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CONSEQUENTIAL IMPLICATIONS OF PROCESS FACTORS ON TANNIN YIELD FROM MANGROVE BARK

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KeyWords
Mangrove bark, Tannin, methanol, solvent extraction, particle size, solvent volume, extraction time and FTIR

ABSTRACT

This study is aimed at exploring the tannin content in Bonny Island mangrove bark, The effect of particle size, solvent volume and extraction time on the extraction of tannin from the mangrove bark was investigated using solvent extraction method.50% concentration of methanol was used and the time varies at 5, 10, 15, 20, 25 and 30 mins with particle sizes of 6, 8, 10, 12 BSS and solvent volume of 25,50,75 and 100mls. The extracted tannin was further characterized using Fourier Transform Infrared (FTIR) spectrometer and the functional group were reviewed as O-H stretch, C=O stretch, N-H bend and C-F stretch. However, the variation in the process factor affected the tannin yield in the extract. Smaller particle size produced higher yield due to the greater surface area for mass transfer exhibited when the particle is finer. Tannin yield increased significantly as the solvent volume and time keeps increasing due to the solvent having greater chance penetrating all the active sites of the sample and the cell structure are destroyed with time. High amount of solvent (100mls) gave better yield of tannin at the longest extraction time of 30 mins producing high percentage of tannin (9.26%) from particle size of 10 BSS and mass of 10grams mangrove bark. The generated model showed the Lack of Fit was quadratic. Using ANOVA, the P-value from the model is less than 0.0001 which indicates the model term are significant. This study proofs tannin presence of tannin in mangrove bark. Particle size, time and solvent volume plays a vital role in tannin extraction.

INTRODUCTION

Tannins are among the substance s of polyphenol group, including lignans, flavonoids, etc (Tuuti, 2019). Tannins exhibit astringent property causing a dry and pucker feeling in the mouth following the consumption of unripe fruits, red wine or tea with a molar weight ranging from 500 to over 3000g/moles. Tannin compound is usually found in gall, roots, bark or in the leave of the mangrove species (Romani et al,2012; Banerjee et al,2008). Tannin is often present in a variety of plant- based food such as fruit examples are cranberries, blueberries], nuts [walnut], spices [vanilla, cinnamon and beverages such as coffee, Lipton tea, wine. Antioxidant and anti- inflammatory content of tannin provides multiple health benefits. Tannin also plays important role in protecting plants from harmful organisms and help to maintain the growth of plant. Tannins are classified into two types: condensed tannin and hydrolyzed tannin. Condensed tannin is the most considered in tannin industry since it does not contain much residue sugar and exhibiting stronger resistance against microbial degradation leading to more value-added ability in bioactivities, such as antibacterial, antiviral, and antifungal properties.

Mangrove are coastal forest that grow where ocean, freshwater and land meet. They are among the most productive and complex ecosystem on the planet. Mangrove bark are thick and broad plates of thin scales of the mangrove tree which are gray to brown in color. Rhizophora Mangle, Rhizophora stylosa, Rhizophoral apiculata and Rhizophoral Mucronata are commonly found mangrove species (Duke et al, 2002; Sauki et al, 2018). The plant extract contains phytochemicals that are of significant value in pharmaceuticals, for wood industry adhesive (Pizzi,2006), insulating foams (Tondi et al,2009), mineral industry, wine production industry, animal nutrition, oil industry (Pizzi, 2019) water treatment plants (Combs,2016) and metal protection from corrosion (Ostovari et al,2009).

The implementation of the agricultural and forest industries related products for production of renewable energy and materials

has been a topic of considerable interest for many years both in the western and developing countries. This becomes convenient owing to the need to enhance raw material of agricultural and pharmaceutical industries contributing to the general shift away from the use of synthetic chemicals in food processing and their likes. Mangrove plants are good source of tannin, saponin, alkaloids, and flavonoid (Sharaf et al., 2002). Mangrove evaluated phytochemically, will be of a great value to the discovery of new natural chemotherapeutic that will be a sure one way to improve mangrove resources. The medicinal potential of mangrove can also provide other raw material such as tannin for leather industries, food industries etc. (Bandaranayake, 2002).

Tannins can be applied in food preservation, materials packaging, and as well as in food enhancement because of their protective nature. They are secondary metabolites found in large quantities in plant-based food products.

