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Dhanush C

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Naveen Kumar TD

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Kiran Kumar BT

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Sathish BN

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Hareesh TS

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Gajendra CV

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Ashwath MN

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Corresponding Author:**Dhanush C**

Department of Forest Products and Utilization, College of Forestry, Ponnampet, UAHS, Shimoga, Karnataka, India

Extraction and chemical characterization of leaf and flower dye from *Lantana camara* Linn.

Dhanush C, Naveen Kumar TD, Kiran Kumar BT, Sathish BN, Hareesh TS, Gajendra CV and Ashwath MN

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Abstract

To fulfill the increasing demand for dyes and pigments by the textile industries syntactic dyes comes into existence, which caused a serious environmental problem. Natural dyes are one such alternative to this problem. In this regard the present study was conducted to optimize extraction protocol (by using Box-Behnken Design) and chemical characterization of dye from the *Lantana camara*. The optimized point for extraction of flower and leaf dye was achieved at 92°C, 174 minutes with Mass to liquor ratio MLR 2:100, yielded 29.70% dye and 79°C, 36 minutes with MLR 3:100 yielded 26.2% dye respectively. The Methanol and Ethanol extract of flower and leaf dye was subjected to phytochemical screening by using GC-MS and dye extract showed that the presence of n-Hexadecanoic acid and 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) in higher concentration. Most of the chemicals found in the dye extract have antibacterial, antifungal and Anti-Inflammatory properties. Hence, the use of the *Lantana camara* leaf and flower will reduce the pollution and helps in the management of this invasive weed in the Indian forests.

Keywords: *Lantana camara* Linn., flower dye, leaf dye, Phytochemicals

Introduction

From the past two decades, an increase of attention has been directed towards the environment health and forests of India and world. The noticeable growing awareness of the public in general and particularly of the entrepreneurs, concerning the conditions that have a negative impact over the environment, water quality and degradation of natural forests being one of their main preoccupations. India accounts for 12% of the global colorant industry, out of which nearly 2/3rd is exported (IIMS-Ahmedabad, 2012)^[9]. In 2010, India produced ~200,000 tonnes of dyes. Of this, 50% were reactive dyes due to the availability of important raw materials like vinyl sulphone, etc. Nearly 70% of the dyestuff was supplied to the textile industry while leather and paper industries accounted for the remaining (IIMS-Ahmedabad, 2012)^[9]. The present production volume of dyes and pigments across the country was around 370 thousand metric tons in 2019 but it was found that 130 thousand metric tons lower in 2013 (270 thousand metric tons) (Statista Research Department, 2019). This data evident that the demand for the dye and pigments in the India was found to be increasing gradually every year. The sector is dominated by unorganized players and has ~1000 players in the small scale category. There are only 50 large organized units. These units are mainly present in Gujarat and Maharashtra, with the former accounting for almost 80% of capacity (IIMS-Ahmedabad, 2012)^[9]. Per capita consumption of dyestuff is ~50 g compared to the world average of 300 g demonstrating a largely untapped domestic market. India has largely been an exporting country and has emerged as a global supplier of reactive, acid, vat and direct dyes accounting for ~10% of world trade (IIMS-Ahmedabad, 2012)^[9].

Natural dyes are colouring substances obtained from natural sources like plants, animals and minerals. Nature has gifted us more than 500 dye yielding plant species (Mahanta and Tiwari, 2005). It is interesting that over 2000 pigments have been synthesized by various parts of plants, of which only about 150 have been commercially exploited (Purkayastha, 2016)^[16]. Therefore, the usage of natural dyes gaining importance worldwide. Most of the natural dyes derived from the various plant species are safe because of their nontoxic, biodegradable, and non-carcinogenic characteristics (Ali *et al.*, 2009; Swamy *et al.*, 2016; Clarke and Anliker, 1980)^[23, 3]. This characters may help in the reduction of environmental pollution and enhances the human health.

Hence the present study is focused to search an alternative raw material which can yields a good amount of dye and also the availability of raw material should be abundant for the large scale production of dye. With this background for the present study we had selected *Lantana camara* Linn., because it exhibits antimicrobial, fungicidal, insecticidal and nematocidal properties and also which is an considered as an invasive weed species creating a serious the problems in the Indian forests and ecological functions.

Materials and methods

Optimization of dye extraction protocol

Raw material for the extraction of dye (fresh flowers and the leaves of the *Lantana camara*) was collected and shade dried than powered by using a grinder. Optimization of dye extraction was carried out by using Box-Behnken Design of Response Surface Methodology (Sinha *et al.*, 2016) [18] by using three independent variables *viz.*, temperature (T), time (T), and MLR. With the help of deign expert v11 (trial version) software 17 different combinations of three independent variables were achieved for the extraction of dye from the flower and leaf, details are given in Table 1 and 2. Aqueous method of dye extraction was followed for the Optimization of dye extraction protocol (Noor *et al.*, 2015; Hemanthraj *et al.*, 2014) [15, 8]. The dye yield was calculated by using the following expression (Noor *et al.*, 2015; Hemanthraj *et al.*, 2014) [15, 8],

$$\text{Dye yield (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of raw material taken (g)}} \times 100$$

Chemical composition

Preparation of plant dye extracts

The crude extracts of flower and leaf dye was prepared in two solvents *viz.*, Methanol and Ethanol. Two grams of the finely ground flower and leaf powder was boiled in 20 ml of different solvents at 60°C in Soxhlet apparatus (Sox plus v1.4) for 6 hours (Luis *et al.*, 2016). The extract was then dried at room temperature and the dried extracts stored at 4 °C in air tight glass vials in refrigerator.

