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Optimization of Extraction Parameters for Natural Dye from *Pterocarpus santalinus* by using Response Surface Methodology

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Abstract: -
Pterocarpus species has been admired for centuries for its dye, beautiful color, hardness and durability. The present study deals with the extraction of natural dye from *Pterocarpu*s wood materials. Response surface methodology was used to study the optimal conditions for the extraction of dye. Factors such as extraction temperature, extraction time, and solid to liquid ratio were identified to be significantly affecting natural dye extraction efficiency. By using three-level three-factor Box-Behnken design, the optimized conditions for dye extraction by different techniques such as Solvent, Ultrasonic and Microwave extraction method. Microwave assisted extraction method showed the highest natural dye yield percentage which is 50.0 for ethyl acetate solvent and 50.2 for methanol solvent.

Keywords: *Pterocarpus* species, Natural dye, Response surface methodology.

I. INTRODUCTION:

A natural dye is a substance derived from natural sources used to add a color to or change the color of something and considered as sustainable and eco-friendly [1-4]. *Pterocarpus* species wood is renowned for its characteristic timber of exquisite color, beauty and superlative technical qualities yielding a natural dye santalin belong to the molecular class of condensed bioflavonoid. Extraction of bioactive compounds is influenced by various process parameters such as solvent composition, pH, temperature, extraction time and solid to liquid ratio [5, 6].

Solvent extraction is a common form of chemical extraction using organic solvents e.g. hexane, ethyl acetate, ether, chloroform, benzene, ethanol, methanol etc. It is commonly used in combination with other technologies such as solidification/stabilization, precipitation and electro winning. Another typical method called Ultrasonic-assisted extraction (UAE) is a process of high extraction yields of good quality in shorter periods of time using lower amounts of solvent than traditional processes. Among the new extraction techniques, UAE is the most economical and the one with less instrumental requirements. Different plant extracts and bioactive metabolites have been obtained with this technique. Microwave assisted extraction (MAE) is a relatively latest extraction techniques which utilizes microwave energy to heat the solvent and the sample to increase the mass transfer rate of the solutes from the sample matrix into the solvent. Microwave extraction offers better selectivity less extractant use and lower energy input efficiently. MAE offers a rapid delivery of energy to a total volume of solvent and solid target matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously.

Response surface methodology (RSM) used to provide superb statistical tools for design and analysis of experiments aimed at process optimization [7]. Design of Experiments (DOE) deals many RSM designs with options depend on the number of design variables or factors, which can range from one to ten offering Box-Behnken designs (BBD) for three to seven factors require only three levels, coded as −1, 0, and +1 creating designs with desirable statistical properties.

The objective of the present study is to provide an overview of existing research studies on extraction of natural dye from *Pterocarpus* spp. with aid of Response surface methodology.

II. MATERIALS AND METHODS

2.1 Materials:

Sawdust sample were produced from *Pterocarpus santalinus* wood collected from Chemistry of Forest Products and Wood Properties and Engineered Wood at Institute of Wood Science and Technology, Bangalore. The HPLC grade solvents (Ethyl acetate and Methanol) used for extracting metabolites were purchased from Merck Specialties Pvt. Ltd. and HiMediaLaboratories Pvt. Ltd. Mumbai. The instrument Buchi Rota Vapor used for evaporation.

2.2 Methods

2.2.1 Batch Solvent Extraction method (BSEM):

1g of the wood powder was mixed with various volumes of ethyl acetate solvent and methanol respectively to give a solid to liquid ratio ranging from 1:50 to 1:300(g/mL).The flask containing sample powder along with solvent was incubated in thermostatic water bath at various temperatures (30–60◦C) and various time intervals (15–300min). Observe the change in color within solvent and filtering it into filtrate and residue. Extract containing colored filtrate is subjected to evaporation in rotavapor at 60°C of water bath temperature. Metabolite extract is remained in the RB is weighed and transferred to ependoffs for the analysis.

2.2.2 Ultrasonic Assisted Extraction (UAE):

1g of the wood powder was mixed with various volumes of ethyl acetate and methanol solvent respectively to give a solid to liquid ratio ranging from 1:50 to 1:250(g/mL) and allowed for gentle mixing. The beaker was then placed into the ultrasonic bath aided with grill containing distilled water for extraction process. Parameter optimized was ultrasonic temperature for the range of 30-80^{o}C and extraction time for 5-30 minutes. All extracts were filtered and evaporated in rotavapour at 60°C of water bath temperature and is dried to get solid sample, the weight was measured and transferred to ependoffs for the analysis and interpretation procedures.

