Textile Application of Natural Colourant from the Roots of *Eichhornia crassipes* (Mart.) Solms

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Abstract- *Eichhornia crassipes* (Mart.) Solms is a free-floating aquatic macrophyte growing normally to a height of 0.5 m to 1 m. This weed causes, substantial economic and ecological harm, warranting its removal, lest the impacts of the weed should become permanent. From an ecological perspective, the most effective, management approach is to make use of the weed for various purposes. The present study offers the novel scheme for utilization of roots of this invasive weed plant. In this instance the 80% methanol extract and methanol fraction from the roots of this weed were tested for its colouring properties on different textile substrates *viz*. wool, nylon, silk, cotton and polyester. Open beaker and infra red colouring based methods were utilized for colouring of different textile fabric substrates. The colour fastness testing and colorimetric evaluation of the coloured samples has been carried out employing standard methods. The 80% methanol extract as well as the methanol fraction exhibited good colouring property on wool, nylon and silk. The presence of alkaloids, flavonoids, tannins, terpenoids, quinones, phenols and other phytochemicals enables good colour yield/depth of 80% methanol extract and methanol fraction on substrates. Thus, the colour extract from the roots may prove to be effective for conventional colouring and may provide commercial importance of this natural colourant for textile applications.

Index Terms- *Eichhornia crassipes* (Mart.) Solms; natural colour; roots.

I. Introduction

Eichhornia crassipes (Mart.) Solms is a free-floating aquatic macrophyte growing normally 0.5 m to 1 m in height. The plant roots are fibrous, purplish black and feathery. It is a South American native and is thought to have originated in the Amazon basin [1]. The weed has beautiful large purple and violet flowers which make it popular ornamental plant for ponds. The Invasive Species Specialist Group (ISSG) has included this weed in a database of "*100 of the world's worst invasive alien species*". This invasive weed is now found in more than 50 countries in five continents [2].

The weed spread between continents and watersheds is mainly the result of its floating propagules and via human activities [3]. The weed plant populations have been known to double in just 12 days [4]. This aquatic weed reproduces both sexually and asexually, and has high growth rates [3]. Infestations of this weed block waterways, limiting boat traffic, swimming, fishing, causing impaired efficiency of irrigation and hydro power generation, reducing biodiversity and its conservational value by preventing sunlight and oxygen from reaching the water column, and changes the population of vectors of human and animal diseases [3-7].

The aquatic weed grows in shallow temporary ponds, wetlands and marshes, sluggish flowing waters, lakes, reservoirs and rivers. The aquatic weed plants can tolerate extreme fluctuation of water level and seasonal variations in flow velocity, and extremes of nutrient availability, pH, temperature and toxic substances [4]. The average annual productivity of *E. crassipes*, is 50 tons ash-free dry weight basis per hectare per year, which makes it one of the most productive plants in the world [8-9]. Due to its uncontrolled and rapid growth, *E. crassipes* has apparently become a problem in different parts of the world, and therefore there is a need to manage its spread through suitable control measures [9].

Control management strategies for *E. crassipes* can be watershed management (to reduce nutrient supply) and direct weed control (e.g. by introduction of biological control agents) [4]. The main management approaches are physical, chemical and biological control. However, the reality remains persistent that this invasive weed has effectively resisted all attempts of its eradication by chemical, biological, mechanical, or hybrid means [8-10]. Thus, from an ecological perspective, the simplest, and usually least expensive, management approach is to make use of the weed for various purposes like phytoremediation, power alcohol production, biogas production, in compost, in animal fodder/fish feed, etc. [9].

The present work has been undertaken for utilization of root extract of this invasive weed plant as a natural colourant on different textile fabric substrates *viz*. wool, nylon, silk, cotton and polyester, so as to determine its utility for human welfare.

II. Materials and Methods

The plant material of *Eichhornia crassipes* (Mart.) Solms were collected from Harike wetland in The Tarn Taran district of the Punjab state in India. The plants were grown in Hoagland solution. The roots were separated from mature plant and utilized for the present study.