Gas Chromatograph and mass spectrometer (GC-MS) analysis

The chemical composition of the methanolic and ethonolic flower and leaf extract was analyzed using Shimadzu Gas Chromatography and Mass Spectrophotometer - QP 2020 with a SH-Rxi-5 Sil MS Cross Band (similar to 5% diphenyl 95% dimethyl polysiloxane) capillary mid-polar column (30m, ID: 0.25 mm and film thickness of 0.25 µm). Sample size of 1.0µl methanolic and ethonolic extract was injected for analysis and Helium was used as a carrier gas at 1.2 mL/minute. The column oven temperature was programmed from 80 °C to 285 °C (80 °C for 5 min, 4 °C rate 260 °C, and 2 °C rate 285 °C hold for 10 minutes). The MS was set to scan from 45-650Da. The MS also had inbuilt pre-filter which reduces the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum *viz.*, NIST4 and WILEY9 containing more than a million references (Vinothini *et al.*, 2016) [27].

Identification of compounds

Interpretation of mass spectrum of GC-MS is done using the database of National Institute Standard and Technology (NIST 4) and WILEY 9 (Dool and Kratz, 1963) [5]. The spectrum of the unknown component is compared with the

spectrum of the known components stored in the inbuilt library (Priyanka *et al.*, 2016a) [27].

Quaitative phytochemical analysis

The dye extract of flower and leaf was tested for the presences of various bioactive compounds by using standard protocols. Flavonoids was tested by using Folindenis test (Harborne, 1998), tannins and phenols in Ferric chloride test (Dawoud *et al.*, 2015) [4], Anthroquinones and alkaloids by using Mayer's test (Aiyegoro *et al.*, 2010) [1], and steroids/terpenes by using Salkowski test (Ishnava *et al.*, 2013) [10].

Statistical analysis

The total amount of dye extracted was analysed with standard Response surface methodology (RSM) called Box-Behnken Design (BBD) by using Design Expert statistical software (ver. 11.1.2.0) (Sinha *et al.*, 2016) [18].

Results and discussion

Optimization dye yield (%) from flower and leaf of *Lantana camara* using Box-Behnken Design

The Box-Behnken Design (BBD) was used for optimizing the dye extraction from *Lantana camara* flower and leaf considering three factors such as extraction temperature (A) (50 - 90 °C), extraction time (B) (30-180 minutes), and MLR (C) 1-5 i.e. 1:100, 2:100, 3:100, 4:100, and 5:100 respectively (Table 1). The experimental design i.e. Box-Behnken Design points falls within a safe operational limit, within the nominal high and low levels. The design arrangement and responses of actual (experimental) and predicted (theoretical) values of dye yield (%) are shown in Table 2 and 3.

Response surface methodology (RSM) is an empherical statistical technique (Swamy *et al.*, 2014) [23], being used for multiple regression analysis to evaluate the multi-various equations simultaneously without expelling any variable (Stamenković *et al.*, 2018) [20]. RSM is better compared to any kind of conventional methods used for optimizing the factors because, the RSM statistical optimization modelling has the ability to consider the non-linear effects as well as the interactions among the variables for optimizing multi-variables and processes multi-level (Noor *et al.*, 2015) [15].

The analysis of variance (ANOVA) for response surface quadric model for flower and leaf dye yield (%) presented in Table 4 and 5 respectively. The model F value flower and leaf dye was 8.89 and 13.55 respectively implies the model is significant. P values less than 0.0044 and 0.0012 for flower and leaf dye respectively indicates the model terms are significant table 4 and 5.

The Fishers's F value flower and leaf dye was 8.89 and 13.55 respectively with a very low probability 0.0044 and 0.0012 respectively implies that model was significant (Table 4 and 5). The goodness of fit of the model for both flower and leaf dye was examined by determination of coefficient $R^2 = 0.9195$ and $R^2 = 0.9457$ respectively, which implies that variation attributed by flower and leaf dye samples were 91.95% and 94.57 statistically significant respectively and only 9.05% (flower dye) and 6.43% (leaf dye) of the total variance could not be explained by the model. The adjusted determination coefficient (Adj. $R^2 = 0.8161$ (flower dye) and Adj. $R^2 = 0.8759$ (leaf dye)) and the predicted determination coefficient (Pred. $R^2 = 0.3676$ (flower dye) and Pred. $R^2 = 0.5626$ (leaf dye)) were also satisfactory to confirm the significance of model. A low value of coefficient of variance (CV = 14.35% (flower dye) and CV = 12.06% (leaf dye))

described that the experiments conducted were precise and reliable. Hence, this model can be used to navigate the design space for optimized dye extraction.

The lack of fit F value of 1.03 (flower dye) and 1.09 (leaf dye) implies the Lack of fit was not significant relative to the pure error. There is a 46.8% (flower dye) and 44.6% (leaf dye) chance that a lack of fit F value, this large could occur due to noise. Non-significant lack of fit was good and hence model was completely fitted (Table 4 and 5).