2.2.3 Microwave Assisted Extraction (MAE):

1g of sawdust samples were sieved and was mixed with various volumes of ethyl acetate and methanol solvent respectively to give a solid to liquid ratio ranging from 1:50 to 1:250 (g/mL) and allowed for gentle mixing in container and placed in micro treatment chamber (oven) with variable extraction time for range 5-30 mins at ranging temperature of 30-60°C respectively and microwave power of 800W was used for the extraction work. Further it is filtered, filtrate was evaporated in rotary evaporator at 60°C of water bath temperature and is dried to get solid sample, and the weight was measured and transferred to ependoffs for the analysis and interpretation procedures.

2.2.4. Statistical screening and optimization design of experiments:

A Box-Behnken model for three factors or variables was adopted in this study as the experimental design model using Design Expert software (version 6.0.8.Stat Ease Inc., Minneapolis, MN, USA). This method is preferred as design model since relatively few combinations of the variables are adequate to estimate potentially complex response function. In total 17 experiments are needed in each sample of individual solvent to calculate its 10 coefficients of the second order polynomial equation which was, fitted on the experimental data.

Percentage recovery of dye was taken as response of the system while the three process parameter i.e., temperature, extraction time and solid to liquid ratio were taken as input independent variables with respect to Solvent, Microwave and Ultrasonic extraction methods.

The system was stated by the following equation:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_{12}AB + b_{13}AC + b_{23}BC$$

Where b_0 is the intercept; b_1, b_2, and b_3 are linear coefficients; b_{11}, b_{22}, and b_{33} are squared coefficients; b_{12}, b_{13}, and b_{23} are interaction coefficients and the experimental variables are temperature (A), Extraction time (B) and Solid to liquid ratio (C).The model adequacies were checked in terms of the values of R^2 and Analysis of variance (ANOVA) was engaged to determine the significance of the models [9].

III. RESULTS AND DISCUSSION

3.1. Box-Behnken Analysis:

In this study, BBD was used for three process variables (extraction temperature, extraction time, and solid to liquid ratio) at three levels. The design points fall within a safe operational limit, within the nominal high and low levels, as BBD does not contain any points at the vertices of the cubic region. Design arrangements and responses of Experimental and Predicted values of Ethyl acetate (EtOAc) and Methanol (MeOH) solvents for Solvent Extraction method, Ultrasonic assisted extraction and Microwave assisted extraction method were generated are given in Table No.1.

3.2. Statistical Analysis:

Multiple regression analysis of the data yielded, the following equation for the recovery of natural dye using Ethyl acetate and Methanol recovery of Batch Solvent Extraction Method in terms of coded factors:

$$Y_1 = +25.72 - 0.46*A + 0.17*B + 2.01*C + 0.65*A^2 + 0.23*B^2 + 0.15*C^2 - 0.35*A*B - 0.22*A*C - 0.70*B*C$$

$$Y_2 = +34.46 - 0.76*A + 0.55*B + 2.41*C + 0.82*A^2 + 0.70*B^2 + 0.57*C^2 - 0.17*A*B - 0.95*A*C - 0.13*B*C$$

The following equation for the recovery of natural dye using Ethyl acetate and Methanol recovery of Ultrasonic extraction method in terms of coded factors:

$$Y_1 = +29.50+0.038*A+2.81*B+2.15*C-0.14*A^2-3.34*B^2+0.44*C^2-0.28*A*B-0.55*A*C-1.00*B*C$$

$$Y_2 = +39.52+0.038*A+2.08*B+1.51*C-0.22*A^2-2.40*B^2+0.78*C^2+0.15*A*B+0.33*A*C-1.50*B*C$$

The following equation for the recovery of natural dye using Ethyl acetate and Methanol recovery of Microwave extraction method in terms of coded factors:

$$Y_1 = +44.70+0.36*A+1.38*B+1.29*C+1.64*A^2-0.59*B^2+1.44*C^2+0.050*A*B-0.68*A*C-0.100*B*C$$

$$Y_2 = +49.70+0.73*A+1.35*B+1.07*C+0.012*A^2-1.69*B^2-0.29*C^2+0.075*A*B-0.63*A*C-0.025*B*C$$

Where Y_1 is Ethyl acetate response variable and Y_2 is Methanol response variable. The student t-distribution and the corresponding p-values along with the f-values of EtOAc and MeOH responses are listed in Table No.2 respectively.

In ANOVA for Response Surface Quadratic Model of Ethyl Acetate recovery of Batch Solvent Extraction method, the Model F-value of 4.36 implies the model is significant and there is only a 3.26% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case Solid to Liquid feed ratio (C) are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 1.58 implies the Lack of Fit is not significant relative to the pure error.

The values for the coefficient of determination R^2=0.8486 and Adjusted R^2 =0.6539 represents the proportion of variation in the yield or response in the model. A negative Pred R-Squared= -0.4215 implies that the overall mean is a better predictor of obtained response than the current model.

