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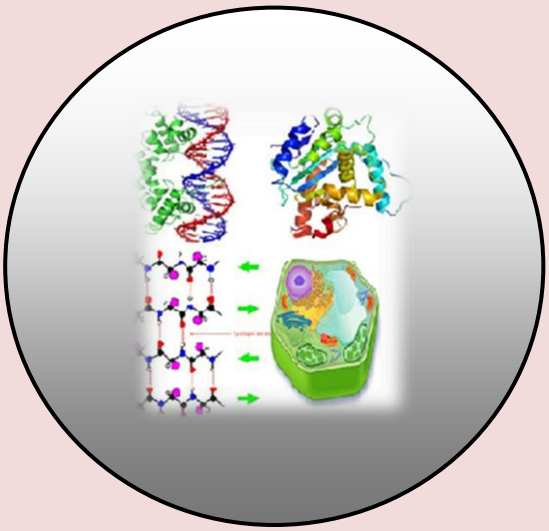

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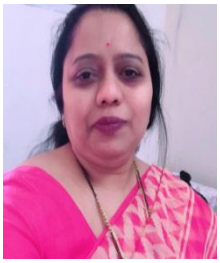

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A Study of Dyeing Characteristics and Antioxidant Activity of Natural Dye Extracted from *Nerium indicum* Flowers

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ABSTRACT

There is a considerable inclination towards natural dyes worldwide in order to avoid hazardous effects of synthetic dyes on the environment and human health. Hence, in search of new natural source, an attempt has been made to extract the dye from Nerium indicum flowers. The spectrometric and antioxidant activity of natural colourant from Nerium indicum flowers have been analyzed in the present study. GC-HRMS profile of the dye extract showed the presence of 20 major bioactive components with varied area percentage which may be responsible for the various biological activities and the colour. DPPH radical scavenging activity of the dye extract and its statistical analysis revealed the promising antioxidant activity when compared with standard ascorbic acid. It was also statistically confirmed that there is a positive relationship between concentration of dye extract and percentage of scavenging activity. The colour fastness properties of cotton fabric dyed with natural dye were also investigated and it was found that this natural dye exhibited very good colour fastness to light and dry rubbing, while good colour fastness was rated on the scale for washing and wet rubbing. Therefore, inheritance of significant antioxidant and colour fastness properties in the natural dye obtained from Nerium indicum flowers provides a huge potential for industry usage.

Keywords: Antioxidant activity, Colour fastness, GC-HRMS analysis, Natural dye, Nerium indicum flowers.

INTRODUCTION

Natural dyes are non-toxic, non-carcinogenic and non-allergic and their non-polluting as well as biodegradable nature makes them environment friendly whereas synthetic dyes uses petrochemical sources which releases harmful carcinogenic, mutagenic and allergic substances.

Hence, natural dyes have gained significant importance over artificial or synthetic dyes in recent times because of health and environmental conscious attitude of the society. This has necessitated the searching of plants which can be used as dyes. Besides this, various biological properties such as anti-allergic, antioxidant, antifungal and antibacterial properties of the dyes extracted from plants confer more value to them. In this context, research efforts have been carrying out all over the world in finding a dye yielding plant with novel biological activities. The different biochemical compounds present in the plant plays an important role in their biological activity and other characteristics such as colour, fragrance *etc.* These biochemical constituents can be better investigated through the spectral studies. Patil et al. 2019 have mentioned *Nerium indicum* as a dye yielding plant in a checklist of dye yielding resources of Maharashtra. *N. indicum* belonging to family *Apocyanaceae,* is an evergreen shrub which is often cultivated as an ornamental plant along roadsides, parks or gardens. The bright red to pink coloured flowers grow in clusters at the end of each branch providing attractive look to the plant. Previous studies by many researchers have reported the therapeutic value and medicinal usage of *N. indicum.* It is used as traditional medicine in different parts of the world for the treatment of skin cancer, psoriasis, epilepsy, warts, ringworm, dermatitis, sores, eczema, corns, abscesses, herpes, scabies and warts (Dey and Chaudhari, 2014). Tantiado (2012) undertook a survey on exploiting medicinal plants in Iloilo, Philippines and found that *N. indicum* has been used to cure headache, fever and dermatological problems. In Kenya, hot aqueous extract of the seeds and leaves is used to cureupper respiratory tract and gastrointestinal infections (Nanyingi *et al.,* 2008). Local community of Kancheepuram district of Tamil Nadu, India has traditionally utilizes stem bark extract of the plant for relieving ear ache (Muthu *et al.,* 2006). The plant possesses antimalarial and antidiabiatic activities and therefore, local people in Calabria, southern Italy uses the plant for treating malaria (Tagarelli *et al.,* 2010) whereas it has been used as antidiabetic in Morocco (Bnouham *et al.,* 2002). This potency of *N. indicum* as a remedy for several diseases is attributed to its innate bioactive components. Very scanty literature was found on use of *N. indicum* in yielding natural dye. Therefore, the work in the present paper was undertaken to find out antioxidant activity and presence of various bioactive components in the dye extracted from *N. indicum* flowers and also to understand its colour fastness when applied on cotton fabric.