2.1 Preparation of crude extract

The dried roots were chopped into small pieces and extraction from roots was carried out thrice in 80% methanol. The dried roots were soaked in a 100:1 (v/m) ratio of 80% methanol and shaken continuously for 2 days on a shaker platform, at 25 °C temperature. The supernatant was collected and filtered with Whatman number 1 filter paper. The extract

filtrate was dried in rotary evaporator under vacuum, weighed and stored in dry solid form until required.

2.2 Polarity wise separation of crude extract

The separation of crude extract was carried out, from lower to higher polarity solvents. The separation was performed with diethyl ether, chloroform and methanol by separating contents of crude extract dissolving in respective solvents.

Out of the crude extract and its fractions, the crude and methanol fraction was utilized as a natural colourant, due to its higher polarity, amount and comparatively better dissolving property in deionised water.

2.3 Phytochemical screening of crude extract and methanol fraction [11-12]

2.3.1 Alkaloids

2.3.1.1 Mayer's test: About 0.5-1 ml of extract/fractions was treated with few drops of Mayer's reagent. Formation of cream coloured precipitates confirms the presence of alkaloids.

2.3.2 Flavonoids

2.3.2.1 NaOH test: About 0.5-1 ml of extract/fractions was treated with aqueous NaOH and HCl. Formation of yellow orange colour precipitates confirms the presence of flavonoids.

2.3.2.2 H_2SO_4 test: A fraction of extract/fractions was treated with concentrated H_2SO_4. The presence of flavonoids was confirmed by the formation of orange colour.

2.3.3 Tannins

2.3.3.1 $FeCl_3$ test: About 0.5-1 ml of extract/fractions was treated with 0.1% $FeCl_3$ Formation of brownish green or blue black colour confirms the presence of tannins.

2.3.4 Terpenoids

2.3.4.1 Salkowski Test: About 0.5-1 ml of extract/fraction was treated with $CHCl_3$ and few drops of concentrated H_2SO_4 and shaken vigorously. Formation of yellow colour at lower layer confirms the presence of terpenoids.

2.3.5 Anthocyanins

2.3.5.1 NaOH test: About 0.5-1 ml of extract/fractions was treated with 2 M aqueous NaOH. The presence of anthocyanin was detected by the formation of blue green colour precipitates.

2.3.6 Quinones

2.3.6.1 HCL test: A fraction of extract/fractions was treated with concentrated HCl. Formation of yellow colour precipitates confirms the presence of quinones.

2.3.7 Phlobatanins

2.3.7.1 HCL test: A fraction of extract/fractions was boiled with 1% aqueous HCl. The red coloured precipitates formation confirms the presence of phlobatanins.

2.3.8 Phenols

2.3.8.1 $FeCl_3$ test: A fraction of extract/fractions was treated with 5% $FeCl_3$ Formation of deep blue/black colour confirms the presence of phenols.

2.3.8.2 Liebermanns test: A fraction of extract/fractions was heated with sodium nitrite, treated with H_2SO_4 solution, diluted with water and excess of dilute NaOH was added. The presence of phenols was confirmed by the formation of deep red or green colour.

2.3.9 Saponins: A small amount of extract/fractions was mixed with water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion confirms the presence of saponins.

2.4 Colouring procedure:

The colouring characteristics of crude and methanol fraction on different substrates like wool, nylon, silk, cotton and polyester were carried out employing open beaker colouring and IR-heated lab colouring using Infra-colour (RBE, Mumbai) machine. The colourant bath was prepared using crude extract and methanol fraction in deionised water.

2.4.1 Open beaker colouring

Colouring of substrates in open beaker was performed on water bath. The colourant bath was maintained at pH 4 by using glacial acetic acid. Temperature was raised to 100 °C and colouring was carried for one hour. Subsequently, substrate samples were rinsed in cold water. The samples were washed in a bath of liquor to material ratio of 20:1 using 0.5 g/l nonionic detergent at 60 °C for 15 minutes and dried at ambient temperature.