Whereas ANOVA for Response Surface Quadratic Model of Ethyl Acetate recovery of Ultrasonic extraction method, the Model F-value of 3.34 implies there is a 6.28% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case Extraction time, Solid to Liquid (Feed) ratio and Squared Extraction time coefficients are significant model terms. The values for the coefficient of determination R^2=0.5687 and Adjusted R^2 =0.8113 represents the proportion of variation in the yield or response in the model. A negative Pred R-Squared=-2.0188 implies that the overall mean is a better predictor of obtained response than the current model. In case of Microwave extraction method the Model F-value of 2.87 implies there is an 8.94% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case Extraction time and Solid to liquid (feed) ratio are significant model terms. The values for the coefficient of determination R^2= 0.7866 and Adjusted R^2 = 0.5122 represents the proportion of variation in the yield or response in the model. A negative Pred R-Squared= -2.4147 implies that the overall mean is a better predictor of obtained response than the current model.

In ANOVA for Response Surface Quadratic Model of Methanol recovery of Batch Solvent Extraction method, the Model F-value of 18.21 implies the model is significant. There is only a 0.05% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob>F" less than 0.0500 indicate model terms are significant. In this case Extraction temperature, Extraction time, Solid to liquid (Feed) ratio, squared temperature coefficient and Temperature-Solid to liquid interaction coefficients are significant model terms.

The "Lack of Fit F-value" of 0.56 implies the Lack of Fit is not significant relative to the pure error. The values for the coefficient of determination R^2= 0.9590 and adjusted R^2 = 0.9064 represents the proportion of variation in the yield or response in the model. The "Pred R-Squared" of 0.7602 is in reasonable agreement with the "Adj R-Squared" of 0.9064.

Whereas ANOVA for Response Surface Quadratic Model of Methanol recovery, the Model F-value of 2.98 implies there is an 8.22% chance that a " A negative Pred R-Squared= -1.3706 implies that the overall mean is a better predictor of obtained response than the current model and ANOVA for Response Surface Quadratic Model of Methanol recovery, the Model F-value of 4.94 implies the model is significant. There is only a 2.34% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case Extraction time, Solid to liquid ration and squared Extraction time coefficients are significant model terms.

3.3. Plot Response Surface Design

To visualize the relationship between response and experimental levels of the independent variables for the natural dye extraction, three dimensional (3D) surface plots were constructed according to the quadratic polynomial model equation.

The variation of Ethyl acetate recovery and Methanol recovery with Extraction time and Temperature are graphically presented in above Fig No.1. As the Extraction time of both solvents increases and Temperature were decreased, the Natural dye recovery increased significantly with curvature contour lines.

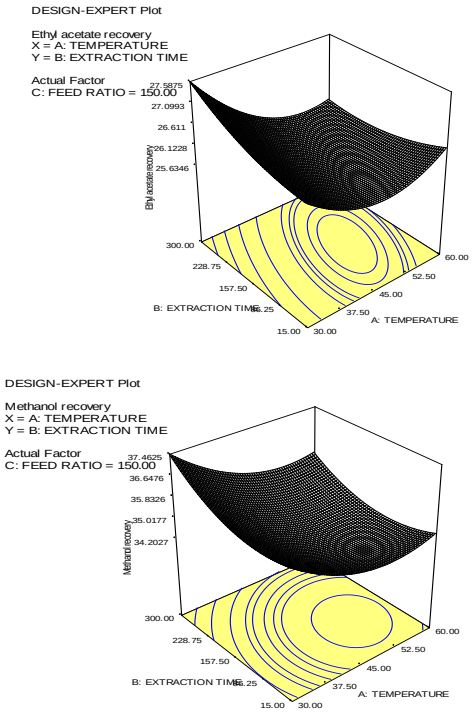
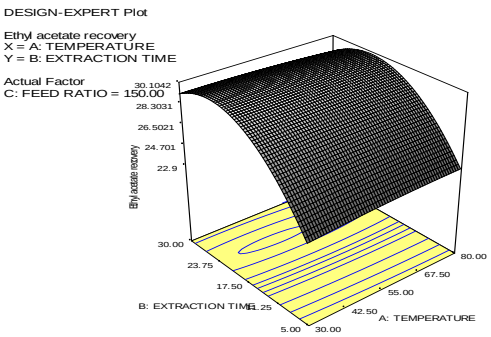


Fig No.1: Extraction time Vs Temperature of Batch Solvent Extraction Method for Sample