MATERIAL AND METHODS

Dye extraction

Conventional extraction

Fresh flowers of *Nerium indicum* were collected from Nagpur region. The collected flowers were dried in shade and crushed using grinder to obtain fine powder. The dye extraction was carried out for 60 minutes at boiling temperature with material to liquor ratio (MLR) 1:20 under acidic condition.

Soxhlet Extraction

The powdered sample of flowers was initially subjected to defatting using petroleum ether (60^{o}C-80^{o}C) which is followed by extraction of dye using Soxhlet extractor with methanol as a solvent. The methanolic dye extract was filtered using Whatman filter paper No. 1 and kept in open Petri plate at room temperature to get dry dye extract by evaporating methanol. 100mg/ml stock solution of the dye extract was prepared and used for GC-HRMS analysis and assessment of antioxidant activity.

Dyeing of Fabric

Plain weaved cotton fabric was selected for dyeing. The fabric was scoured prior to dyeing in order to remove any non-cellulosic particles. The dyeing was carried out at acidic pH in a dye bath for 60 minutes with MLR 1:20.

Evaluation of Colour Fastness Properties

Colour fastness to light, washing and rubbing was evaluated on the basis of degree of colour removal after light exposure, washing and rubbing treatment to the dyed fabric and the grades were assigned according to the grey scale as described in ISO-105-AO2 (Trotman, 1984).

Colour fastness to light

Dyed fabric was exposed to the direct sunlight for total 24 hours and colour fastness to light was assessed by comparing the colour fading due to sunlight exposure.

Colour fastness to washing

Dyed fabric was inserted in the soap solution (liquor to material ratio 1:50) for 30minutes. It was washed thoroughly with water and air dried under shade. After drying, the colour fastness was assessed.

Colour fastness to rubbing

Dry and wet rubbing fastness was determined. In dry rubbing test, the dyed fabric piece was rubbed 10 times with the finger covered with white cotton fabric. Same procedure was followed with wet white or undyed cotton fabric for wet rubbing test.

GC-HRMS analysis of dye extract

The methanolic dye extract was run in gas-chromatograph coupled with mass spectrometer to investigate the bioactive chemical compounds.HP5 column with dimension 60m × 0.32mwas used. Helium gas was used as carrier gas at a flow rate of 1 mL/min. The oven temperature was raised from 80^{o}Cupto 290^{o}Cwith the rate of 5^{o}C/ min. The total GC run was carried out for 35 minutes. The Mass Spectra was taken at 70 eV. Mass spectral range was 10-2000 amu. This analysis was carried out at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. The compounds were identified by comparing the spectrum of compounds in their library with the probable name, molecular weight and structure.