2.4.2 Infra-red colouring

High temperature colouring was performed on polyester using Infra-colour (RBE, Mumbai) machine. The colourant bath was maintained at pH 4. Process was carried out by raising the colourant bath temperature from 20 °C to 130 °C at 4 °C/min, for 60 min and rapidly cooling (9.9 °C/min) to 50 °C as shown in **Figure 1**. The coloured samples were rinsed with cold water and reduction cleared at 70 °C for 15 minutes. Subsequently, samples were rinsed successively with hot water detergent solution (70 °C, 5 min) and cold water. The samples were air dried at ambient temperature.

The colouring was carried out by open beaker colouring method and Infra-red colouring based method with colouring conditions and sample codes as shown in **Table 1**.

2.5 Fastness testing and colorimetric evaluation:

The coloured samples were tested according to standard methods. The specific tests were as follows: ISO CO6 C2S, colour fastness to washing test; IS-766-88, colour fastness to rubbing; and ISO 105-B02, colour fastness to light (xenon arc) [13]. The colorimetric evaluations of coloured samples were determined using Spectraflash 600 colorimeter (Datacolor International) using CIELAB, 1976. Colour space, D_{65} illuminant, 10° observer [14].

2.5.1 Colour fastness to washing

Standard method for testing colour fastness to washing (ISO 105 CO6 C2S) was performed on coloured samples. ECE reference detergent (4 g/l), sodium perborate (1 g/l) and steel balls were employed in the test and pH 10.5 was maintained using sodium carbonate if required. Coloured samples strips of 10×4 cm dimension were cut and stitched through the short end of SDC's multifibre test fabric. The test was performed on Washtech (RBE, Mumbai) at 60 °C for 30 minutes. Then samples were rinsed with cold deionised water and air dried at ambient temperature.

2.5.2 Colour fastness to rubbing

Standard method testing for colour fastness to rubbing (IS-766-88) was performed on coloured samples using crockmeter with dry white piece and wetted white piece of cotton fabric. The

amount of colour fastness and staining was assessed against grey scale by colorimetric analysis.

2.5.3 Colour fastness to light

Standard method testing for colour fastness to light (ISO 105-B02) was performed on coloured samples by xenon arc fading lamp test using blue wool reference sample.

2.5.4 Absorbance and colour strength evaluation

The absorbance of the colour baths was recorded before and after colouring on a Systronics PC based double beam spectrophotometer 2202. The colour strength (*K/S*) values of the coloured samples were evaluated by light reflectance technique and the values were assessed using spectraflash 600 colorimeter (Datacolor International).

2.5.5 Colorimetric analysis

The colorimetric analysis of coloured samples was evaluated at D_{65} illumination, 10° observer using spectraflash 600 colorimeter (Datacolor International).

2.6 Toxicity testing

Kirby-Bauer Disk Diffusion Susceptibility Test of crude extract was carried out on wild strain of *Escherichia coli* and *Bacillus subtilis* [15-16] with ampicillin as positive control. The filtrates were obtained from dyed fabric, using a liquor ratio of 1g fabric/20 ml of sterilized H_2O at 20 °C, shaken and incubated for 18 h at 37 °C [17]. Preparation of overnight bacterial culture was carried out using autoclaved Luria Broth (LB) medium (2%) in deionised water on shaker at 37° C for bacterial growth. The turbidity of the medium justifies the growth of the bacterial strains. The autoclaved LB-agar medium (LB-2% and agar-1.5%) was prepared and poured into autoclaved and sterilized petri dishes (90 mm) in a laminar hood. The medium was allowed to come at room temperature. The autoclaved top agar medium (agar and NaCl) was prepared and subsequently used for inoculating and spreading of bacterial strains (100 µl petridish^{-1}) on to solidified LB-agar medium. Disks of 4 mm diameter were prepared by Watman number 1 filter paper, autoclaved and sterilized. The appropriate filtrate-impregnated sterilized disks were placed on the surface of the LB-agar, using sterilized forceps. Once all disks were placed, incubation was carried out in a 37° C air incubator for 24-48 h. Following incubation, zone sizes were measured to the nearest millimeter using a ruler.