Central composite design (CCD) and Box-Behnken (BBD) designs are the most powerful tool in RSM; suits well in fitting the quadratic surface model, thereby the number of effective factors were optimized with minimum number of experiments and experimental runs, effective factor interactions by considering more than two factor at a time, and reduces the time, labour requirement in conducting experiment; also provides the precise and very accurate results (Mishra and Aeri, 2016)^[14].

Plot response surface design - interaction effect of factors

To visualize the relationship between response and

experimental levels of independent variables of dye yield, a three dimensional (3D) surface plots were constructed according to quadratic model equation.

The variation in flower and leaf dye yield with extraction temperature and time are graphically represented in Figure 1a and 1e respectively. As the extraction time and temperatures increases the flower and leaf dye yield similarly, with respect to extraction time to MLR (Fig. 1b and 1d), extraction temperature and MLR (Fig. 1c and 1f).

The Figure 1a and 1d represents effect of time (A) and temperature (B) on flower and leaf dye yield (%) under fixed MLR (material to liquor ratio) respectively. At a definite extraction time, the dye yield increased with extraction temperature from 50 to 100 °C and nearly reached a peak.

Table 1: Experimental factors and their levels for extraction of flower and leaf dye

Factor	Units	Low	High
(A) Temperature	°C	50	100
(B) Time	Minutes	30	180
(C) (MLR)	-	1	5

Table 2: Flower dye extraction by using Box-Behnken experimental design with factors and response

Runs	Factors			Response	
	Time (minutes)	Temperature (Deg. C)	MLR	Actual Dye Yield (%)	Predicted Dye yield (%)
1	105	75	3	13.36	15.96
2	105	75	3	19.86	15.96
3	180	100	3	31.00	31.06
4	105	50	5	13.14	15.72
5	30	75	5	28.70	26.18
6	105	75	3	13.36	15.96
7	105	100	1	34.50	31.92
8	105	75	3	13.36	15.96
9	180	50	3	23.86	23.02
10	30	50	3	29.70	29.64
11	180	75	5	21.32	19.58
12	105	75	3	19.86	15.96
13	105	100	5	30.92	32.60
14	30	75	1	32.60	34.34
15	180	75	1	28.60	31.12
16	30	100	3	33.43	34.27
17	105	50	1	37.80	36.12

Table 3: Leaf dye extraction by using Box-Behnken experimental design with factors and response

Runs	Factors			Response	
	Time (min)	Temp. (°C)	MLR	Actual Value yield (%)	Predicted Value yield (%)
1	105	75	3	14.14	16.14
2	105	75	3	15.00	16.94
3	180	100	3	15.00	16.94
4	105	50	5	15.00	16.94
5	30	75	5	19.86	16.94
6	105	75	3	19.86	16.94
7	105	100	1	21.32	19.70
8	105	75	3	23.86	23.48
9	180	50	3	27.00	25.28
10	30	50	3	28.60	30.32
11	180	75	5	29.70	29.42
12	105	75	3	30.00	30.27
13	105	100	5	30.92	32.26
14	30	75	1	32.60	34.22
15	180	75	1	33.43	33.81
16	30	100	3	33.50	31.50
17	105	50	1	37.80	36.46

Table 4: ANOVA response surface quadric model for flower dye yield (%)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1027.58	9	114.18	8.89	0.0044	S
A-TIME	48.27	1	48.27	3.76	0.0938	S
B-Tem.	80.33	1	80.33	6.25	0.0410	S
C-MLR	194.24	1	194.24	15.12	0.0060	S
AB	2.91	1	2.91	0.2263	0.6488	NS
AC	2.86	1	2.86	0.2223	0.6516	NS
BC	111.09	1	111.09	8.65	0.0217	S
A ²	158.03	1	158.03	12.30	0.0099	S
B ²	231.27	1	231.27	18.00	0.0038	S
C ²	137.70	1	137.70	10.72	0.0136	S
Residual	89.92	7	12.85			
Lack of Fit	39.22	3	13.07	1.03	0.4683	NS
Std. Dev. 3.58; Mean = 25.01; CV = 14.35%; R ² = 0.9195, Adjusted R ² = 0.8161, Predicted R ² = 0.3676						

Table 5: ANOVA response surface quadric model for leaf dye yield (%)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	897.71	9	99.75	13.55	0.0012	S
A-TIME	44.89	1	44.89	6.10	0.0429	S
B-TEMP	62.44	1	62.44	8.48	0.0226	S
C-MLR	191.30	1	191.30	25.99	0.0014	S
AB	1.45	1	1.45	0.1973	0.6703	NS
AC	0.7056	1	0.7056	0.0959	0.7659	NS
BC	111.09	1	111.09	15.09	0.0060	S
A ²	118.13	1	118.13	16.05	0.0052	S
B ²	206.71	1	206.71	28.08	0.0011	S
C ²	111.21	1	111.21	15.11	0.0060	S
Residual	51.53	7	7.36			
Lack of Fit	23.18	3	7.73	1.09	0.4492	NS
Std. Dev. 2.71; Mean = 25.26; CV = 12.6%; R ² = 0.9457, Adjusted R ² = 0.8759, Predicted R ² = 0.5626						

at the highest extraction time (105 minutes). However, upon increasing to 90 °C, there was a decline in the response, and extraction time over 105 minutes did not show any obvious effect on dye yield. This result may be attributed by the fact that under increased temperature, polarity of solvent got reduced, which in turn unable to extract sufficiently polar extractives such as tannins, phenols, flavonoids from bark (Mishra and Aeri, 2016) [14].