Antioxidant activity of dye extract

The antioxidant activity in the methanolic dye extract was studied using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging assay. Sample solutions of three concentrations *viz.,*100µg/ml, 200µg/ml and 300µg/ml were prepared in methanol from 100mg/ml stock solution of the dye extract. Ascorbic acid was used as standard with same concentration as that of sample solution. 0.5 ml of 0.1 mM DPPH solution prepared in methanol was added in each sample and also to standard, and final volume was made to 3ml with methanol. All the sample solutions and standard were incubated at room temperature in dark for 30 minutes. Absorbance was read thrice in spectrophotometer at 517nm after incubation. DPPH solution served as control. The DPPH radical scavenging activity was calculated using following formula:

$$\textbf{DPPH radical scavenging activity (\%)} = \frac{\mathbf{Ac - As}}{\mathbf{Ac}} \times \mathbf{100}$$

Where,

Ac = Absorbance of control.

As = Absorbance of sample/ standard with DPPH radical.

Statistical analysis of antioxidant activity

Correlation analysis was carried out to study the relationship between concentration of the dye extract and percentage scavenging activity. The significant antioxidant activity of the dye extract with respect to standard ascorbic acid was determined by hypothesis testing with t-Test: paired two samples for means.

RESULTS AND DISCUSSION

Dyeing of fabric and evaluation of colour fastness properties

Grey colour was imparted to the cotton fabric after dyeing with natural dye obtained from *Nerium indicum* flowers. The dyed cotton fabric was assessed for the colour fastness properties. The light fastness grade was 4/5 on grey scale and thus exhibited very good fastness to light. Similar result was observed for dry rub fastness whereas wet rub fastness and wash fastness were good with grade 4 on grey scale.

GC-HRMS analysis of dye extract

The bioactive compounds present in the extract can be easily detected through Gas Chromatographic- High Resonance-Mass Spectrophotometric (GC-HRMS) technique. The nature and structure of the various compounds present in the extract can be better investigated through the mass spectroscopic analysis of the different biocomponents eluted at different times (Jha *et al.,* 2015). GC-HRMS analysis of the dye extracted from *Nerium indium* flowers revealed the presence of twenty different compounds with different retention time (Figure 1). Their peak area %, molecular weight, formula and structure are represented in Table 1.Pregnan-3-ol-16-one, 20-[4-carboxypentanoyl] was showing highest peak area percentage 32.25 %.

Antioxidant activity of dye extract

The absorbance and percent scavenging activity for standard and sample dye extract at 100µg/ml, 200µg/ml and 300µg/ml were tabulated in Table 2.

Statistical analysis of antioxidant activity

Correlation analysis was done to find out the direction of the relationship between the concentration of the dye extract and the DPPH radical scavenging activity. On applying the correlation analysis to the standard and sample, coefficient of correlation between the dye concentration and percentage of scavenging for standard ascorbic acid and dye extract of *Nerium indicum* flowers was found to be 0.950 and 0.887 respectively (Table 3). Since the values coefficient of correlation in both cases were found to be positive and also near to 1, it is evident that there exist a strong positive correlation and hence it is inferred that the % of scavenging will increase with increasing concentration of the extract.

Further, an important objective of conducting this study was to gauge whether the antioxidant activity in case of dye extract of *Nerium indicum* flowers is significant or not when compared with standard. For statistically examine the same, a hypothesis testing was carried out as follows.

Hypothesis

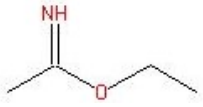
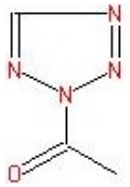
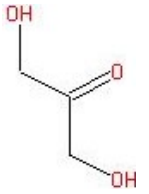
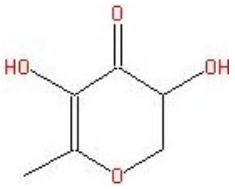
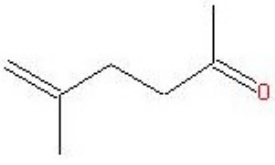
H_0: There is no significant difference in the antioxidant activities of dye extract of *Nerium indicum* and ascorbic acid.