III. Results and Discussion

The crude extract yield was found to be 6% of total dry weight of roots. The methanol fraction yield out of crude extract was found to be over 50% of total weight of crude extract. The colour of crude extract appears to be black brown and methanol fraction appears to be reddish brown. The results of the presence/absence of the various phytochemicals in the crude extract and methanol fraction of the roots of *Eichhornia crassipes* (Mart.) Solms are shown in the **Table 2**. The presence of different phytochemicals enables good colour yield/depth of crude extract and methanol fraction on substrates, which may be attributed by synergistic effect of mixture many innate compounds within plant roots.

Initially the colouring on substrates like wool, nylon, silk, cotton and polyester were carried out to select fabric with maximum and constant colour yield. Colouring of substrates *viz*. wool, nylon, silk and cotton were initially performed by open beaker colouring method. Resulted samples showed maximum colour yield on wool and nylon, moderate on silk and minimum on cotton substrate.

High temperature colouring was performed on polyester using Infra-colour (RBE, Mumbai) machine by IR-heated lab colouring method. Resulted sample showed different hues on substrate but with good colour yield. Therefore, polyester was not employed for further colouring process due to unevenness of colour on the sample.

The results showed that colour diffusion into wool, nylon and silk was maximum, which may be due to the presence of functional groups *viz*. $–NH_2$, –COOH and –CO–NH– in the fiber. Considering the results, wool, nylon and silk were preferred for colouring.

The fastness properties of the coloured fabrics are shown in **Table 3**. The results of colour fastness to washing in terms of grey scale ratings showed good fastness properties for WCF and SCF, moderate rating for NMF, average ratings for WMF and NCF and below average rating for SMF. The results of colour staining to washing showed good fastness properties except WCF and NCF which showed moderate fastness ratings on multifibre strips towards acrylic component and worsted wool component respectively.

The results of colour fastness to light showed moderate rating for WMF and WCF and average rating for SMF and SCF on grey scale. The results of colour fastness to rubbing showed moderate to good fastness properties on grey scale ratings. The results of colour staining to rubbing showed good to excellent fastness results on grey scale ratings.

The colourant bath made from crude extract and methanol fraction were evaluated for its absorbance before and after colouring of samples as shown in the **Figure 2**. The absorbance graphs clearly show the good colour uptake by fabric samples. Also, **Figure 3** shows the *K/S* graphical representation of coloured samples after colouring from crude extract and methanol fraction, in which continuous line represents wool, square bulleted line represents nylon and dotted line represents silk. The *K/S* graph clearly represents that nylon samples show maximum absorbance of colour whereas wool and silk shows minimum absorbance in case of colouring from crude extract and from methanol fraction respectively.

The evaluations of colour-coordinates of coloured samples are shown in **Table 4**. All colour-coordinates are positive with respect to brightness L*, red-green a*, yellow-blue b*, chroma C* and hue h. The colour yield on wool and nylon appears to be yellowish light brown and light brown respectively; simultaneously, the colour yield on silk appears to be golden yellowish brown for methanol fraction and golden light brown for crude extract, as shown in **Figure 4** and **5**.

The filtrates from dyed fabrics were found to show no toxic effect on both bacterial strains used (*B. subtilis* and *E. coli*) in disc diffusion assay as shown in **Fig. 6** and **7**. This study shows that the dyes were not toxic to both gram positive and gram negative bacterial strains.

IV. Conclusion

It may be concluded that the colouring from the crude extract of the roots of *Eichhornia crassipes* (Mart.) Solms appears to be simple and cost effective management approach to make use of this weed for textile applications. The colouring effect may be attributed due to the synergistic effect of alkaloids, flavonoids, tannins, terpenoids, quinones, phenols and other phytochemicals present within the plant roots. The coloured substrates showed better fastness properties especially on wool substrate, however all substrates showed good colour depth/yield. The work may provide basis for utilization of roots of this invasive plant on commercial scale.