Though increase in temperature promotes the discolouration of pigments, this phenomenon can be attributed to better solubility of colour component under high temperatures. In fact heat or temperature renders the cell walls to get more permeable, decreases the viscosity of solvent, thus temperature facilitates its passage through solid substrate mass (Tan *et al.*, 2011). Higher temperature turned to damage pigment by loss of protection from membrane, very quickly the colourant self oxidises (Chen and Wu, 2009) [2].

Figure 1c and 1e indicates the effect of material to liquor ratio MLR (C) and extraction temperature (B) on dye yield. As the MLR increases the content of dye yield decreases, which could be due to overheating of the material, dilution of extractives in the flower and leaf and degradation of colouring substances at high temperatures (Goula, 2013) [7].

With higher MLR, the dilution resulted in the loss of colour strength, and effective ooze out of colourant into water medium (solvent) would be ceased. With lower MLR colourant may not be properly extracted into liquor (Lazar *et al.*, 2016) [12]. Hence, optimum yield of dye was achieved by considering the varied levels of factors ranging from the least contributing one to the highest contributing factor to yield higher extraction efficiency and better extract quality (Zhang *et al.*, 2011) [29].

Figure 1b and 1d shows the effect of the interaction of time (A) and MLR (C) on the dye yield. The results indicated that

the lower MLR and time yielded lower dye yield. Whereas the highest dye yield was achieved at 105 minutes as the extraction time with 3 material to liquor ratio (MLR - 3:100). The effect of extraction duration can be considered with higher contact time of solvent with bark powder which grasped more colouring component into the solution but further increase resulted in decrease in colour strength and which might be due to decomposition of colouring component at higher/prolonged contact time and temperature (Umbreen *et al.*, 2008; Elksibi *et al.*, 2014) [25, 6].

At lower times the colourant was not sufficiently extracted while at higher times may result in degradation of extracted colourant due to exposure to oxygen i.e. oxidation (Spyroudis, 2000) [19]. Higher time extraction lead to decreased colour strength, due to degradation of major polyphenolic compounds in dyes, and phenols are weak acids having -OH groups in their structure (Uslu and Bamufleh, 2016; Yusuf *et al.*, 2017) [26, 28]. The maximum yield was achieved under long extraction duration but, after certain point of time the colour strength decreases. This was attributed due to the oxidation of phenolic compounds under prolonged exposure and also thermal degradation of endogenous enzymes in plant cells leads to varied colours or extractive yield (Kuljarachanan *et al.*, 2009) [11].

Optimization of dye extraction using desirability function

In this numerical optimization the desired goal was preferred for each variable and response (R1) from the menu. A minimum and maximum level was considered for each independent parameter (temperature, time and MLR) provided. A level of extraction temperature within range 50 - 90°C, extraction time 30-180 minutes, and material to liquor ratio (MLR) 1-5 respectively, were set to maximum desirability. Later, ramp desirability was developed from 5

optimum points via numerical optimization (Figure 2). By seeking from 5 starting points in response surface changes the best local maximum value for dye yield were found to be at the extraction temperature of 92 °C (flower dye) and 80 °C (leaf dye), extraction time of 174 min (flower dye) and 36 min

(leaf dye), and MLR (material to liquor ratio) 2 (flower dye) and 3 (leaf dye) respectively (Fig. 2a (flower dye) and fig. 2b (leaf dye)). The efficiency on the extraction of dye under these optimized conditions (theoretical or predicted) was found to be 29.34% (flower dye) and 26.34% (leaf dye)

