Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/21135
DOI URL: http://dx.doi.org/10.21474/IJAR01/21135

ISSN NO. 2320-5407

RESEARCH ARTICLE

EVALUATION OF COCOS NUCIFERA (COCONUT) STARCH AS AN ALTERNATIVE TO SERUM SEPARATOR TUBES FOR ALANINE AMINOTRANSFERASE AND FASTING BLOOD SUGAR

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Manuscript Info

Manuscript History
Received: 08 April 2025
Final Accepted: 11 May 2025
Published: June 2025

Key words:-
Serum Separator, Coconut Starch, ALT, FBS, Laboratory Diagnostics

Abstract

The need for sustainable and biodegradable alternatives in laboratory diagnostics has prompted interest in natural polymers for serum separation. While synthetic serum separator tubes (SSTs) are widely used for biochemical analyses, concerns over their environmental impact and potential interference with test results necessitate exploration of alternative materials. However, limited studies have examined the viability of plant-based polymers in this application. This study aimed to evaluate the potential of Cocos nucifera (coconut) starch gel as a natural substitute for SSTs in alanine aminotransferase (ALT) and fasting blood sugar (FBS) testing. Experimental trials were conducted using starch gel at varying concentrations (25%, 50%, 75%, and 100%) to assess serum separation efficiency and its influence on laboratory parameters. The results demonstrated that higher concentrations of starch gel improved serum separation, with 75% starch gel achieving optimal performance, minimizing hemolysis, and yielding clear serum samples. ALT levels remained stable across all concentrations, indicating no significant interference. However, FBS levels exhibited notable variability, suggesting possible interactions between the starch gel and glucose measurement. These findings highlight the potential of Cocos nucifera starch gel as an eco-friendly alternative to conventional SSTs, reducing reliance on synthetic materials. Nevertheless, further refinement is required to enhance its stability and ensure its reliability for clinical applications. Future research should explore its impact on additional biochemical parameters and optimize its formulation to align with existing laboratory standards.

Introduction:-

In today's advancement of technology, serum separator tubes (SST) are viewed as crucial in the blood collection routine in every medical laboratory due to their effectiveness in serum separation from cellular elements. Gel

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barriers—often composed of synthetic polymers such as silica—fill these tubes. They improve the quality of the material and assist in rapidly separating serum (Ayala-Lopez et al., 2021). Located within the tubes, the gel is a barrier separating the serum from the cellular layers, preventing mixing and facilitating the serological collecting after centrifugation. This element is mainly connected to the thixotropic property of the gel, which enables surface division (Babakhani et al., 2018). Although SST is produced from synthetic polymers presently, demand for alternative materials—including plant-based starch—is developing. Various research studies have shown the thixotropic characteristics of various polymers and starches, which qualify them as a suitable replacement in gel formulation and possibly used in serum separator tubes.

Chemical analysis in medical laboratories is crucial in testing a patient's health status and is usually required by a practitioner. It is typically performed on serum, attained through blood clotting. Fasting Blood Sugar (FBS) and Alanine Aminotransferase (ALT) serve as standard indicators for liver function. Alanine Aminotransferase is primarily confined to the liver and released into the bloodstream following liver damage. Fasting Blood Sugar measures glucose levels in the bloodstream and is a diagnostic marker for diabetes. An elevated level of Alanine Aminotransferase (ALT) and Fasting Blood Sugar (FBS) might point to liver impairment (Lala et al., 2023). Serum separator tubes are usually necessary to properly separate serum from cellular components in sample collecting for medical tests, enhancing the analysis's accuracy.

The coconut palm is of paramount significance for its many uses, growing from the coconut tree under the scientific name Cocos nucifera. It originates from several Southeast Asian parts, including the Philippines, Indonesia, and Malaysia. Among other names, the coconut tree is called coco-da-bahia, niyog, and coco. Well-known for their many applications, particularly in the domains of nutrition and medicine, Cocos nucifera supplies a variety of goods like coconut oil, coir fiber, and coconut water. Local businesses and customs find use for every coconut palm component, highlighting its worth as a botanical and medicinal source.

Recently, a growing emphasis has been on developing biodegradable materials utilizing natural polymers and their derivatives. Despite the widespread availability of coconut, research focusing on utilizing its starch properties remains limited. Notably, a research gap exists regarding applying coconut starch as an alternative to commercial serum separator gels used in clinical laboratories. By addressing this gap, researchers aim to expand current knowledge and understanding regarding the applications of ALT and FBS in biodegradable materials, specifically within clinical diagnostics

Research Design

This study will utilize a proper experimental research design, which is any inquiry that uses at least one experimentally manipulated independent variable and at least one dependent variable.

To obtain the extensive data and results required for this experimental research, the researchers will apply purposive sampling to assess the impact of Cocos nucifera starch gel on the efficiency of serum separation and its effect on Alanine Aminotransferase (ALT) and Fasting Blood Sugar (FBS). Twelve (12) healthy human subjects, ranging in age from 18 to 45, will be sampled through convenience sampling. This method guarantees rapid data-collecting application and supports the timely implementation of the data-gathering process by efficiently compiling information from conveniently available participants.

