolites present in this extract can be considered for further analysis. The activity shown by the extract could be possibly due to tannis for its antimicrobial activity along with alkaloids and flavonoids.

Since multiple components are present in papaya having the potential to act as bioactive component for improving blood cell's quality; separation, purification and detection of the effect of each component on blood cells individually is required to confirm the bioactive components affecting blood cells in a positive manner.

5. Conclusion

In this study it has been demonstrated that currently used anticoagulants for the storage of blood and its components have a very limited life span and there is still scope to improve the quality of blood cells in storage and increase the shelf life 5 days in case of platelet. It will be advantageous to prolong the shelf life of blood cells for situations like disaster where stockpiling is necessary, or for the individuals with rare blood type, it can also act as an insurance/ reserve for irregular supply. So keeping this thought in mind an effort was made to access the effect of *Carica papaya* leaf extract (in different solvent systems) on blood cells already preserved in anticoagulant CPDA against control, remarkable results were observed. TLC profiling of *Carica papaya* leaves confirmed the presence of phytochemical components and then by phytochemical analysis presence of compounds like alkaloids, glycosides, saponins, tannins, flavonoids, phenolic compounds was confirmed. Different Rf values of the compounds in different solvent system provide information about their polarity. GC-MS results further confirmed that Papaya leaf extracts contain compounds which show medicinal properties like antimicrobial, antioxidant, antiinflammatory, analgesic, anti-atherogenic, antithrombotic, anticoagulant, neuroprotective, antiviral, immune-modulatory, cell membrane-stabilizing and antiproliferative activities. These medicinal properties of extracts help in maintaining cellular viability of blood cells during storage.

In case of Platelet more specific conclusion can be made and we can say that leaf extract in solvent system hexane showed better results. Platelet storage lesions in hexane were controlled as compared to control and other extracts. Microbial contamination was absolutely zero. Discs prepared from these stored platelets showed no microbial growth. No growth in liquid broth was confirmed by spectrophotometer.

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