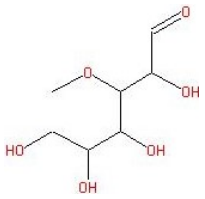
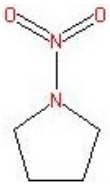
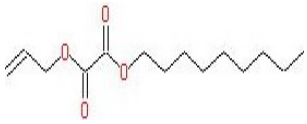
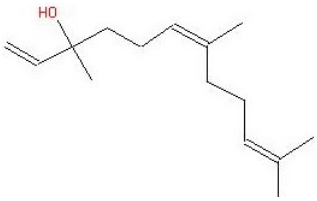
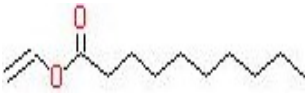
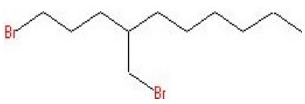
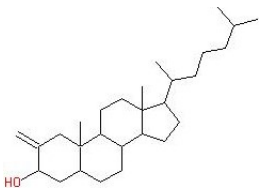
H_1: There is a significant difference in the antioxidant activities of dye extract of *Nerium indicum* and ascorbic acid.

On running the t-Test to the data of table 2, the t-calculated (1.41) was less than the t critical (4.3) at significance level of 5% with two degrees of freedom (Table 4). Therefore, the null hypothesis is to be accepted which implies that there is no significant difference in the antioxidant activities of dye extract and ascorbic acid. Hence, the statistical analysis has revealed that the dye extract of *Nerium indicum* flowers has significant antioxidant activity as there was no difference found in this respect when compared with the standard (ascorbic acid).

Jadav and Ninge Gowda (2017) studied antioxidant activity of cotton fabric dyed with natural colourant extracted from *Araucaria columnaris* bark peel and found that it has potential application for textile dyeing owing to strong antioxidant property. Previous studies have reported that *Nerium indicum* flowers' dye extracts have noteworthy antibacterial activity against *Bacillus subtilis* and *Salmonella abony* with zone of inhibition 24mm and 14mm respectively (Dongare *et. al.*, 2019). Hence, dye extracted from *Nerium indicum* flowers can be efficiently used to dye textiles due to their significant antibacterial and antioxidant activities.

Table 1. GC-HRMS analysis of dye extract.

Sr. No.	Retention Time (min.)	Peak area %	Name of the Compound	Molecular weight	Molecular formula	Molecular structure
1	6.97	0.82%	Ethyl acetimidate	87	C_4H_9NO	
2	7.21	0.68%	1-Tetrazol-2-ylethanone	112	$C_3H_4N_4O$	
3	7.98	0.81%	Dihydroxyacetone	90	$C_3H_6O_3$	
4	13.13	1.03%	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	144	$C_6H_8O_4$	
5	15.78	0.93%	5-Methyl-5-hexen-2-one	112	$C_7H_{12}O$	

6	24.94	1.51%	n-Decanoic acid	172	$C_{10}H_{20}O_2$	
7	25.22	21.17%	3-O-Methyl-d-glucose	194	$C_7H_{14}O_6$	
8	30.07	0.10%	1-Nitropyrrolidine	116	$C_4H_8N_2O_2$	
9	30.37	0.18%	Oxalic acid, allylnonyl ester	256	$C_{14}H_{24}O_4$	
10	31.13	0.27%	n-Capric acid isopropyl ester	214	$C_{13}H_{26}O_2$	
11	31.83	1.45%	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-[S-(Z)]	222	$C_{15}H_{26}O$	
12	32.44	5.11%	Vinyl decanoate	198	$C_{12}H_{22}O_2$	
13	33.62	0.83%	1-bromo-4-bromo methyl decane	312	$C_{11}H_{22}Br_2$	
14	34.04	1.71%	1-chloroeicosane	316	$C_{20}H_{41}Cl$	
15	34.34	5.54%	5α-Cholestan-3β-ol,2-methylene	400	$C_{28}H_{48}O$	