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Table Captions
Table 1 Colouring conditions for different samples
Table 2 Qualitative estimation of phytochemicals in the crude extract and methanol fraction of the roots of *Eichhornia crassipes* (Mart.) Solms
Table 3 Colour fastness data[a] of the colour on samples
Table 4 Colorimetric data for coloured samples

Figure captions
Fig. 1 Colouring profile
Fig. 2 Absorbance of colourant bath before and after colouring of substrates
Fig. 3 *K/S* graphical representation of coloured substrates after colouring
Fig. 4 Colour of the samples from methanol fraction
Fig. 5 Colour of the samples from crude fraction
Fig. 6 Disk Diffusion assay on *Bacillus subtilis*
Fig. 7 Disk Diffusion assay on *Escherichia coli*

Table 1

Sample	Sample codes	Colouring method
Wool (methanol fraction)	WMF	Conventional colouring (at boil)
Nylon (methanol fraction)	NMF	Conventional colouring (at boil)
Silk (methanol fraction)	SMF	Conventional colouring (at boil)
Wool (crude extract)	WCF	Conventional colouring (at boil)
Nylon (crude extract)	NCF	Conventional colouring (at boil)
Silk (crude extract)	SCF	Conventional colouring (at boil)

Table 2

S. No.	Phytochemicals	Tests	Crude extract	Methanol fraction
1.	Alkaloids	Mayer's test	+	+
2.	Flavonoids	NaOH test	+	+
		H_2SO_4 test	+	+
3.	Tannins	$FeCl_3$ test	+	-
4.	Terpenoids	Salkowski test	+	+
5.	Anthocyanins	NaOH test	-	-
6.	Quinones	HCl test	+	+
7.	Phlobatannins	HCl test	-	-
8.	Phenols	$FeCl_3$ test	+	+
		Libermann's test	+	+
9.	Saponins	Froth test	-	-

Table 3

Sample code	Washing fastness							Light fastness	Rubbing fastness			
	Colour fastness	Colour staining							Colour fastness		Colour staining	
		SCA[b]	BUC[c]	N[d]	P[e]	A[f]	WW[g]		Dry	Wet	Dry	Wet
WMF	3	4	4-5	5	5	4-5	4	3-4	4	4	5	4
NMF	3-4	4-5	4-5	5	5	4-5	4-5	2	4	3-4	5	5
SMF	2-3	4	4-5	5	5	5	4-5	3	3-4	3-4	5	5
WCF	4	4	4	4-5	4-5	3-4	4	3-4	3-4	3-4	5	5
NCF	3	4-5	4-5	4-5	4-5	4-5	3-4	2	3-4	4	5	4-5
SCF	4	4-5	5	5	5	5	4	3	4	4	5	4-5

[a]Grey scale ratings; [b]Secondary cellulose acetate; [c]Bleached unmercerised cotton; [d]Nylon 66; [e]Polyester; [f]Acrylic; [g]Worsted wool.

Table 4

Sample code	L*	a*	b*	C*	h	x	y
WMF	70.42	4.53	19.42	19.94	76.87	0.3656	0.3719
NMF	67.65	6.67	22.72	23.67	73.63	0.3790	0.3783
SMF	74.35	3.76	17.90	18.29	78.14	0.3588	0.3677
WCF	74.03	3.54	21.97	22.25	80.85	0.3672	0.3769
NCF	66.76	6.67	24.11	25.02	74.53	0.3828	0.3820
SCF	67.45	4.91	20.72	21.29	76.68	0.3712	0.3759