Specimen Collection

A Comprehensive approach is necessary to investigate the influence of Cocos nucifera starch gel serum separation efficiency on Fasting Blood Sugar (FBS) and Alanine Aminotransferase (ALT) levels. The participants will receive two copies of the Informed Consent Form (ICF): one for their records and one to be retained by the researchers. Participants will have at least half an hour to read the ICF. The researchers will then articulate the ICF's content to the participants following this period. After completing this discussion, the participants and researchers will properly sign both copies of the ICF. First, twelve (12) healthy subjects will be selected based on predeterminedinclusion and exclusion criteria to have a balanced representation of gender and various demographic strata. Inclusion criteria for the study include individuals aged between 18 and 45 years who have passed comprehensive preliminary screening tests. Such tests involve an intense scrutiny of medical history and a thorough physical examination conducted by a competent healthcare practitioner to ascertain optimal health status.

Additionally, participants must avoid taking medications that profoundly affect the physiological parameters under study. Exclusion criteria are applied to elderly individuals and those with pre-existing disorders. These criteria ensure consistency and reliability in the study's outcomes by targeting specific age and health conditions.

Research Locale

The preparation of Cocos nucifera starch extract will be carefully conducted within the controlled environment of the designated laboratory facility at the esteemed National University - MOA in Pasay City. Specimens from human subjects will then be responsibly procured at the same laboratory. Following procurement, all experimentation and subsequent testing of ALT and FBS levels will be meticulously conducted at Globalife Medical Laboratory & Polyclinic, also in Pasay City. Under the strict guidance of a licensed medical technologist, each step of the experimental process will be monitored closely to ensure the highest level of scientific integrity and ethical practice.

Data Gathering Procedure

The data gathering of this study follows established procedures and includes basic principles based on the output of previous researchers in the field. This systematic process includes key steps like Plant Collection and Authentication, Plant Preparation, Extraction of Cocos nucifera starch, Gelatinization and Settling of Cocos nucifera starch, Human Blood Sample Collection, Laboratory Experimentation, and finally, Scoring and Interpretation. Using the knowledge and findings of previous researchers, this process follows a robust and systematic approach to data collection and analysis for our study.

Collection and Authentication of Cocos nucifera

Fifteen (15) coconut samples will be collected from a farm in Silang, Cavite. The fruit samples will be strictly identified and authenticated at the Institute of Biology, Jose Vera Santos Memorial Herbarium (PUH), located in the College of Science of the University of the Philippines, Diliman, Quezon City. They will issue a certificate identifying the fruit samples.

Cocos nucifera Preparations

Strictly using aseptic methods in manipulating coconuts is crucial in maintaining cleanliness and preventing contamination hazards in experimental procedures. First, all equipment, utensils, and work surfaces must be washed and disinfected using distilled water to provide a sterile environment. Researchers will utilize sterile gloves, aprons, and masks to prevent contaminating the environment. Second, the outer surface of the coconut will be washed using distilled water to eliminate any visible dirt or debris. Sterile instruments on sanitized cutting mats will be utilized to open the coconut carefully to preserve the sterility of the inner flesh. At this point, strict compliance with aseptic methods will be maintained to prevent the introduction of pathogenic bacteria or other disease-causing microorganisms.

Extraction of starch from Cocos nucifera

The starch extraction from Cocos nucifera is carried out using conventional extraction procedures. Mixing five hundred grams of coconut flour with distilled water facilitates starch extraction. The mixture is filtered using cheesecloth to separate the solid residue from the starch. The solid residue containing starch is dried at 80°C for an hour to reduce moisture content. The coconut fraction is dried and ground into fine powder after drying to achieve uniformity. Excess supernatant or excess liquid is removed, and purified coconut starch is obtained. The starch powder is then moved to a refrigerated condition for 24 hours to facilitate starch precipitation.

Gelatinization and settling of Cocos nucifera starch

According to the procedure given by Macugay et al. (2018), Cocos nucifera starch extract is gelatinized to produce starch gel separators. The extracted starch of coconut is stored in the refrigerator. The starch extract will be weighed in specific concentrations of 25%, 50%, 75%, and 100%. Combining different amounts of starch extract with Normal Saline Solution (NSS) allows the maintenance of cellular integrity by attaining concentration.

The mixture will be under a controlled water bath for two hours at specific temperature and pH conditions to allow gelatinization, hence improving the functional properties of the starch gel. 12mL of sodium hydroxide (NaOH) will be added midway through the heating procedure; consistent stirring will follow until the two-hour process is complete. The resultant mix will cool before being put into evacuated tubes for blood collecting. After gelatinization, 8mL of each concentration of starch gel will be pipetted into conical tubes; three consecutive washes with NSS using a decantation method will remove any remaining contaminants. Subsequently, one gram of the

gelatinized starch will be placed into red-top tubes. The samples will then be centrifuged to allow the settling of the starch at the bottom of the tube, ensuring stable and uniform gel consistency for use in serum separation.

Human Blood Specimen Collection

The research aims to determine the effectiveness of Cocos nucifera starch gel on ALT and FBS activities of human blood samples. The blood samples would be drawn from twelve (12) healthy subjects using the venipuncture with a syringe method. Every volunteer would be asked to provide 12mL of blood per test for three tests per subject. Taking blood samples will involve two needle punctures of a 10-cc and a 5-cc syringe and 3mL of blood, filling each of the five collection tubes with experimental starch gel or control solution. The five collection tubes would have distinct identification labels showing the student's school name and the collection sequence as his/her subject ID.