Tannins is an effective agent and resistant against methicillin-resistant Staphylococcus aureus (MRSA) and also highly effective antimicrobial ability towards Staphylococcus aureu which causes contamination in food industry. (Hintz and Mathew, 2015), Another potential antiviral agent used to manufacture food products, keep them safer and preserve them to last for a very long time with the aid of natural compounds is hydrolysable tannin.

The utilization of numerous natural substances, such as tannins, which are polyphenolic base secondary metabolites that can be expressed in microorganisms by metabolic engineering and can be obtained from fruits, vegetables, or plants. Numerous studies have conclusively demonstrated the role of tannins as a natural antioxidant in the prevention of degenerative diseases such atherosclerosis, cardiovascular disease, neurogenerative disease, and some types of cancer by functioning as an antioxidant and antibacterial agent. Because of the great level of environmental and health hazards resulting from the application of synthetic materials in the market to wrap the packing materials which are plastic, polyethylene, and low-density polyethylene (LDPE) because of their lightness, inertness, and easy availability. Hence leading to the concept of natural and active packaging materials from biological sources like, chitosan, starch, gelatin, tannins and methycellulose (Missio et al. 2018).

Tannins are applied as membrane pump inhibitors to fight the ATCC 43300 and MRSA clinical strains, and the way in which the method of operation was observed using Next Generation sequencing (NGS) to have rooted understanding of the antibacterial structures at the level of the genome, transcriptome, and protein synthesis. This investigation observed that tannins primarily interfere with protein synthesis methods by causing significant alterations in ribosome pathways. These changes affect translation processes in MRSA cells, which ultimately results to a decrease in bacterial growth. Therefore, tannins have the ability to be utilized as weapons against anti-MRSA medicines in clinical settings, especially for antibacterial cream and antiseptic body treatments. Tannins' antibacterial potential is investigated in both plants and animals, in addition to animals. Numerous bacteria destroy various fruit, vegetable, and plant species on a widespread scale, resulting in significant monetary loss.

Tannins can equally be used to transform animal hide into leather in the leather company. In the leather industry, tannins are primarily utilized to preserve leather from microbe and heat related deterioration. Tannins have the capacity to unite with skin proteins, protecting petrification resulting to rise in their antibacterial properties. Collagen, the major protein that makes up skin, and the tannins found in the vegetable components have formed a chemical bond that gives them these antibacterial properties. Tannins obtained from various plant components can be used in the manufacture of leather. Tannins from plant offer many usefulness, including the production of high-quality, thermally stable leather goods.

Due to the presence of tannin in mangrove bark, thus this study focused on the extraction of tannin from the plant bark using solvent extraction which is one of the effective ways of extracting phenolic compounds.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

The plant used in this experiment was obtained from Bonny Island Port Harcourt Rivers State, Nigeria. The mangrove bark sample was washed with distilled water to remove impurities, sun dried for somedays to a constant weight and then ground into powder using a mechanical grinder and sieved with analytical sieve shaker.

2.2 Characterization of the Mangrove Bark Sample

2.2.1 Test for Alkaloid

Using wegners reagent.

The wegners reagents was prepared by dissolving 1.3g of iodine crystal and 2g of potassium iodide in water in a 100ml volumetric flask and made up to 100ml mark. 1ml of Wegner's reagent was added separately to 1ml of each of the extract. The solution was monitored and observation taken.

2.2.2 Test for Saponin (Emulsion Test)

3 drops of olive oil were added to 3mls of each extract in 3 different test tube and shaken. The resultant solution was monitored and observation taken.

2.2.3 Test for Quinone

Using 0.1M acidified (HCL) KI i.e., potassium iodine solution acidified with HCL. 1ml of the extract was added in a test tube with 0.1m acidified (HCL) KI. It was observed and the result noted.

2.2.4 Test for Glycoside

1ml of the extract was mixed with 1ml of 2% solution of 3,5 dinitrosalicilin acid (3,5 DNS) in methanol and 1ml of 5% aqueous

NaOH. The solution was also monitored and observation taken.

2.2.5 Test for Tannin

5g of 5% Ferric chloride solution was dissolved and made up to 100ml with distilled water. 0.5ml of 5% Ferric chloride was separately added to 1ml each of the extract in two different test tube. The resultant solution was also monitored and observed.