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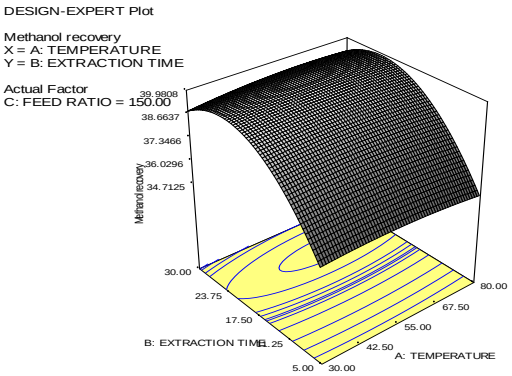
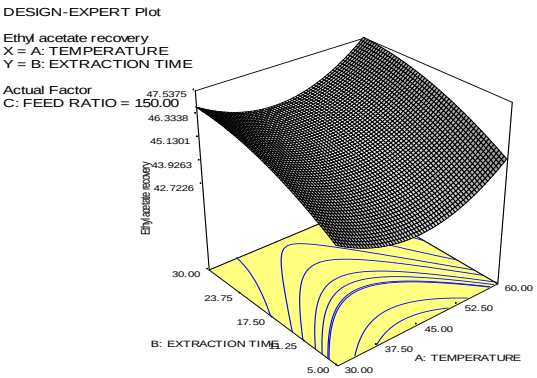


Fig No.2: Extraction time Vs Temperature of Ultrasonic Assisted Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Extraction time and temperature are graphically presented in above Fig No.2. As the Extraction time of both solvents increases and temperature were decreased, the Natural dye recovery increased significantly with curvature contour lines.



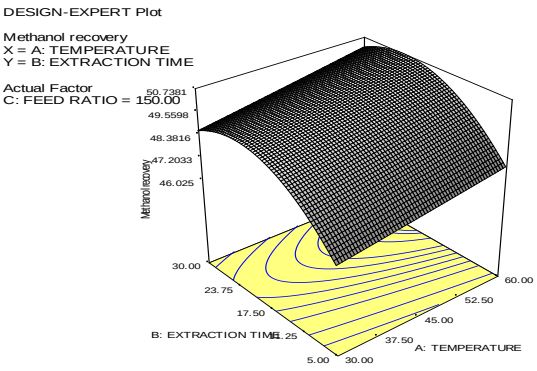


Fig No.3: Extraction time Vs Temperature of Microwave Assisted Extraction Method for Sample

Table No.1: Box-Behnken Design arrangement and Responses for Batch Solvent, Ultrasonic Assisted and Microwave extraction method

			Batch Solvent Extraction							Ultrasonic Assisted extraction							Microwave Assisted extraction			
A	B	C	Exp (%)	Pred (%)	Exp (%)	Pred (%)	A	B	C	Exp (%)	Pred (%)	Exp (%)	Pred (%)	A	B	C	Exp (%)	Pred (%)	Exp (%)	Pred (%)
°C	min	gm/ml	EtOAc		MeOH		°C	min	g/ml	EtOAc		MeOH		°C	min	g/ml	EtOAc		MeOH	
30	15.00	150.00	26.2	26.54	36.1	36.01	30	5.0	150	20.5	22.90	34.2	34.94	30	5.0	150	45.5	44.0	46.7	46.02
60	15.00	150.00	25.4	26.31	34.8	34.84	80	5.0	150	22.1	23.52	33.5	34.71	60	5.0	150	45.3	44.6	48.1	47.33
30	300.00	150.00	28.5	27.59	37.5	37.46	30	30.	150	30.5	29.07	40	38.79	30	30.0	150	46.1	46.7	47.8	48.58
60	300.00	150.00	26.3	25.96	35.5	35.59	80	30.	150	31	28.60	39.9	39.16	60	30.0	150	46.1	47.5	49.5	50.17
30	157.50	50.00	24.5	24.75	33.5	33.25	30	17.5	50	28.3	27.06	39.6	38.85	30	17.5	50	45.8	45.4	47.6	47.00
60	157.50	50.00	24.6	24.27	34	33.63	80	17.5	50	28.5	28.24	39.5	38.27	60	17.5	50	48.7	47.5	50.2	49.70
30	157.50	250.00	28.9	29.23	39.6	39.98	30	17.5	250	32.2	32.46	40	41.22	30	17.5	250	48.2	49.3	49.9	50.40
60	157.50	250.00	28.1	27.85	36.3	36.55	80	17.5	250	30.2	31.44	41.2	41.95	60	17.5	250	48.4	48.7	50	50.60
45	15.00	50.00	23.8	23.21	32.3	32.64	55	5	50	21.8	20.64	32.8	32.81	45	5.00	50	41	42.7	44	45.28
45	300.00	50.00	24.3	24.96	33.7	33.99	55	30	50	25.6	28.26	38	39.96	45	30.0	50	46	45.7	48.2	48.03
45	15.00	250.00	29.3	28.64	38	37.71	55	5	250	29.6	26.94	40.8	38.84	45	5.0	250	45.3	45.5	47.3	47.47
45	300.00	250.00	27	27.59	38.9	38.56	55	30	250	29.4	30.56	40	39.99	45	30.0	250	49.9	48.1	51.4	50.13
45	157.50	150.00	25.1	25.72	34.1	34.46	55	17.5	150	29.5	29.50	39.4	39.52	45	17.5	150	44.7	44.7	49.7	49.70
45	157.50	150.00	26.7	25.72	35.7	34.46	55	17.5	150	29.5	29.50	38.5	39.52	45	17.5	150	44.7	44.7	49.7	49.70
45	157.50	150.00	25.1	25.72	34.1	34.46	55	17.5	150	29.5	29.50	38.5	39.52	45	17.5	150	44.7	44.7	49.7	49.70
45	157.50	150.00	25	25.72	34.2	34.46	55	17.5	150	29.5	29.50	39.4	39.52	45	17.5	150	44.7	44.7	49.7	49.70
45	157.50	150.00	26.7	25.72	34.2	34.46	55	17.5	150	29.5	29.50	41.8	39.52	45	17.5	150	44.7	44.7	49.7	49.70