Following collection, the blood samples will be allowed to stand up to 30 minutes before separation into serum via centrifugation at 3500 revolutions per minute (rpm) for 20 minutes. The extent of serum separation and integrity of the sample will be observed and documented. Participants will be compensated with 300 pesos as an incentive for participation. Ethical clearance will be obtained from the National University – MOA before the sample collection is initiated. Inclusion criteria will be utilized to randomly select participants aged 18 to 45 who are assumed to be healthy. Exclusion criteria will be participants above the age of 45 years and volunteers with existing medical conditions. All-rounded health screening tests will be performed to determine eligibility and status of the volunteers' health.

Laboratory experimentation

The study design involves the collection of 12mL blood from each subject per trial, which will be allocated into different experimental groups totest the efficiency of serum separation. The categorization of the experimental groups is as follows:

a.Group I: The group will use routine commercial serum separator tubes containing 3mL of blood collected from the subjects.

b.Group II: 3mL blood samples will be treated with a solution of 25g of Cocos nucifera starch extract, 90mL of NSS, and 10mL of NaOH, which will give a 25% starch extract concentration, and only 1g of starch extract will be used.

c.Group III: Three mL of blood samples will be treated with 50g of a starch extract solution of Cocos nucifera, 90 mL of NSS, and 10 mL of NaOH, with a 50% starch extract concentration and using only 1g of starch extract.

d.Group IV: 3mL blood samples will be treated with a solution of 75g of Cocos nucifera starch extract, 90mL of NSS, and 10mL of NaOH, which is 75% starch extract concentration and the use of only 1g of starch extract.

e.Group V: 3mL of the blood samples shall be treated with a 100g Cocos nucifera starch extract and 10mL of NaOH solution, which carries a 100% starch concentration, with merely 1g of starch being used.

The medical technology faculty's supervision and specialized expertise will be necessary to guarantee accuracy and adherence to accepted protocols throughout the experimental processes.

Waste Disposal

Appropriate waste management was rigorously maintained throughout experimentation in compliance with laboratory security and biohazard control standards. Following collection, blood sample tubes containing residual blood, which could pose a risk, were disposed of properly. Following institutional guidelines, blood, and hazardous debris were put in specifically designed biohazard garbage containers. These properly marked storage containers made distinguishing regular garbage from hazardous waste simple.

Strict procedures have been implemented to mitigate any possibility of dangerous chemical exposure, especially concerning bloodborne illnesses. Emphasizing the need to follow safety rules and manage biohazardous items appropriately, the laboratory personnel underwent extensive instruction on appropriate waste management. They were given protective clothing and gloves to handle waste, adding further protection layers.

The facilities were regularly maintained and monitored to ensure waste disposal functioned adequately. This included routine checks to guarantee appropriate sealing of biohazard containers, hence avoiding leaks or exposure to dangerous chemicals. In addition, rigorous adherence to safety recommendations established by the institutional

biosafety committee was maintained, guaranteeing a safe experimental environment and prioritizing all participants' health and safety.

Scoring and Interpretation

The data obtained throughout the starch extraction, gelatinization, and serum separation efficiency processes are thoroughly examined. Unanticipated findings or observations are considered and analyzed to provide further details. The researchers will not disclose the results to the participants to uphold the study's integrity and avoid bias.Employing the systematic evaluation technique, the researchers determined the efficacy of Cocos nucifera starch gel for serum separation. This type of systematic evaluation—as described in Table 3.1 below—assigns ratings from Excellent (4) to No Significance (0) that correspond to different performance categories for serum separation.

Table 3.1:- Criteria for the Serum Separation Evaluation of Cocos nucifera Starch Gel.

Score	**Excellent (4)**	**Good (3)**	**Fair (2)**	**Poor (1)**	**No Significance (0)**
Separation	The concentration of the *Cocos nucifera* starch gel contributes to the separation of serum and plasma, with little to no red blood cells mixed into the serum.	The concentration of the *Cocos nucifera* starch gel contributes to the separation of serum and plasma but with a few red blood cells visibly mixed into the serum.	The concentration of the *Cocos nucifera* starch gel contributes to the separation of serum and plasma, but a more significant amount of red blood cells is mixed into the serum.	The concentration of the *Cocos nucifera* starch gel fails to contribute to the separation of serum and plasma as many red blood cells are mixed into the serum.	The concentration fails to separate the serum and plasma at all.
Visible Hemolysis	The tube is mixed correctly with the starch gel and filled adequately, which does not lead to hemolysis.	The tube is mixed correctly with the starch gel but may be filled inadequately, which is at risk of hemolysis.	The tube is not mixed correctly with the starch gel and is slightly inadequately filled, which puts it at risk of hemolysis.	The tube is not mixed correctly with the starch gel and is inadequately filled, which leads to hemolysis.	The tube had visible hemolysis, and the starch gel did not work.

Under the "Separation" category, the Cocos nucifera starch gel's performance in separating serum from plasma and thereby preventing red blood cell contamination establishes its practicality. "Excellent" represents effective separation without red blood cell inclusion in the serum. Nonetheless, when the serum's red blood cell content increases, the scores decline until a final "No Significance" grade is obtained, signifying complete separation failure.

The "Visible Hemolysis" subsection describes how the gel could stop hemolysis—a process in which erythrocytes lyse and release their internal contents into the serum. A "Good" grade indicates correct mixing and sufficient gel filling without apparent hemolysis. Usually, lacking enough gel or incorrect mixing causes lower scores, which raises the risk of hemolysis. This causes apparent hemolysis, and the gel is not successful in preventing it.