2.2.6 Test of Phenol

This was done by adding 1ml of the extract to 1ml of water and 5 drops of 5% NaOH. It was monitored and observation noted.

2.2.7 Test for Flavonoid

2 drops of 10% NaOH solution was added separately to 1ml of each of the extract in two different test tube. Subsequently, 2 drops of $AlCl_3$ were added to the solution followed by addition of concentrated H_2SO_4. A colour changes was observed.

2.2.8 Test for Steroids

(Lieberman – Richard test)

1ml acetic anhydride was mixed with 2ml of concentrated H_2S0_4 and were separately added to 1ml of each extract in different test tube. The solution was observed and noted.

2.3 Solvent Extraction OF TANNIN

Phytochemical screening of the mangrove bark was done to know the bioactive constituents of the plant part. Solvent extraction of the bark sample was performed and the process factors observed were particle size, solvent volume and extraction time. Percentage yield of the extract was calculated and the data analyzed using the Design Expert Program.

100ml of 50% methanol was introduced into a beaker containing 10g of the mangrove bark sample. The beaker was placed in a water bath and shakes continuously with mechanical shaker and extracted for various extraction time at varying particle size and solvent volume at ambient temperature. The precipitated solution was filtered in a glass filter, dried in an oven to gather the solid material. The weight different was calculated and the percentage yield of the extract from the solvent was measured.

Tannin extract was measured in weight% by distilling off the methanol from the extract.

$$\% \text{ yield} = \frac{weight\ of\ total\ dried\ extract}{weight\ of\ oven\ dry\ sample} \text{ x } 100 \qquad (1)$$

2.3 Effect of Process Variables

2.3.1 Effect of particle size

While other variables such as time and solvent volume were held constant at 1hr, 1g:100ml solute /solvent ratio, at ambient temperature with varying particle sizes of 6BSS, 8BSS,10BSS, and 12BSS respectively. The effect of particle size was examined. The quantity of tannin extracted was calculated using the method in equation 1.

2.3.2 Effect of time

At various time intervals of 5, 10, 15, 20, 25,30 mins, the effect of time on tannin yield was examined. Other process parameters were constant at 1g:100ml solute/solvent ratio, 8 BSS (2830microns) and at ambient temperature. The amount of tannin extracted was calculated using the method in equation 1.

2.3.3 Effect of solvent volume

The effect of solvent volume was examined at varied ratios of 25ml, 50ml, 75ml, 100ml. the conditions maintained at ambient temperature, 1hr and 8BSS. The amount of tannin extracted was calculated at the conclusion of the extraction. was calculated using the method in equation 1.

2.4 Characterization of The Extracted Tannin

The tannin from the mangrove bark was characterized using the colour standard chart. The developed colour was noted using Axis Gear Standard Colour Chart as reference point. Tannin in the extract was determined using drops of Iron (III) chloride and the subsequence change in colour indicates the presence of tannin

2.4.1 Using Fourier Transform Infrared (FTIR) Spectrometer.

The chemical composition of the extracted tannin was confirmed using Fourier transform infrared (FTIR) spectroscopy and the functional groups studied. The extracted tannin was placed directly on the diamond crystal or light path, the reflectance spectra were collected. The FTIR were recorded at the wave number range of 4000 to 650cm^{-1}. The spectrum was interpreted to determine the functional group.

2.5 Optimization

To maximize the process variable for tannin extraction from mangrove, bark, Box-Behnken experimental design was used. Optimization techniques were used for solvent extraction processes. Design expert trial version was employed to investigate the best combination of extraction variables for the extraction of tannin from mangrove bark. Extraction time, particles size, solvent volume were the independent variables selected to be optimized for the extraction. The range of independent variables and their level with three dimensional factors separating the processes, gave a total of twenty experimental runs for the extraction.