Exp EtOAc: Experimental Ethyl acetate, Pred EtOAc: Predicted Ethyl acetate, Exp MeOH: Experimental Methanol, Pred MeOH: Predicted Methanol.

Table No. 2: ANOVA for Response Surface Quadratic Model of Ethyl Acetate and Methanol Recovery

Source	F Value						Prob > F					
	BSEM		UAE		MAE		BSEM		UAE		MAE	
	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH
Model	4.36	18.21	3.34	2.98	2.87	4.94	0.0326	0.0005	0.0628	0.0822	0.0894	0.0234
A	1.71	11.84	2.208E	3.399E	0.51	4.43	0.2323	0.0108	0.9638	0.9551	0.4981	0.0734
B	0.24	6.16	12.42	10.41	7.34	15.36	0.6359	0.0421	0.0097	0.0145	0.0302	0.0058
C	32.38	118.54	7.26	5.53	6.44	9.74	0.0007	<0.0001	0.0309	0.0510	0.0388	0.0168
A^2	1.79	7.21	0.016	0.063	5.48	6.930E	0.2226	0.0313	0.9040	0.8090	0.0517	0.9797
B^2	0.22	5.18	9.21	7.31	0.71	12.63	0.6549	0.0570	0.0190	0.0304	0.4287	0.0093
C^2	0.098	3.48	0.16	0.77	4.22	0.37	0.7635	0.1043	0.7027	0.4096	0.0789	0.5640
AB	0.49	0.31	0.059	0.027	4.855E	0.024	0.5067	0.5939	0.8145	0.8737	0.9464	0.8820
AC	0.20	9.19	0.24	0.13	0.88	1.65	0.6664	0.0191	0.6409	0.7314	0.3782	0.2404
BC	1.96	0.16	0.79	2.72	0.019	2.634E	0.2044	0.7019	0.4050	0.1431	0.8931	0.9605
Lack of Fit	1.58	0.56		2.89			0.3268	0.6670		0.1656		
Cor Total		18.21	3.34	2.98	2.87	4.94		0.0005	0.0628	0.0822	0.0894	0.0234

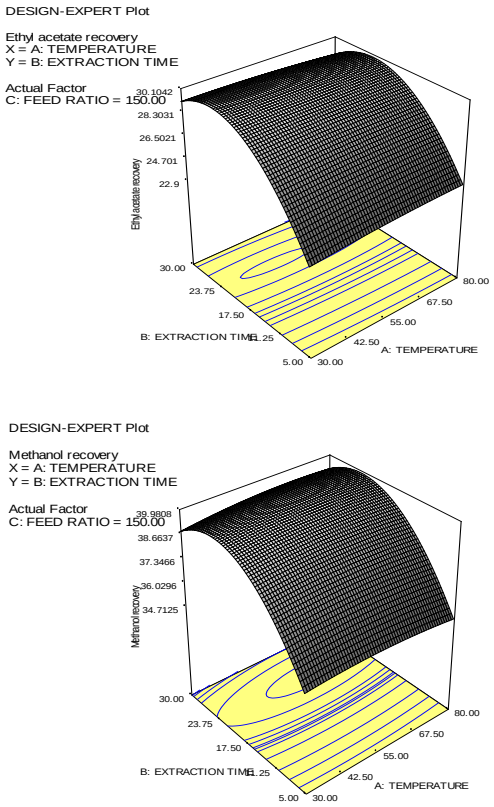
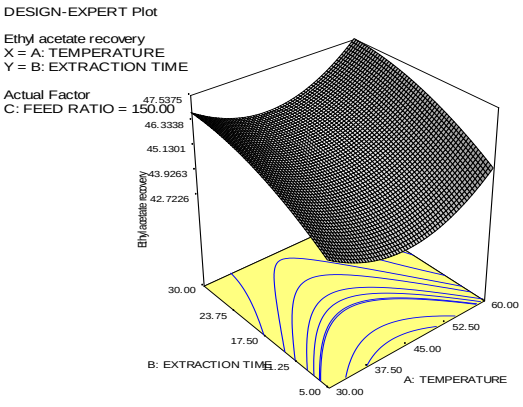


Fig No.2: Extraction time Vs Temperature of Ultrasonic Assisted Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Extraction time and temperature are graphically presented in above Fig No.2. As the Extraction time of both solvents increases and temperature were decreased, the Natural dye recovery increased significantly with curvature contour lines.