The criterion assessment table serves as a comprehensive tool for scientifically evaluating the performance of Cocos nucifera starch gel in serum separation and laboratory testing.

Ethical Considerations

As the experiment commenced, ethical and safety considerations were carefully examined to ensure the highest levels of research ethics while protecting each participant's safety. The National University-MOA Research Ethics Board carefully examined and approved the study plan to guarantee strict adherence to ethical standards and values,

including human welfare. The careful process underlines the significance of doing research with human beings by emphasizing how crucial it is to conduct it ethically and responsibly.Post-study, the findings will be shared in scholarly publications, and infographics will be published on social media.
Aside from ethical concerns, other safety measures were taken to counteract any harm from the research practices. Some examples include careful supervision of laboratory safety measures instituted to prevent accidental exposure to harmful chemicals or substances used in the research. Scientists also must follow safety measures to prevent harm to individuals involved and themselves.

The researchers ensure that all the ethical standards are fulfilled, particularly concerning conflicts of interest, which can arise when personal, financial, or professional interests could influence or appear to influence the research process. All the researchers in this study have no interest that could bias the results. No researcher has received any financial payment or incentives from any institution or individual that could influence the study design. Study is independent and free from external pressures or influence from sponsors or other interested individuals. Complete transparency is maintained during the research process. All possible conflicts are revealed and addressed according to institutional and ethical standards. An independent ethics committee has reviewed and approved the study to ensure the absence of conflicts of interest and adherence to all ethical standards.

Before the commencement of data collection, informed consent will be given to all those who are to be involved in the research. Participants will be informed of the purpose and objectives of the study, the nature of the data to be collected, and data handling methods. Personal identifiers shall be deleted or encrypted as a participant confidentiality measure, with each participant receiving a unique number to ensure data anonymity. All collected data will be securely kept in password-protected digital databases. In contrast, physical documents (e.g., consent forms) will be kept in securely locked cabinets in areas where access is highly controlled. All such data access will be controlled by authorized personnel whose authorizations shall be based on role and requirement, thus ensuring data integrity and confidentiality. Human data will be retained until April 2025 and destroyed following the applied third-party protocol.

Also emphasized is the requirement for proper handling procedures to avoid spillage or accidents that may result in injuries, like puncture wounds. To avoid inaccuracies, researchers have to handle resources and instruments cautiously. Moreover, the integrity and safety of the study project depend on following appropriate standards. Researchers must follow all biosafety guidelines for the experimental activities and get the necessary permits and approvals from authorities.

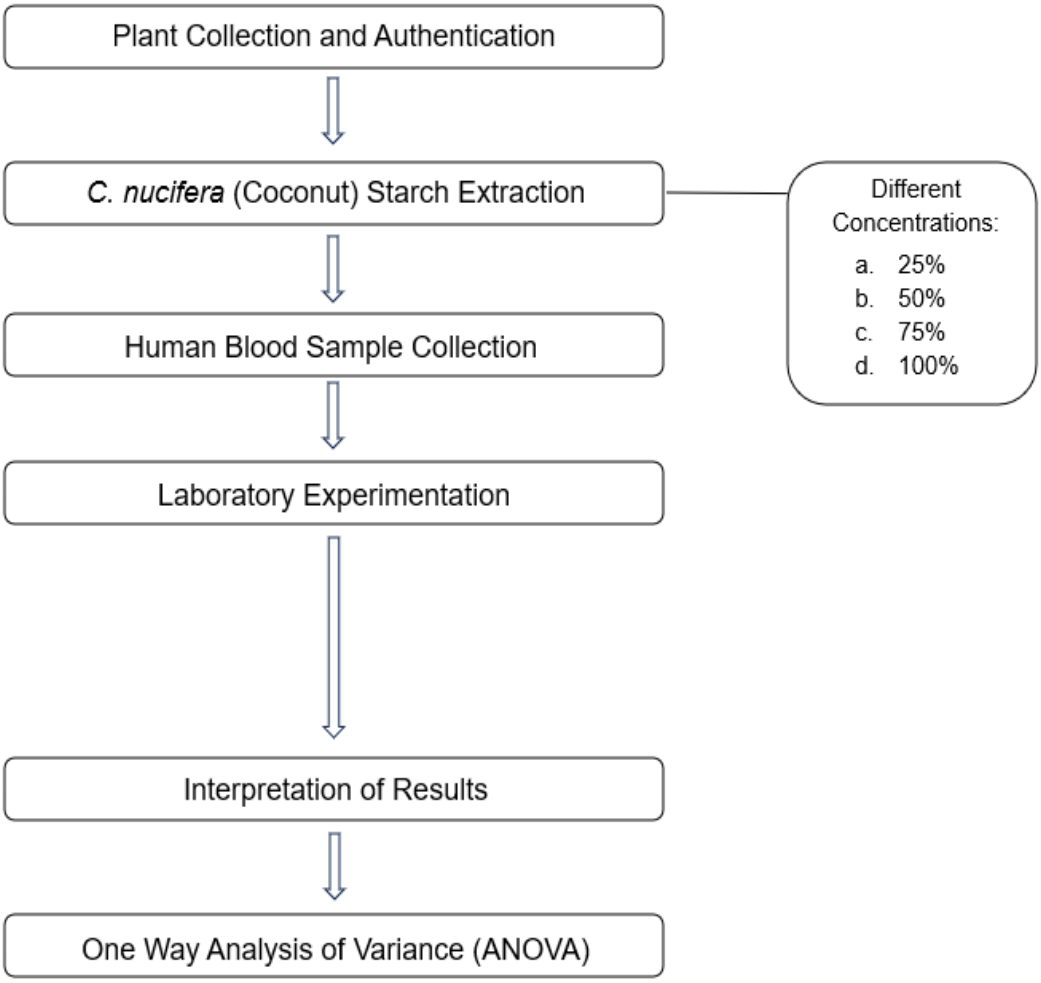
Appropriate waste disposal techniques should also be used to guarantee the safety of laboratory employees and minimize the effect on the surroundings. To lower contamination and risks, the researchers must properly separate the waste products and dispose of them in compliance with approved policies. These safety precautions depend on maintaining moral values and guaranteeing the welfare of every study subject. Researchers demonstrate their dedication to carrying out moral research activities and safeguarding the health of all study participants by getting the required permissions and following the guidelines and regulations.