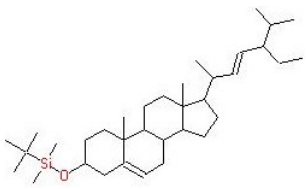
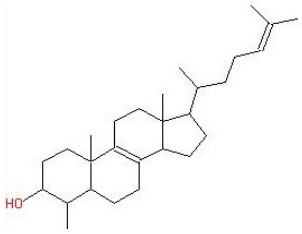
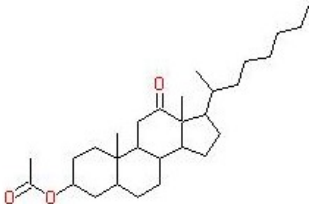
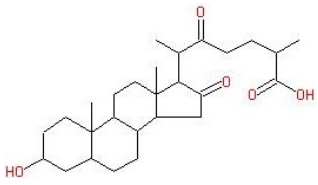
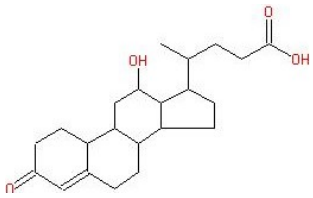
16	34.71	2.34%	Stigmasteroltbdms	526	$C_{35}H_{62}OSi$	
17	34.96	0.45%	4- Methyl cholesta-8,24-dien-3-ol	398	$C_{28}H_{46}O$	
18	35.42	21.05%	Pregnan-12-one,3-(acetyloxy)-20-hexyl-(3α)	444	$C_{29}H_{48}O_3$	
19	36.05	32.25%	Pregnan-3-ol-16-one,20-[4-carboxypentanoyl]-	440	$C_{27}H_{42}O_5$	
20	36.85	3.73%	12-Hydroxy-3-keto-bisnor-4-cholenic acid	360	$C_{22}H_{32}O_4$	

Table 2. DPPH radical scavenging assay of the natural dye extract.

Sr. No.	Particulars	Concentration (µg/ml)	Absorbance at 517 nm			% Scavenging			Mean of % Scavenging
1.	DPPH (control)	-	1.242	1.242	1.242	-	-	-	-
2.	Ascorbic acid (standard)	100	0.217	0.221	0.200	82.53	82.21	83.90	82.88
		200	0.085	0.085	0.086	93.15	93.15	93.07	93.12
		300	0.059	0.059	0.059	95.25	95.25	95.25	95.25
3.	Dye extract (sample)	100	0.364	0.364	0.364	70.69	70.69	70.69	70.69
		200	0.091	0.092	0.092	92.67	92.59	92.59	92.62
		300	0.077	0.077	0.077	93.80	93.80	93.80	93.80

Table 3. Correlation analysis between dye concentration and % scavenging.

1	Ascorbic acid (standard)		Dye concentration (µg/ml)	% Scavenging
		Dye concentration (µg/ml)	1	
		% Scavenging	**0.950066**	1
2	*Nerium indicum* dye extract		Dye concentration (µg/ml)	% Scavenging
		Dye concentration (µg/ml)	1	
		% Scavenging	**0.887861**	1

Table 4. t-Test: Paired Two Sample for Means CCC.

	Ascorbic acid (standard)	***Nerium indicum* dye extract**
Mean	90.41666667	85.36888889
Variance	43.73523333	162.7940704
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t calculated	1.413490286	
t Critical two-tail	4.30265273	

CONCLUSION

This study enlightens *Nerium indicum* flowers as a natural source of dye owing to its significant antioxidant and overall good colour fastness properties. Nowadays, natural dyes are gaining significant importance due to their soothing colours, biodegradability and higher compatibility with the environment and thus these dyes with inherent biological activities further enhance the quality of the product to be dyed. In the present study, the substantial antioxidant property of the dye extracted from *Nerium indicum* flowers has been found and furthermore, the spectral study reveals 20 major bioactive compounds in the dye extract which may account for the beneficial properties of the herbal dyes. Thus, in depth insight regarding the antioxidant and colour fastness properties of *Nerium indicum* flowers dye extract along with details of major bioactive compound will be useful in creating new venture for the potential application of the natural colourants.

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