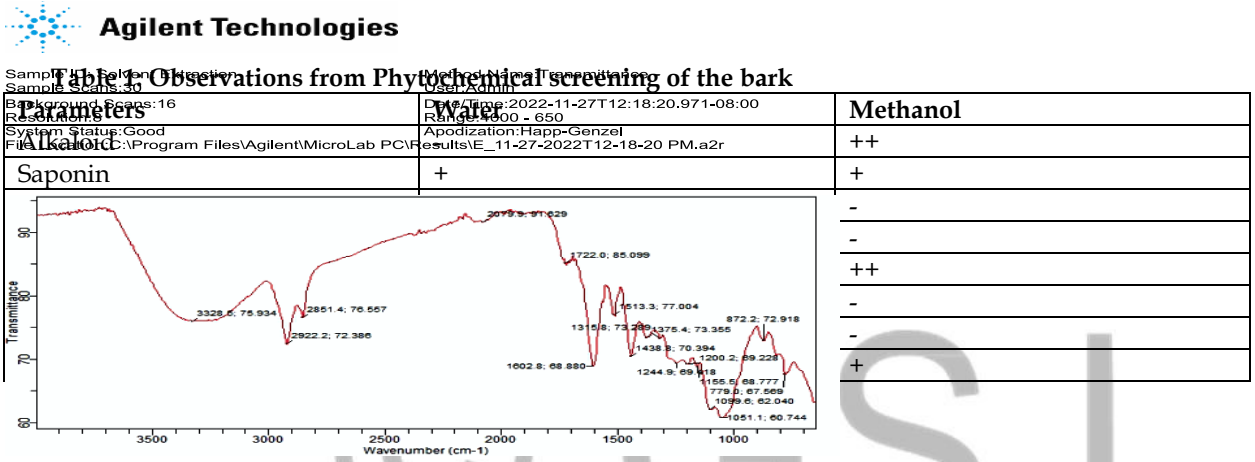
3.0: RESULTS AND DISCUSSION

3.1 Phytochemical Results

The phytochemical screening revealed strong presence of some of bioactive compounds in mangrove bark.
Alkaloid was observed to be highly present in the extract with methanol with light reddish precipitate but negative when extracted with water.
Saponin was observed with a slightly stable emulsion present when shaken for both water and methanol as extracting solvent.
Steroid was slightly positive, with a slight dirty brown coloration when methanol was used as extracting solvent but negative with water.
Quinone was completely negative for both methanol and water as extracting solvent.
Glycoside, no indication of glycosides presence for both methanol and water as extracting solvent.
Tannin was observed to be strongly present in the extraction with both methanol and water with a greenish coloration which is an indication of highly presence of tannin in the mangrove bark sample.
Phenol, no indication of phenol presence for both methanol and water as extracting solvent.
Flavonoid, no indication of flavonoid presence for both methanol and water as extracting solvent.
Steroid was slightly positive, with a slight dirty brown coloration when methanol was used as extracting solvent but negative with water.

Table 1: Observations from Phytochemical screening of the bark

Parameters	Water	Methanol
Alkaloid	++	++
Saponin	+	+
Steroid	+	-
Glycoside	-	-
Tannin	++	++
Phenol	-	-
Flavonoid	-	-
Steroid	+	+



3.2 Analysis of Extracted Tannin using FTIR Spectroscopy

Extracted tannin was used for FTIR analysis to determine the functional group. The FTIR spectra were recorded at the wave number range of 4000-650 CM^{-1}. The spectrum was interpreted to determine the functional group of the extractive and the matching functional groups were found in the tannin extraction by IR spectra showing OH stretch from phenolic and aliphatic structure (hydroxyl group), c = 0 stretch from carbonyl group c-H bend from aromatic, CH3C-H bend from aromatic methoxyl groups, N-H bend from Amine group.
Tannin showed bands absorption at peaks 3328.5, 2922.2, 2851.4, 2079.9, 1438.8, 1722.0, 1602.8, 1513.3, 1375.4, 1315.8, 1244.9, 1200.2, 1155.5, 1099.6, 1051.1, 1,779.0 all in CM^{-1}.

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Figure 3.1 Fourier Transform Infrared plot for Solvent Extraction of Tannin

3.3 Percentage Yield of Tannin

3.3.1 Effect of Particle Size

The result on particle size from Figure 3.2, below, showed that the highest yield of tannin for solvent extraction is at sieve size 10BSS (12.9%) which is an indication that lesser particle size has great surface area for mass transfer (Romer et al, 2020) but as the particle size began to reduced, tannin yield began to decline due to the solvent having hard time passing through the sample because of its compactness leading to low extraction efficiency. However, the very small particles are more prone to float in the solvent of extraction and therefore, the interaction with the solvent was limited Paira C. L. et al, (2015).

Figure 3.2: Effect of particle size on tannin yield.