Treatment of Data
Regarding conventional approaches, the researchers will employ a one-way ANOVA to fully evaluate the efficiency of Cocos nucifera starch gel as a serum separator. Therefore, one-way ANOVA will provide a detailed study of variation within and across groups, resulting in significant information on the performance of the starch gel at various concentrations and its use in clinical diagnostics.

Results and Discussion:-
This chapter presents the results of the experimental study that tested the effectiveness of Cocos nucifera starch gel as a substitute for serum separator tubes. The findings include data from four concentrations (25%, 50%, 75%, and 100%), with a commercial-grade serum separator tube as the baseline control.

Analysis of Data
The statistical analysis included the Kruskal-Wallis H Test to ascertain if variances between the four concentrations for serum separation efficiency and visible hemolysis were significant. Subsequently, the Mann-Whitney U Test was conducted as a post hoc analysis to find the precise variations between every concentration in pairs.

Research Flowchart



Presentation of Data

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Separation	180	2.8667	1.25694	.00	4.00
VisibleHemolysis	180	3.1333	1.36762	.00	4.00
Group	180	3.0000	1.41816	1.00	5.00

Table 4.1:- Descriptive Statistics of the Separation and Visible Hemolysis Cocos nucifera as an Alternative for Serum Separator Tubes.

Table 4.1 presents data on the number of samples (N), mean, standard deviation, minimum and maximum values obtained for separation, visible hemolysis, and group classification.

With an average separation value of 2.87 (±1.26), Cocos nucifera starch gel appeared to have primarily moderate separation over all examinedconcentrations. While the most significant value was 4.00, signifying total and effective separation, the lowest reported separation value was 0.00, indicating no separation. Likewise, visible hemolysis exhibited an average value of 3.13 (±1.37), ranging from 0.00 to 4.00, indicating that hemolysis levels changed with concentration applied. With values between 1.00 and 5.00, corresponding to the several concentrations of Cocos nucifera starch gel used in the experiment, the grouping variable had a mean of 3.00 (±1.42).

The findings indicate that separation efficiency exhibited huge variability regarding concentrations used, with some samples not separating at all while others wholly separated. Additionally, the extent of visible hemolysis was inconsistent, suggesting that some concentrations of the starch gel may have disrupted red blood cells considerably more than others.

Kruskal-Wallis Test

Ranks

	Group	N	Mean Rank
Separation	25%	36	40.22
	50%	36	59.26
	75%	36	101.72
	100%	36	88.79
	Total	144	
VisibleHemolysis	25%	36	61.04
	50%	36	82.22
	75%	36	80.38
	100%	36	66.36
	Total	144	

Test Statistics[a,b]

	Separation	VisibleHemolysis
Chi-Square	61.353	11.140
df	3	3
Asymp. Sig.	.000	.011

a. Kruskal Wallis Test

b. Grouping Variable: Group

Table 4.2:- Kruskal-Wallis H Test Results for Separation and Visible Hemolysis.

Kruskal-Wallis H Test was employed to compare mean ranks between different Cocos nucifera starch gel concentrations, depending on how they influence separation and visible hemolysis. As shown in Table 4.2, the Asymp. Sig. (p-value) determines whether there is a statistically significant difference among the groups. If the p-value is less than 0.05, it indicates asignificant difference. The results show a significant difference in separation ($p = 0.000$) and visible hemolysis ($p = 0.011$) among the different starch gel concentrations. A post hoc test was performed to identify which specific concentrations differ significantly.

To identify these differences, the Mann-Whitney U Test was performed for pair-wise comparisons. However, a constraint of this test is that it only allows for comparing two concentrations at a time. Consequently, six pair-wise comparisons were conducted to evaluate the effectiveness of different starch gel concentrations in separating red blood cells and minimizing visible hemolysis. The results of these comparisons are presented in six separate Mann-Whitney U Test tables, providing further statistical insights. The pair-wise comparisons are the following:

a.25% vs. 50%
b.25% vs. 75%
c.25% vs. 100%
d.50% vs. 75%
e.50% vs. 100%
f.75% vs. 100%

Table 4.3:- Mann-Whitney U Test Results of Comparison of 25% vs. 50% Cocos nucifera Starch Gel Concentrations.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Separation	25%	36	31.28	1126.00
	50%	36	41.72	1502.00
	Total	72		
VisibleHemolysis	25%	36	31.29	1126.50
	50%	36	41.71	1501.50
	Total	72		

Test Statistics[a]

	Separation	VisibleHemolysis
Mann-Whitney U	460.000	460.500
Wilcoxon W	1126.000	1126.500
Z	-2.795	-2.689
Asymp. Sig. (2-tailed)	.005	.007

a. Grouping Variable: Group

Table 4.4:- Mann-Whitney U Test Results of Comparison of 25% vs. 75% Cocos nucifera Starch Gel Concentrations.