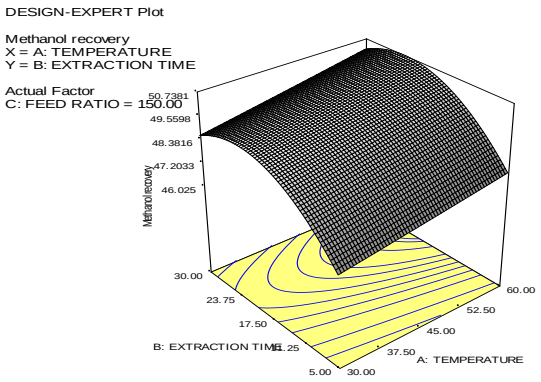


Fig No.3: Extraction time Vs Temperature of Microwave Assisted Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Extraction time and temperature are graphically presented in above Fig No.3. As the Extraction time of both solvents increases and temperature were decreased, the Natural dye recovery increased significantly with curvature contour lines

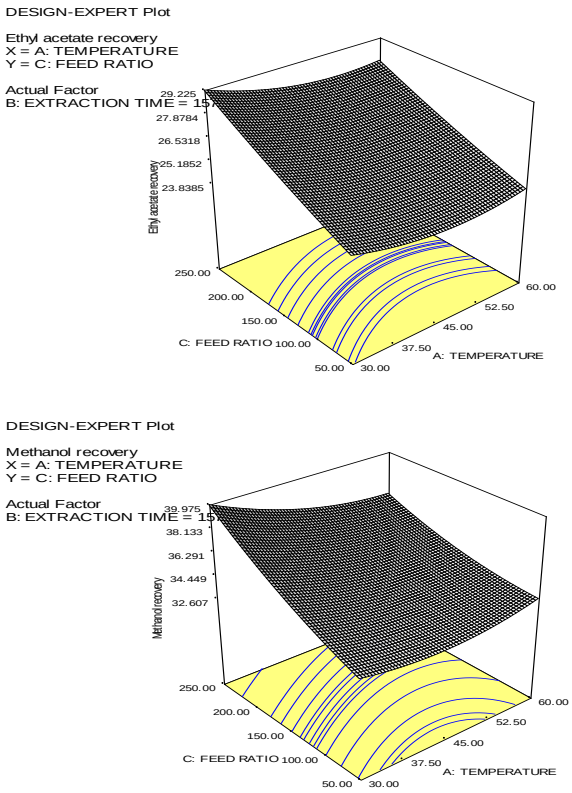
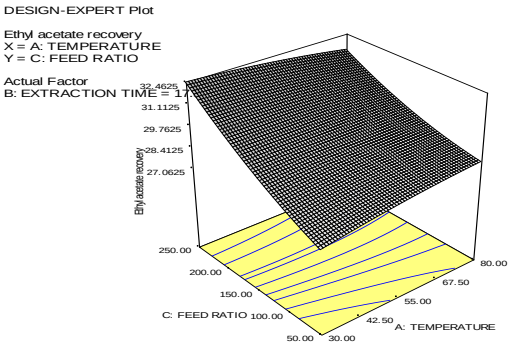


Fig No.4: Feed ratio Vs Temperature of Batch Solvent Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Solid to Liquid Feed ratio

and Temperature are graphically presented in above Fig No.4. As the Solid to Liquid Feed ratio of both solvents increases and Temperature were decreased, the Natural dye recovery increased significantly with curvature contour lines.

The variation of Ethyl acetate recovery and Methanol recovery with Solid to Liquid Feed ratio and temperature are graphically presented in above Fig No.5. As the Solid to Liquid Feed ratio of both solvents increases and temperature were decreased, the Natural dye recovery increased non-significantly with linear contour lines.