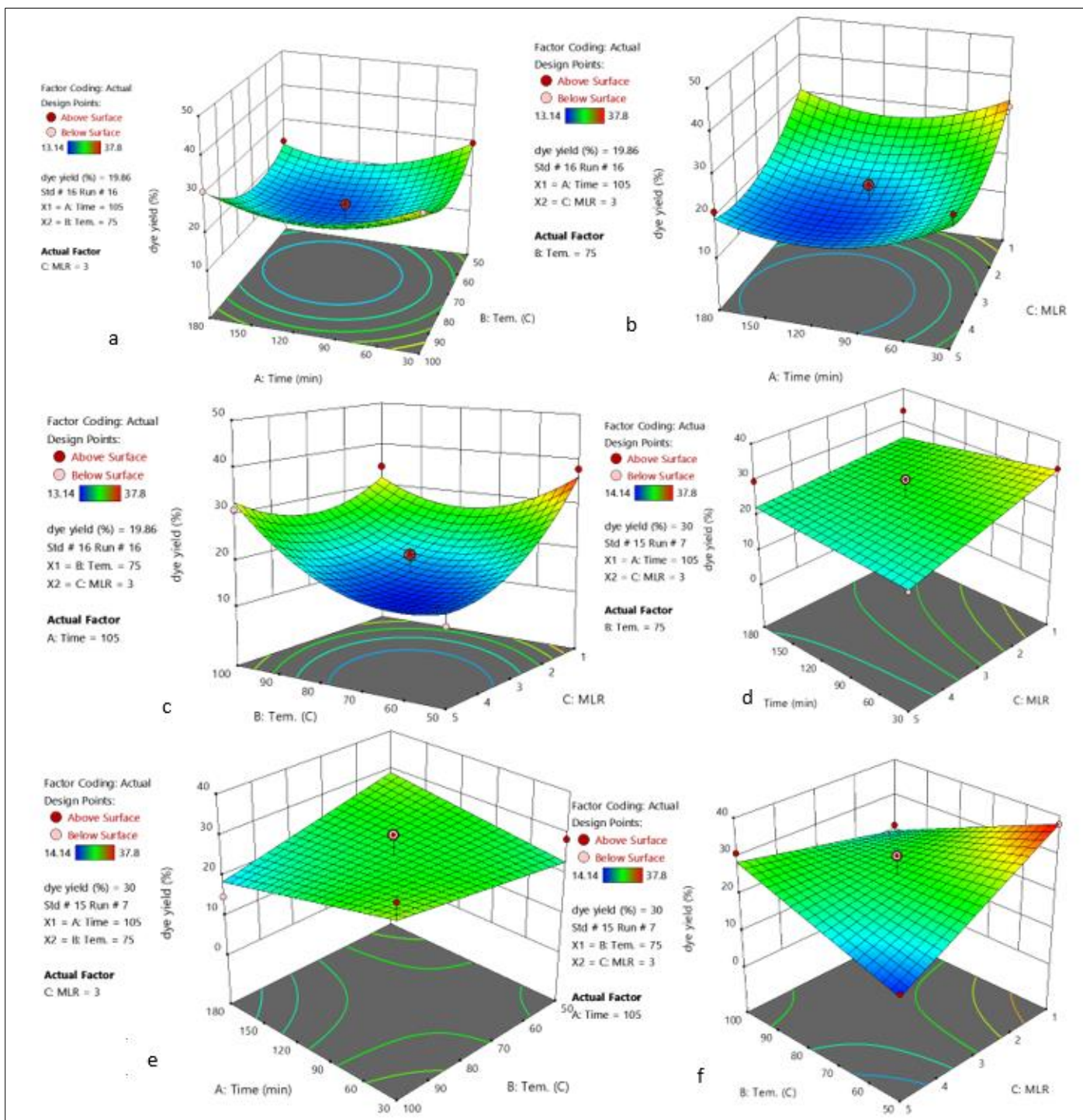


Fig 1: Effect of time and temperature on flower (a) and leaf (e), time and MLR on flower (b) and leaf (d), time and MLR on flower (c) and leaf (f) on dye yield (%)