Test Statistics[a]

	Separation	VisibleHemolysis
Mann-Whitney U	112.000	475.500
Wilcoxon W	778.000	1141.500
Z	-6.687	-2.435
Asymp. Sig. (2-tailed)	.000	.015

a. Grouping Variable: Group

Ranks

	Group	N	Mean Rank	Sum of Ranks
Separation	25%	36	21.61	778.00
	75%	36	51.39	1850.00
	Total	72		
VisibleHemolysis	25%	36	31.71	1141.50
	75%	36	41.29	1486.50
	Total	72		

Table 4.3 shows the results of the Mann-Whitney U Test for comparing the 25% and 50% Cocos nucifera starch gel concentrations regarding their effectiveness in separation and visible hemolysis. Based on the Mann-Whitney U Test results, the p-values for separation (p = 0.005) and visiblehemolysis (p = 0.007) are lower than the 0.05 significance level. This indicates that the difference between the two concentrations is statistically significant. The mean ranks indicate that the 50% concentration has higher values for separation (41.72 vs. 31.28) and visible hemolysis (41.71 vs. 31.29) than the 25% concentration. These findings indicate that the 50% starch gel concentration is significantly more effective in red blood cell separation and preventing visible hemolysis than the 25% concentration.

The table above shows the statistical comparison of starch gel concentrations at 25% and 75% by the Mann-Whitney U Test in the same manner as the comparison between 25% and 50%. The results indicate a statistically significant difference between the two concentrations since p-values at separation were 0.000 and at visible hemolysis were 0.015, indicating that the differences obtained were unlikely to be due to random chance.
Besides, mean ranks for 75% starch gel concentration are greater than those of the 25% concentration for both separation (51.39 vs. 21.61) and visible hemolysis (41.29 vs. 31.71). The findings show that a higher starch gel concentration of 75% enhances separation efficiency and reduces visible hemolysis than a lower concentration of 25%.

Table 4.5:- Mann-Whitney U Test Results of Comparison of 25% vs. 100% Cocos nucifera Starch Gel Concentrations.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Separation	25%	36	24.33	876.00
	100%	36	48.67	1752.00
	Total	72		
VisibleHemolysis	25%	36	35.04	1261.50
	100%	36	37.96	1366.50
	Total	72		

Test Statistics[a]

	Separation	VisibleHemolysis
Mann-Whitney U	210.000	595.500
Wilcoxon W	876.000	1261.500
Z	-5.655	-.682
Asymp. Sig. (2-tailed)	.000	.495

a. Grouping Variable: Group

The findings reveal a statistically significant difference in separation efficiency between the two concentrations (p = 0.000), while no significant difference was observed in visible hemolysis (p = 0.495).

The higher mean rank for separation in the 100% concentration (48.67) compared to the 25% concentration (24.33) suggests that a 100% starch gel concentration demonstrates superior separation performance. Conversely, the absence of a significant difference in visible hemolysis indicates that both concentrations exhibit similar outcomes in this parameter, rendering further comparison unnecessary.

Table 4.6:- Mann-Whitney U Test Results of Comparison of 50% vs. 75% Cocos nucifera Starch Gel Concentrations.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Separation	50%	36	25.67	924.00
	75%	36	47.33	1704.00
	Total	72		
VisibleHemolysis	50%	36	36.99	1331.50
	75%	36	36.01	1296.50
	Total	72		

Test Statistics[a]

	Separation	VisibleHemolysis
Mann-Whitney U	258.000	630.500
Wilcoxon W	924.000	1296.500
Z	-4.974	-.316
Asymp. Sig. (2-tailed)	.000	.752

a. Grouping Variable: Group

The table illustrates the statistical comparison between 50% and 75% starch gel concentrations. The computed p-values for separation and visible hemolysis are 0.000 and 0.752, respectively, indicating a significant difference in separation between the two concentrations. In contrast, no significant difference is observed in visible hemolysis.
Given that separation exhibits a significant difference, the analysis focuses on this parameter. The mean rank for separation is higher in the 75% concentration (47.33) than in the 50% concentration (25.67), suggesting that 75% starch gel concentration is significantly more effective in separation. However, as there is no significant difference in visible hemolysis between the two concentrations, they yield comparable results, making further comparisons unnecessary for this parameter.

Table 4.7:- Mann-Whitney U Test Results of Comparison of 50% vs. 100% Cocos nucifera Starch Gel Concentrations.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Separation	50%	36	28.88	1039.50
	100%	36	44.13	1588.50
	Total	72		
VisibleHemolysis	50%	36	40.53	1459.00
	100%	36	32.47	1169.00
	Total	72		

Test Statistics[a]

	Separation	VisibleHemolysis
Mann-Whitney U	373.500	503.000
Wilcoxon W	1039.500	1169.000
Z	-3.554	-2.162
Asymp. Sig. (2-tailed)	.000	.031

a. Grouping Variable: Group

The table above shows the statistical comparison between 50% and 100% starch gel concentrations. The computed p-values for separation and visible hemolysis are 0.000 and 0.031, respectively, indicating a significant difference between the two concentrations in both parameters.