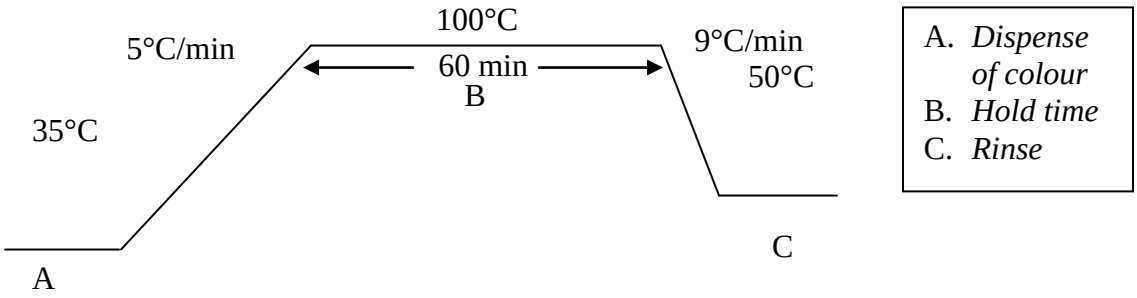
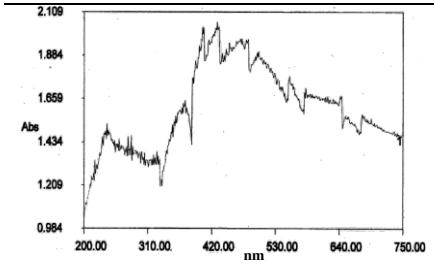
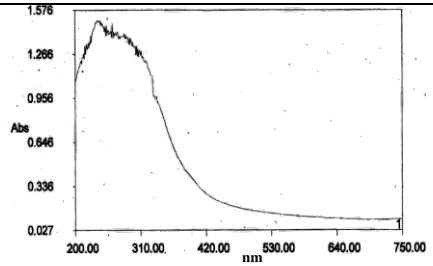

100°C
5°C/min
9°C/min
60 min
B
50°C
35°C
C
A
A. Dispense of colour
B. Hold time
C. Rinse

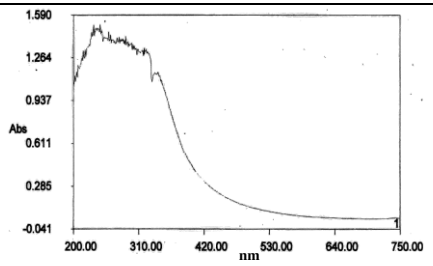
Fig. 1



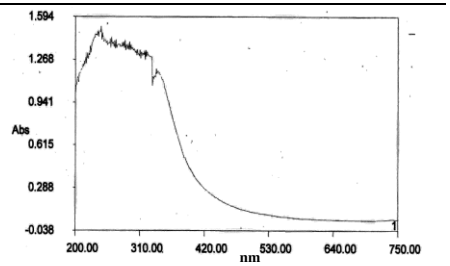
Absorbance of colourant bath made from crude extract before colouring



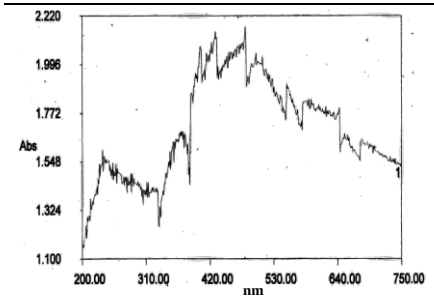
Absorbance of crude colourant bath left after colouring on wool substrate



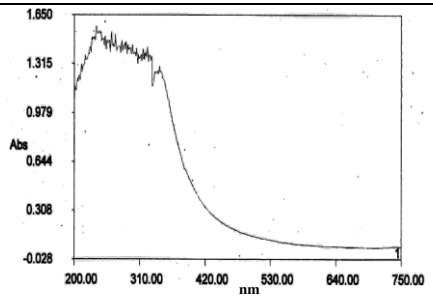
Absorbance of crude colourant bath left after colouring on nylon substrate



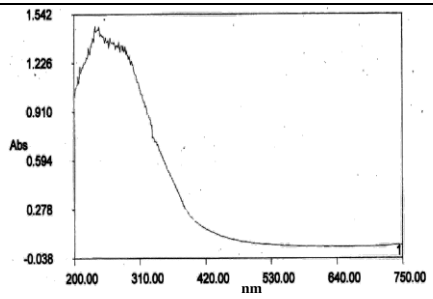
Absorbance of crude colourant bath left after colouring on silk substrate



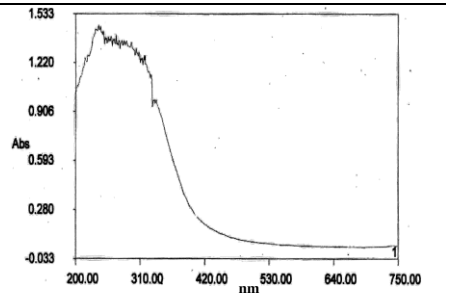
Absorbance of colourant bath made from methanol fraction before colouring