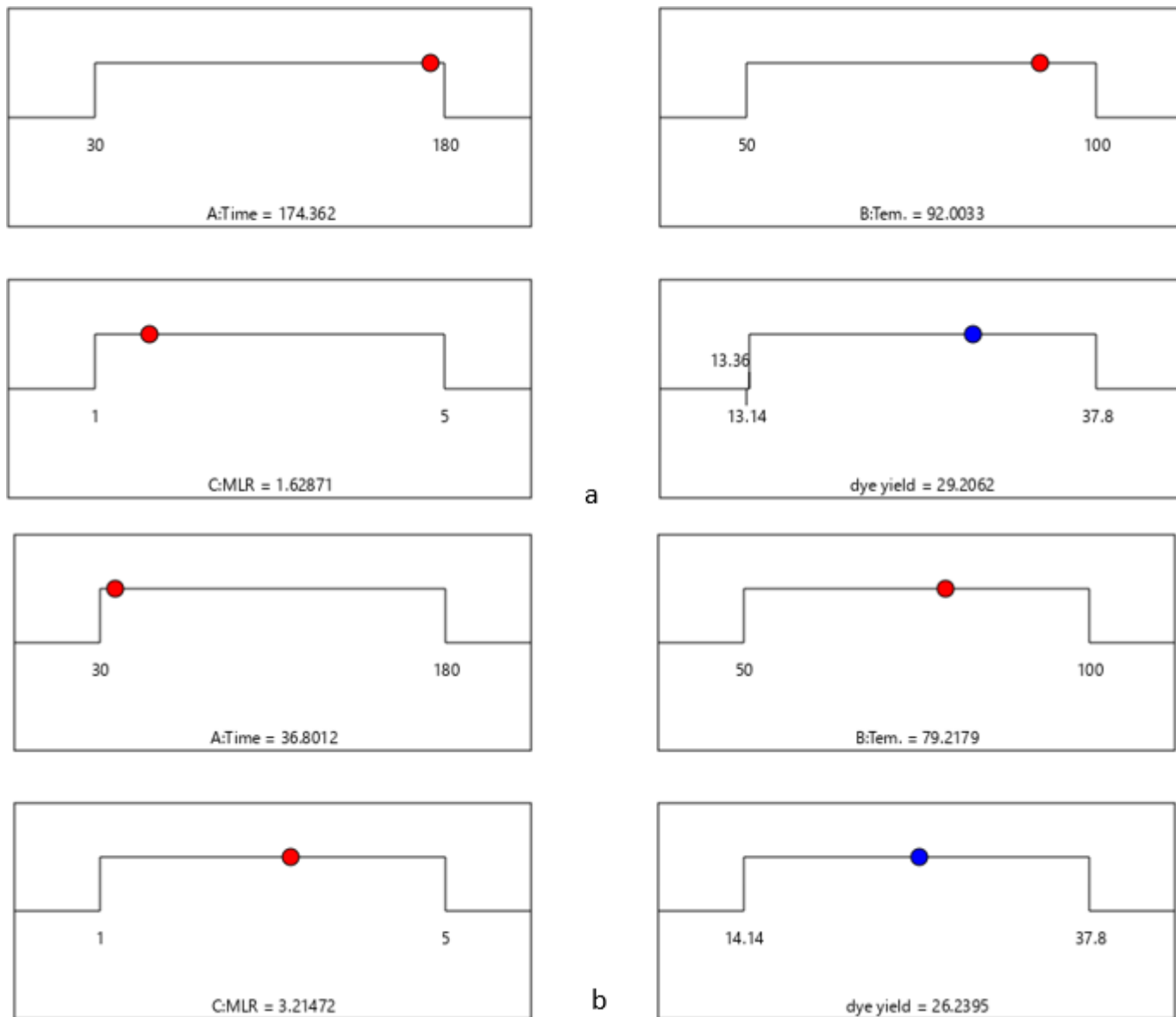


Fig 2: Numerical optimization of flower dye (a) and leaf dye (b) yield using desirability function.

and experimental (actual) value was 19.96% (flower dye) and 30.00% (leaf dye). The per cent deviation of the experimental and theoretical results is 9.38% (flower dye) and 3.66% (leaf

dye). This suggests that response surface methodology (RSM) model optimized and represented the experimental results.

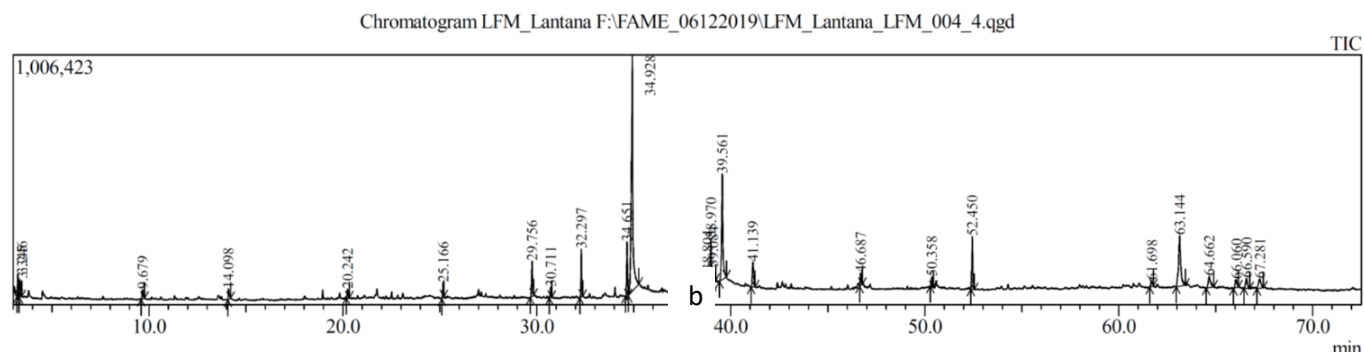


Fig 3: GC-MS Chromatogram of methanolic extract of Lantana flower dye

Table 6: Phytochemical profiling of methanolic extract of Lantana flower dye

Peak#	R. Time	Name	Area%
1	3.246	1-Butanol, 2-amino-3-methyl-, (+/-)-	1.52
2	3.345	Styrene	1.66
3	9.679	Glycerin	1.05
4	14.098	Caprolactam	1.00

5	20.242	3-Nitrobenzyl iodide	0.86
6	25.166	1-Heptadecene	0.99
7	29.756	Tetradecanoic acid	3.03
8	30.711	1-Nonadecene	0.75
9	32.297	1,2-Benzenedicarboxylic acid, bis(2-methy	3.48
10	34.651	Dibutyl phthalate	3.84
11	34.928	n-Hexadecanoic acid	33.95
12	38.804	9,12-Octadecadienoic acid (Z,Z)-	1.63
13	38.970	9-Octadecenoic acid, (E)-	5.32
14	39.084	Oleic Acid	2.08
15	39.561	Octadecanoic acid	11.95
16	41.139	1,8-Diazacyclotetradecane-2,9-dione	2.78
17	46.687	Hexadecanoic acid, 2-hydroxy-1-(hydroxy	1.16
18	50.358	Dotriacontane	0.88
19	52.450	Squalene	4.51
20	61.698	Stigmasterol	0.79
21	63.144	.gamma.-Sitosterol	9.61
22	64.662	1,8-Diazacyclotetradecane-2,9-dione	2.35
23	66.060	Nonacosane-6,8-dione	1.32
24	66.590	.gamma.-Sitostenone	1.54
25	67.281	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,	1.97
			100.00