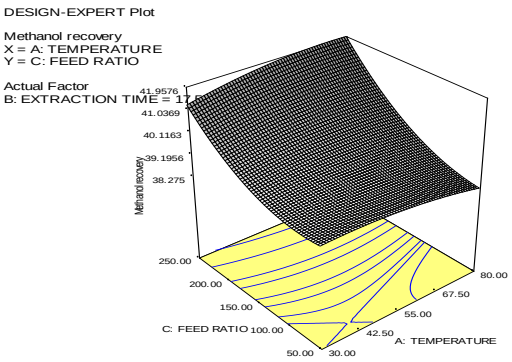
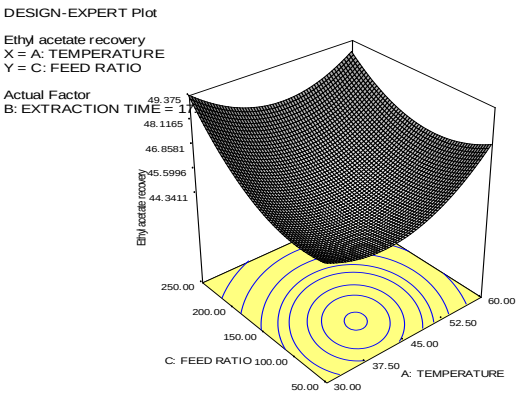


Fig No.5: Feed ratio Vs Temperature of Ultrasonic assisted Extraction Method for Sample



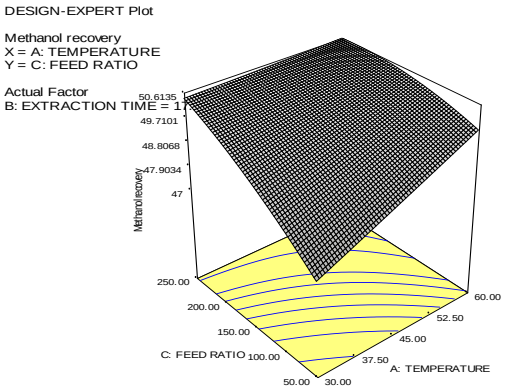
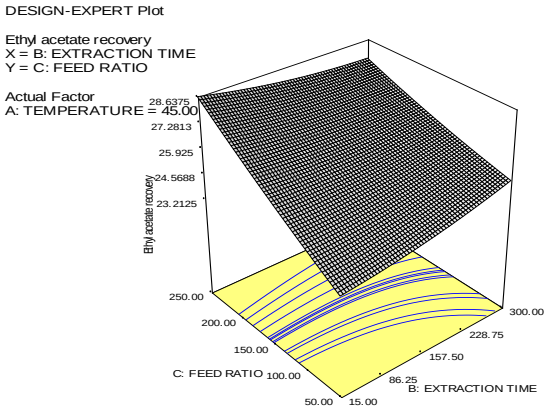


Fig No.6: Feed ratio Vs Temperature of Microwave assisted Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Solid to Liquid Feed ratio and temperature are graphically presented in above Fig No.6. As the Solid to Liquid Feed ratio of both solvents increases and temperature were increased, the Natural dye recovery increased significantly with curvature contour lines.



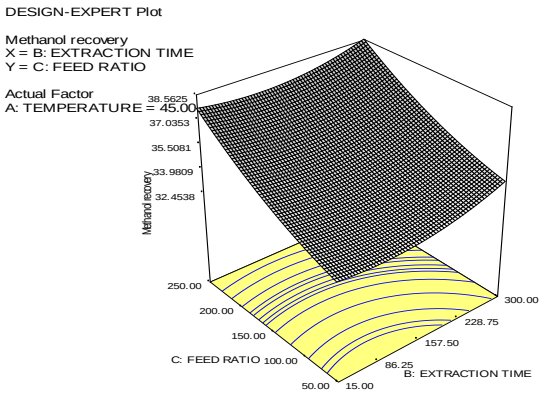
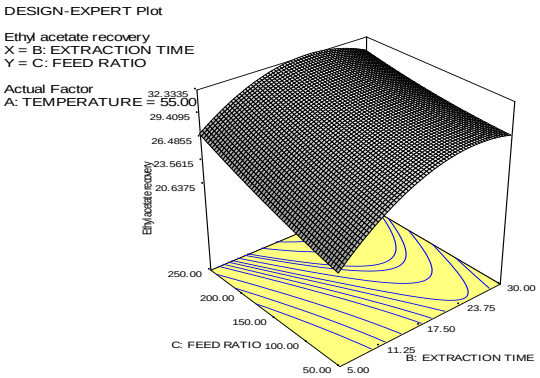


Fig No.7: Feed ratio Vs Extraction time of Batch Solvent Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Solid to Liquid Feed ratio and Extraction Time are graphically presented in above Fig No.7. As the Solid to Liquid Feed ratio of both solvents increases and Extraction Time were decreased, the Natural dye recovery increased significantly with curvature contour lines.