For separation, the mean rank is higher in the 100% concentration (44.13) than in the 50% concentration (28.88), suggesting that 100% starch gel concentration is more effective in achieving separation. However, in visible hemolysis, the 50% concentration (40.53) has a higher mean rank than the 100% concentration (32.47), indicating that 50% starch gel concentration results in more significant visible hemolysis.

These findings suggest that while 100% concentration is superior for separation, 50% concentration leads to increased visible hemolysis, highlighting a trade-off between the two parameters.

Table 4.8:- Mann-Whitney U Test Results of Comparison of 75% vs. 100% Cocos nucifera Starch Gel Concentrations.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Separation	75%	36	40.00	1440.00
	100%	36	33.00	1188.00
	Total	72		
VisibleHemolysis	75%	36	40.07	1442.50
	100%	36	32.93	1185.50
	Total	72		

Test Statistics[a]

	Separation	VisibleHemolysis
Mann-Whitney U	522.000	519.500
Wilcoxon W	1188.000	1185.500
Z	-1.757	-1.881
Asymp. Sig. (2-tailed)	.079	.060

a. Grouping Variable: Group

Table 4.8 demonstrates the statistical comparison between 75% and 100% starch gel concentrations. The computed p-values for separation and visible hemolysis are 0.079 and 0.060, respectively. Since both values exceed 0.05, the results indicate no statistically significant difference between the two concentrations.

Given the absence of a significant difference, it can be inferred that 75% and 100% starch gel concentrations yield comparable results in separation and visible hemolysis. Consequently, further comparative analysis between these two concentrations is unnecessary.

Table 4.9:- Descriptive Statistics of Fasting Blood Sugar (FBS) and Alanine Aminotransferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT) Across Different Concentrations of Cocos nucifera Starch Gel.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
FBS (mmol/L)	0%	36	4.7622	.56433	.09405	4.5713	4.9532	4.05	6.00
	25%	36	3.8536	.55453	.09242	3.6660	4.0412	2.83	5.66
	50%	36	3.8900	.50478	.08413	3.7192	4.0608	3.16	5.61
	75%	36	3.7767	.49351	.08225	3.6097	3.9436	3.00	5.22
	100%	36	4.0500	.76041	.12673	3.7927	4.3073	2.89	6.05
	Total	180	4.0665	.68013	.05069	3.9665	4.1665	2.83	6.05
SGPTUperL	0%	36	33.6944	23.47256	3.91209	25.7525	41.6364	3.00	109.00
	25%	36	30.6389	20.22467	3.37078	23.7958	37.4819	15.00	109.00
	50%	36	29.3889	16.55342	2.75890	23.7880	34.9898	15.00	77.00
	75%	36	28.9494	15.50482	2.58414	23.7034	34.1955	15.00	70.00
	100%	36	28.6389	17.47349	2.91225	22.7267	34.5511	15.00	80.00
	Total	180	30.2621	18.74622	1.39726	27.5049	33.0193	3.00	109.00

The Descriptive Statistics table presents the distribution of FBS and SGPT levels across the different starch gel concentrations. The table includes the mean, standard deviation, standard error, and confidence intervals to provide an overview of the data variability.

For FBS, the highest mean value was observed at 0% concentration (4.7622 mmol/L), which serves as the control group, while the lowest mean value was recorded at 100% concentration (4.0655 mmol/L). The data indicates a gradual decrease in FBS levels as the starch gel concentration increases, suggesting that higher Cocos nucifera starch gel concentrations might impact FBS levels. With probable group variations, the confidence intervals for all the concentrations reveal minor overlap.

The mean value of ALT/SGPT was determined to be greatest at 0% (33.6944 U/L); the least mean value was observed at 100% (28.6389 U/L). However, large standard deviations and overlapping confidence intervals point to more variability in ALT/SGPT tests and imply that variations in starch gel concentration have little to no impact on ALT/SGPT levels.

Table 4.10:- Fasting Blood Sugar (FBS) and Alanine Aminotransferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT) ANOVA One-Way Analysis for Various Concentrations of Cocos nucifera Starch Gel

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FBSmmolperL	Between Groups	23.212	4	5.803	17.042	.000
	Within Groups	59.589	175	.341		
	Total	82.801	179			
SGPTUperL	Between Groups	613.560	4	153.390	.431	.786
	Within Groups	62290.786	175	355.947		
	Total	62904.346	179			

The one-way ANOVA table shows the mean values of FBS and SGPT at several starch gel concentrations. In the last column, the p-value (Sig.) indicates whether the observed variations are statistically significant.

The FBS parameter gave a p-value of 0.000, less than the significance level of 0.05. This indicates a significant difference in FBS levels between the different concentrations of starch gel. The high F-value of 17.042 also supports the claim that the different concentrations of starch gel significantly affect FBS levels.
Conversely, the ALT/SGPT parameter gave a p-value of 0.786, above the 0.05 mark. This indicates no statistically significant variation in the levels of ALT/SGPT between the groups. The minimal value of F, which is 0.431, indicates that any observable variation in the levels of ALT/SGPT is most likely due to random chance rather than the effect of the starch gel.

Post-Hoc Tukey's Test

Table 4.11:- Post-Hoc Tukey's Test Results for FBS Levels for Different Concentrations of Cocos nucifera Starch Gel (Homogeneous Subset Table).