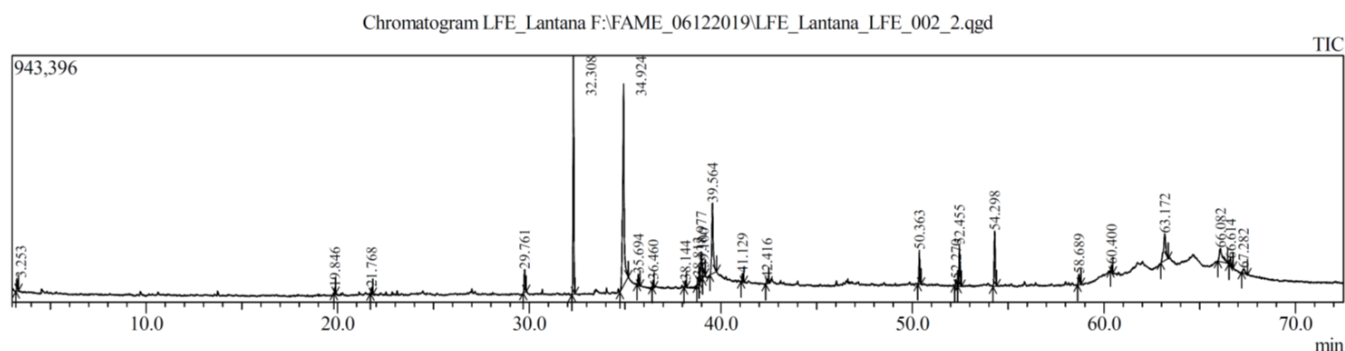


Fig 4: GC-MS Chromatograph of ethanoic extract of Lantana flower dye

Table 7: Phytochemical profiling of ethanoic extract of Lantana flower dye

Peak	R. Time	Name	Area%
1	3.253	(S)-(+)-2-Amino-3-methyl-1-butanol	1.00
2	19.846	Caryophyllene	0.44
3	21.768	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabic	0.58
4	29.761	Tetradecanoic acid	2.05
5	32.308	1,2-Benzenedicarboxylic acid, bis(2-methyl	19.30
6	34.924	n-Hexadecanoic acid	30.30
7	35.694	Hexadecanoic acid, ethyl ester	0.89
8	36.460	Heptadecanoic acid, methyl ester	0.55
9	38.144	9-Octadecenoic acid, methyl ester, (E)-	0.58
10	38.813	9,12-Octadecadienoic acid (Z,Z)-	0.75
11	38.977	Oleic Acid	3.40
12	39.100	Oleic Acid	1.27
13	39.564	Octadecanoic acid	10.20
14	41.129	1,8-Diazacyclotetradecane-2,9-dione	0.71
15	42.416	Glycidyl palmitate	0.54
16	50.363	Dotriacontane	2.91
17	52.270	Dotriacontane	0.68
18	52.455	Squalene	3.93
19	54.298	Dotriacontane	5.82
20	58.689	Dotriacontane	1.30
21	60.400	Triacosane, 1-iodo-	0.66
22	63.172	.gamma.-Sitosterol	6.05
23	66.082	Heptacosane-6,8-dione	4.18
24	66.614	.gamma.-Sitostenone	1.05
25	67.282	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,	0.85
			100.00

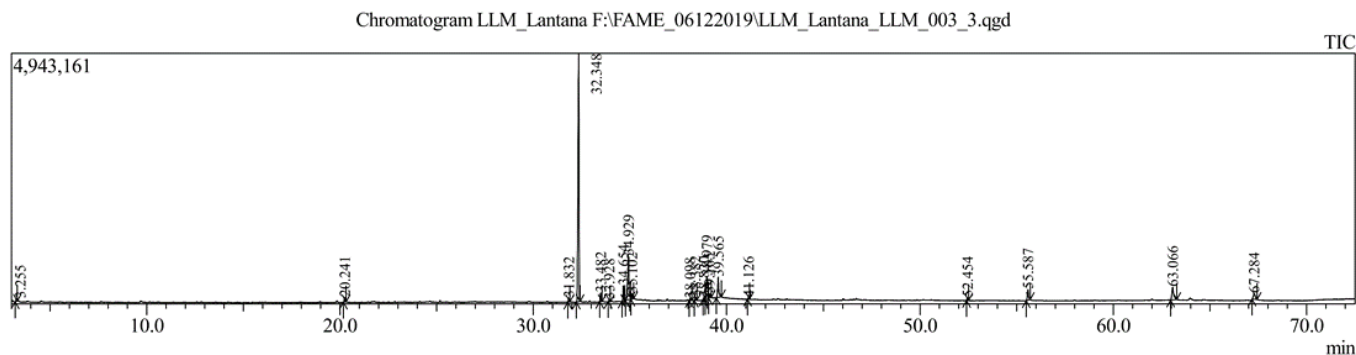


Fig 5: GC-MS Chromatograph of Methanolic extract of Lantana leaf dye

Table 8: Phytochemical profiling of Methonolic extract of Lantana leaf dye

Sl.no.	R. Time	Name	Area%
1	3.255	1-Butanol, 2-amino-3-methyl-, (+/-)-	0.45
2	20.241	Benzene, 1-(bromomethyl)-3-nitro-	0.94
3	31.832	Neophytadiene	0.68
4	32.348	1,2-Benzenedicarboxylic acid, bis(2-methy	52.57
5	33.482	1,2-Benzenedicarboxylic acid, bis(2-methy	1.50
6	33.928	Phthalic acid, hept-2-yl isobutyl ester	0.31
7	34.654	Dibutyl phthalate	2.73
8	34.929	n-Hexadecanoic acid	13.99
9	35.102	Phthalic acid, butyl 2-pentyl ester	1.31
10	38.098	9,12,15-Octadecatrienoic acid, methyl este	0.50
11	38.382	Phytol	0.64
12	38.820	9,12-Octadecadienoic acid (Z,Z)-	0.32
13	38.979	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	6.88
14	39.105	9-Octadecenoic acid, (E)-	0.89
15	39.565	Octadecanoic acid	5.33
16	41.126	1,8-Diazacyclotetradecane-2,9-dione	0.29
17	52.454	Squalene	0.52
18	55.587	Pectolinaringenin	2.75
19	63.066	6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6	4.95
20	67.284	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,	2.46
			100.00