3.3.3 Effect of solvent volume

The result on the solvent volume as shown in Figure 3.3 below, is an indication that the highest percentage of tannin yield of 9.14% was observed for solvent with the biggest solvent volume of 100ml. This shows that continuous mixing of the solvent with the particles helps the extraction process and a good mixing is possible when there is higher solvent to solute ratio. The yield of tannin increased significantly as the volume of solvent keep increasing which shows that increase in solvent, leads to greater chance of solvent penetration to all the active sites of the sample leading to greater yield of tannin. Solvent volume is another important factor to consider in tannin extraction processes (Guo et al., 2020).

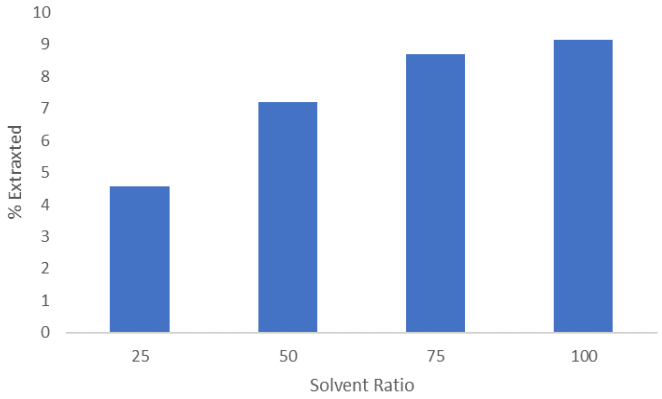

Figure 3.3: Effect of solvent volume on tannin yield

3.3.4 Effect of extraction time

Fig. 3.4 below, shows that a greater extraction time generally led to a higher percentage yield of tannin which could be as a result of the longer period of time the solute and solvent were in contact with each other.

However, the extraction effectiveness and the concentration of extracted tannin depends on how standard the extraction method is (Hussain et al 2020), Luo et al, 2019). Extraction time of 30mins gave the highest percentage tannin yield of 9.20%. While 5mins gave the lowest yield of tannin of 6.65%. This is as a result of the cell structure of the plant part being destroyed with time in the solvent (Petchidurai et al., 2019).

Figure 3.4: Effect of extraction time on tannin yield

3.4 Analysis using Response surface methodology (RSM)

Response surface methodology result of the optimization process is presented in Table 3.2. It shows the interactive effects of process factors (solvent volume, time, particle size and extraction method) on the tannin yield. The relationship between the response (tannin yield) and the process factors of solvent volume, time, particle size is expressed in inform of mathematical model and graphs of the subsequent subsections. Acceptability of the mathematical model largely depends on the outcome of the analysis of variance (ANOVA).

Table 3.2: Experimental Result for RSM

	Run	Solvent vol (mls)	Time (min)	Particle size (BSS)	Tannin Yield (%)
16	1	75	25	10	7.3679
6	2	100	15	12	7.6378
15	3	75	25	10	7.3632
14	6	75	25	14	7.0817
7	8	50	35	12	7.0592
1	9	50	15	8	5.7454
19	11	75	25	10	7.0552
8	13	100	35	12	7.56
3	14	50	35	8	5.8854
17	17	75	25	10	7.2792
20	19	75	25	10	7.3601
13	22	75	25	6	5.6739
12	23	75	45	10	7.1828
18	24	75	25	10	7.287
11	25	75	5	10	6.979
10	26	125	25	10	7.2224
5	30	50	15	12	6.9074
2	31	100	15	8	6.5559
9	32	25	25	10	5.7804
4	36	100	35	8	6.9557

Table 3.3, represents the ANOVA for the model of the tannin yield. The Model F-value of 332.36 implied the model was significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, D, AC, AD, CD, A^2, B^2, C^2 were significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 1.44 implied the Lack of Fit is not significant relative to the pure error. There was a 28.02% chance that a Lack of Fit F-value this large could occur due to noise. The predicted R^2 of 0.9810 was in reasonable agreement with the adjusted R^2 of 0.9884; i.e. the difference is less than 0.2. Adequate precision measured the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 59.875 indicated an adequate signal. This model can be used to navigate the design space.