FBSmmolperL

Tukey HSD[a]

Group	N	Subset for alpha = 0.05	
		1	2
75%	36	3.7767	
25%	36	3.8536	
50%	36	3.8900	
100%	36	4.0500	
0%	36		4.7622
Sig.		.276	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 36.000.

The table presents the outcome of applying Tukey's Honest Significant Difference (HSD) test, which compares variances between Fasting Blood Sugar (FBS) values at various Cocos nucifera starch gel concentrations. The homogeneous subsets presented indicate that the means of FBS at the 25%, 50%, 75%, and 100% concentrations of Cocos nucifera starch gel are statistically equivalent ($p > 0.05$), in contrast to the mean FBS recorded at a 0% concentration of starch gel, which is significantly different from the other concentrations ($p < 0.05$). This proves that Cocos nucifera starch gel significantly affects FBS levels, where the control group (0%) is significantly different from all other concentrations.

The table shows the result of the HSD test between levels of Alanine Aminotransferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT) compared to different concentrations of Cocos nucifera starch gel. All concentrations, including the control (0%) concentration, fall within the same homogeneous subgroup, as determined by the test, which displays no significant differences ($p = 0.787$). This implies that Cocos nucifera starch gel is a viable substitute medium for measurement since it has minimal to no effect on ALT/SGPT levels.

Table 4.12:- Post-Hoc Tukey's Test for ALT/SGPT Levels at Various Concentrations of Cocos nucifera Starch Gel (Homogeneous Subset Table).

SGPTUperL

Tukey HSD[a]

Group	N	Subset for alpha = 0.05
		1
100%	36	28.6389
75%	36	28.9494
50%	36	29.3889
25%	36	30.6389
0%	36	33.6944
Sig.		.787

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 36.000.

Conclusions:-

The present study emphasizes the potential of Cocos nucifera starch gel to induce serum separation, so the gel concentration strengthens its effectiveness in this context. Out of all the concentrations investigated, the 75% starch gel produced the most desired result, showing good red blood cell separation without causing significant hemolysis. The findings show that higher starch gel concentrations improve serum separation, offering a feasible substitute for conventional synthetic separators.

Notably, the concentration of starch gel employed affected the very varied FBS findings. Significantly different FBS levels in the control group—in which no starch gel was used—indicated that the presence of the gel affects glucose measurement outcomes in the experimental groups. This fluctuation suggests the possible impact of Cocos nucifera starch gel on specific biochemical tests, so additional research on its interactions with serum components is rather important.

On the contrary, the ALT results corresponded to every concentration of starch gel. This stability of ALT readings implies that Cocos nucifera starch gel has no impact on the enzyme measurement, so it is a suitable choice for tests requiring precise enzyme activity measurements.

The study indicates that although the starch gel of Cocos nucifera may separate serum, it is not entirely ideal as a substitute for commercially offered serum separator tubes. Although the gel is promising, optimization is necessary to enhance its stability, uniformity, and overall performance in the laboratory. The study reveals that even though the starch gel is a natural and potentially renewable alternative, formulation, and processing optimization is necessary before it can be suggested as a replacement for synthetic separators in clinical practice.

Recommendations:-

The researchers recognized the potential of Cocos nucifera starch gel as a viable serum separator; however, they acknowledge that additional modifications are needed to increase its efficiency and reliability. Concerning the study findings and the challenges realized, the following recommendations are given to enhance future studies in this field further:

1.Optimization of the starch gel composition. The researchers contend that further improvement of Cocos nucifera starch gel will help to enhance its consistency, separation efficiency, and practicality. Among the treatments that could minimize challenges like clot formation, hemolysis, and rapid degradation involve modifications in concentration, methods of preparation, and stabilization addition. Furthermore, NaOH causes red-brown coloring of the starch gel that interferes with serum separation visibility. Future research should focus on improving clarity, such as modifying pH levels or purifying techniques. Although the current innovation showed promise, modifying these parameters will culminate in a more constant, effective, and visually stimulating serum separator.

2.Expansion of testing to other biochemical parameters. Future studies should include a broader spectrum of biochemical testing, including lipid profiles, renal function tests, and enzyme markers, regardless of the majority of the study centered around ALT and FBS. Expanding the range of tests allows researchers to more accurately evaluate the adaptability and possible uses of Cocos nucifera starch gel in clinical laboratory settings. Furthermore, studying its impact on serum stability over time might provide important information on long-term use.

3.Environmental and economic feasibility assessment. Cocos nucifera starch gel has environmental and economic benefits as a natural substitute for synthetic separators. Nevertheless, its commercial sustainability, financial efficiency, and long-term storage are deemed for further study. A critical evaluation of these elements will ascertain their usefulness and viability for laboratory application.

4.Further investigation of other natural separators. Although the Cocos nucifera starch gel worked, albeit with minimal application, other organic substances could have functioned as serum separators. Future research on alternative biodegradable or plant-based products, including other starch sources, polysaccharides, or naturally occurring gelling agents, may be beneficial to ascertain their relative qualities for serum separation.

5.Large sample size and an extended study duration. One of the challenges this study experienced was the rapid perishable nature of coconut starch gel, which limited the testing duration. Future research should include a larger sample size and extended testing to obtain more statistically significant results. Improving storage methods or incorporating stabilizing agents may also prolong the gel's usability, reducing time constraints and ensuring more reliable findings.

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