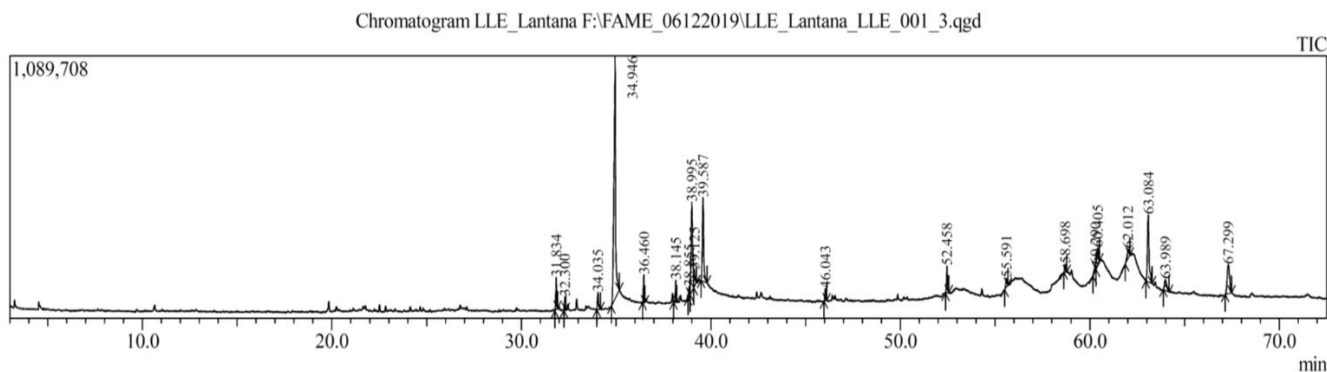


Fig 6: GC-MS Chromatograph of ethanoic extract of Lantana leaf dye

Table 9: Phytochemical profiling of ethanoic extract of Lantana leaf dye

Peak#	R.Time	Name	Area%
1	31.834	Neophytadiene	2.51
2	32.300	1,2-Benzenedicarboxylic acid, bis(2-methyl	0.95
3	34.035	Hexadecanoic acid, methyl ester	1.61
4	34.946	n-Hexadecanoic acid	37.07
5	36.460	Heptadecanoic acid, methyl ester	1.84
6	38.145	9-Octadecenoic acid, methyl ester, (E)-	1.69
7	38.855	cis-9-Tetradecen-1-ol	1.03
8	38.995	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	12.67
9	39.125	Oleic Acid	1.89
10	39.587	Octadecanoic acid	10.04
11	46.043	octadecanoic acid, 3-oxo-, ethyl ester	0.98

12	52.458	Squalene	2.42
13	55.591	Pectolinarigenin	1.01
14	58.698	Tetrapentacontane	0.79
15	60.290	Octanoic acid, 1-(hydroxymethyl)-1,2-etha	0.87
16	60.405	Tetrapentacontane	2.52
17	62.012	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,	1.60
18	63.084	6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)	10.04
19	63.989	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,	2.17
20	67.299	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,	6.31
			100.00

Table 10: Phytochemical screening of *Lantana camara* flower and leaf dye

Name of The Test	FLOWER		LEAF	
	Ethanol	Methanol	Ethanol	Methanol
Flavonoid test	+	+	+	+
Anthroquinones test	-	+	-	-
Alkaloids test	-	-	-	-
Terpenes test	-	-	+	-
Steroids test	-	-	-	-
Phenols	+	+	+	+

(+present and – absent)

Phytochemical screening

The flower and leaf from *Lantana camara* prepared using two solvents such as ethanol and methanol were tested for the presence or absence of Phenols, flavonoids, anthroquinones, alkaloids, terpenes and steroids. Results revealed that the flower and leaf from *Lantana camara* contained phenols and flavonoids in all the two solvents but flower dye extracted in ethanol contains Anthroquinones and leaf dye extracted from methanol contains (Table 10).

GC-MS (Gas Chromatography and Mass Spectrometer) analysis

The GC-MS profiling of bioactive compounds in flower dye revealed that in methanolic extract, 25 compounds were identified and presented in Table 6 and Fig. 3 and in ethanoic extract 20 compounds were identified and presented in Table 7 and Fig. 4. Among the identified compounds n-Hexadecanoic acid and 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) were the major compounds.

The GC-MS profiling of bioactive compounds in leaf dye revealed that in methanolic extract, 25 compounds were identified and presented in Table 8 and Fig. 5 and in ethanoic extract 20 compounds were identified and presented in Table 9 and Fig. 6. Among the identified compounds n-Hexadecanoic acid and 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) were the major compounds.

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