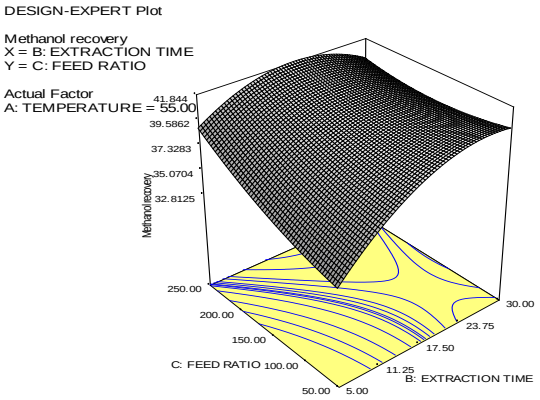
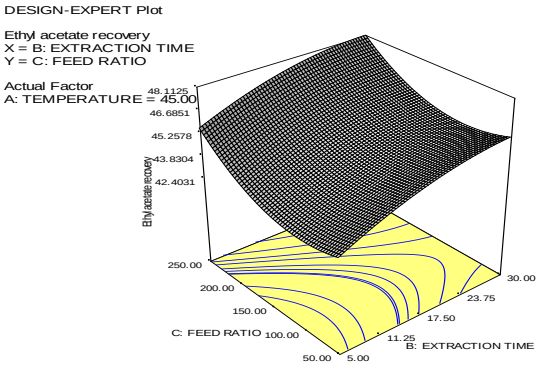


Fig No.8: Feed ratio Vs Extraction time of ultrasonic Assisted Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Solid to Liquid Feed ratio and Extraction Time are graphically presented in above Fig No.8. As the Solid to Liquid Feed ratio of both solvents increases and Extraction Time were decreased, the Natural dye recovery increased significantly with curvature contour lines.



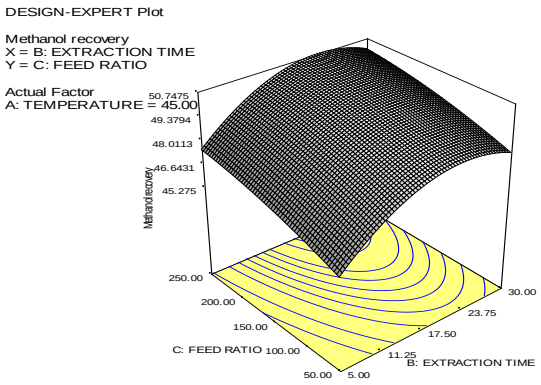


Fig No.9: Feed ratio Vs Extraction time of Microwave assisted Extraction Method for Sample

The variation of Ethyl acetate recovery and Methanol recovery with Solid to Liquid Feed ratio and Extraction Time are graphically presented in above Fig No.4.9.As the Solid to Liquid Feed ratio of both solvents increases and Extraction Time were increased, the Natural dye recovery increased significantly with curvature contour lines.

3.4. Verification of Optimized Condition and Predictive Model

Optimization requires goals to be set for the variables and response where all goals then get combined into one desirability function. To find a good set of conditions that will meet all the goals, the three variables extraction temperature extraction time, solid to liquid, were set within range while ethyl acetate and methanol recovery was set at maximum. For response, the "importance" was set at 5 in order to meet the objective of getting maximum recovery. By applying the desirability function approach, the optimum level of various parameters was obtained as showed in Table No.3 provided with experimental values for obtained optimal conditions.

Table No. 3: Optimum conditions and the predicted and experimental value of responses at the optimum conditions.

Extraction methods	Responses	Temperature (°C)	Extraction Time (min)	Feed Ratio (1gm/ml)	EtOAc recovery (%)	MeOH recovery (%)
Batch solvent	**Predicted**	31.01	57.57	249.67	29.3351	39.6676
	Experimental	31	57	250	29.10	39.50
Ultrasonic Assisted	**Predicted**	58.69	21.01	249.41	32.2277	41.8215
	Experimental	59	21	249	32.0	42.0
Microwave Assisted	**Predicted**	30.00	23.84	250.00	49.845	50.6
	Experimental	30.0	24.0	250.00	50.0	50.2

IV. CONCLUSION

In the present study, response surface methodology was used to optimize the Solvent, Ultrasonic and Microwave assisted extraction of natural dye from *Pterocarpus santalinus* wood. Box-Behnken design was used to determine the optimum process parameters and the multiple regression analysis for predicting responses were obtained. Under optimum condition Microwave assisted extraction method showed the highest natural dye yield percentage which is 50.0 for ethyl acetate solvent and 50.2 for methanol solvent. Microwave assisted extraction method dictates the quality, economics and environmental impact of any processing plant. It shows a highly promising future with drastic reduction in extraction time resulting in higher sample throughput without significant losses in analyte recovery. In this study among three parameters Solid to liquid ratio for Solvent extraction method, Extraction time for Ultrasonic extraction method and extraction time and solid to liquid for microwave assisted extraction method is found to be most prominent factor affecting the efficiency of dye extraction.

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