Table 3.3: ANOVA for the model of the tannin yield

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	88.86	10	8.89	332.36	< 0.0001	Significant
A-Solvent vol	6.35	1	6.35	237.51	< 0.0001	
B-Time	0.1843	1	0.1843	6.89	0.0137	
C-Particle size	8.85	1	8.85	330.96	< 0.0001	
D-Method of Extraction	67.06	1	67.06	2508.30	< 0.0001	
AC	0.1490	1	0.1490	5.57	0.0252	
AD	0.1601	1	0.1601	5.99	0.0207	
CD	0.3106	1	0.3106	11.61	0.0019	
A^2	2.75	1	2.75	102.86	< 0.0001	
B^2	0.1912	1	0.1912	7.15	0.0122	
C^2	4.18	1	4.18	156.50	< 0.0001	

Residual	0.7754	29	0.0267			
Lack of Fit	0.5683	19	0.0299	1.44	0.2802	not significant
Pure Error	0.2071	10	0.0207			
Cor Total	89.64	39				
Std. Dev.	0.1635		R^2			0.9913
Mean	8.19		Adjusted R^2			0.9884
C.V. %	2.00		Predicted R^2			0.9810
			Adeq Precision			59.8755

3.5 Mathematical Models.
Mathematical Equation (in terms of coded factors). It is a quadratic model because the highest power of the variable is two. It also shows the interactive effects of solvent volume, time, particle size and extraction method on the tannin yield. The corresponding models for the extraction process is presented in equation 1. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Where A is solvent volume, B is Time, C is particle size, and D is extraction method.

For the solvent extraction method:
Tannin yield = $-7.23594+0.090420A+0.038417B+1.80076-0.001930AC -0.000374A^2-0.000617C^2$ (1)

3.6 3-D Graph for Interaction Effect

Figures 3.5 present the 3D plots of the interaction effect of particle size and solvent volume on the tannin yield. The yield increased with increase in both particle size and solvent volume till it got to the maximum point, where slight decline in the tannin yield was noticed. It reviewed that the relationship between the response and the considered factors is in parabolic in nature, which subsequently, is a reflection of quadratic model.

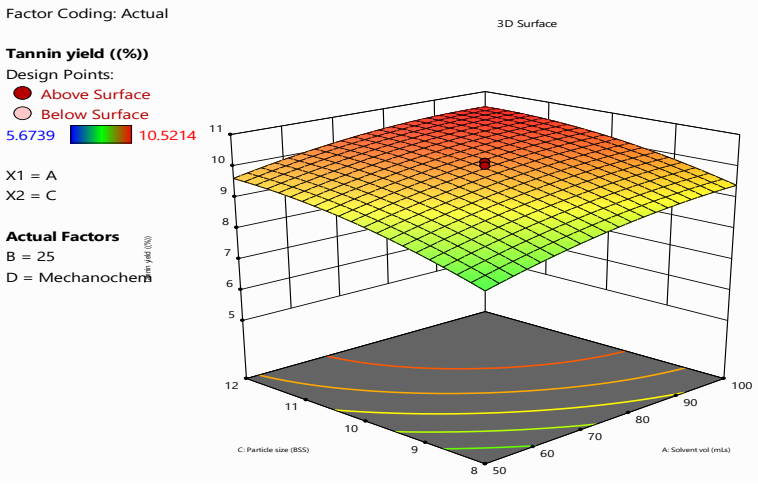

Figure 3.5 The 3D plot of particle size and solvent volume against percentage yield

4. CONCLUSION
The study is a proof that mangrove bark is a good source of tannin and the mangrove bark contains more tannin than its other parts do. Particle size, time and solvent volume plays a vital role in tannin extraction.

Reduced particle sizes, increase the surface area available for mass transfer, and subsequently the tannin yield although finer particle sizes are more likely to agglomerate, which could impede the extraction process. In order to optimize the surface area for mass transfer while avoiding agglomeration, the best particle size for extraction should be maximized.

The effects of the parameters examined in this study—extraction time and solvent volume on the yield of tannin in the extract were confirmed. A statistical investigation revealed that the independent variable interacted in a substantial manner to give increased tannin yield.

The outcome of this investigation supports earlier findings that, in various solvents, the extraction yield increases as the extraction time increases. A longer extraction period would result in improved mass transfer and equilibrium between the mangrove bark and solvent. The best circumstances produced a yield of 9.26% with an optimal solvent concentration of 91.8% and an extraction period of 31.1mins

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