Absorbance of methanol colourant bath left after colouring on wool substrate

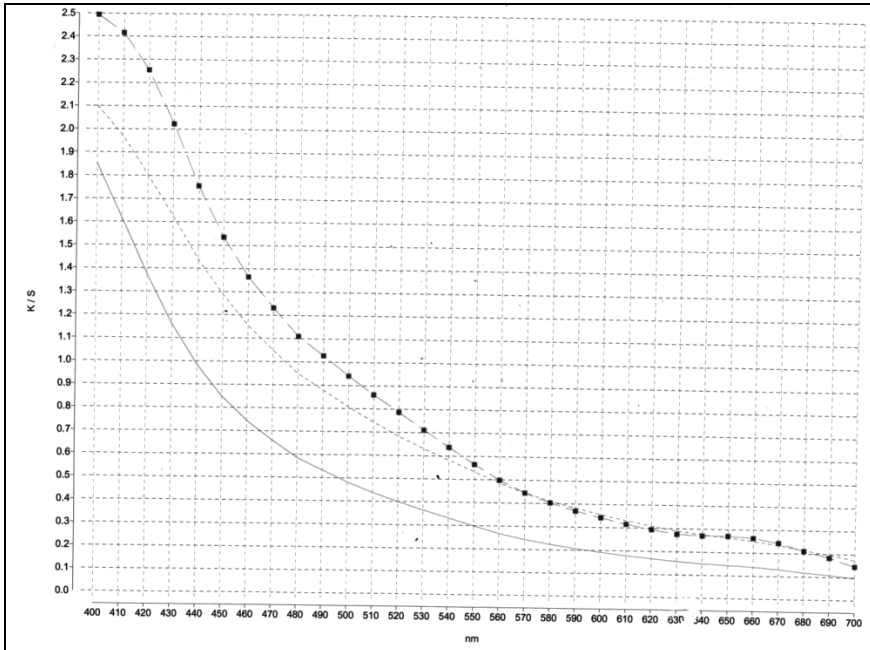


Absorbance of methanol colourant bath left after colouring on nylon substrate

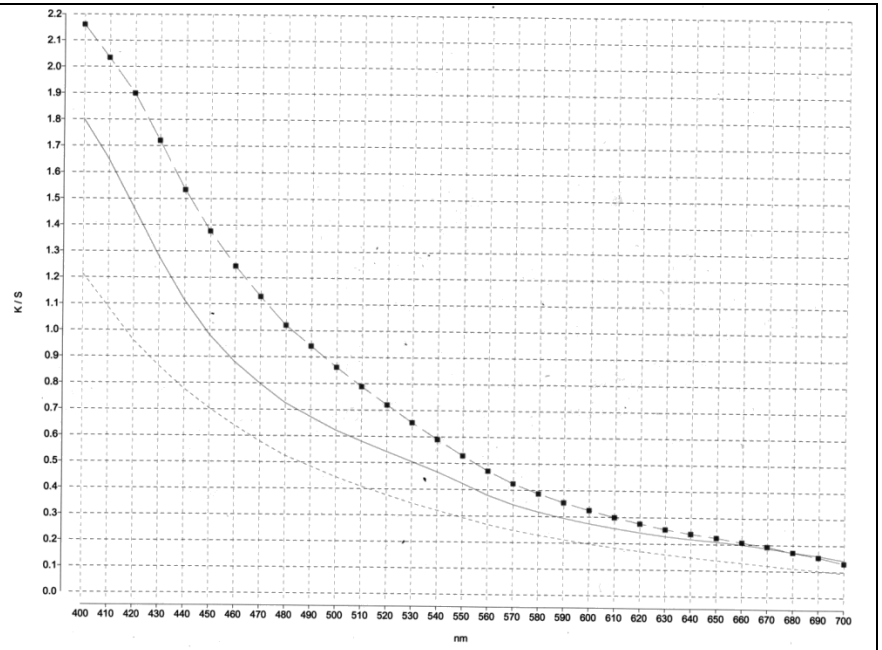


Absorbance of methanol colourant bath left after colouring on silk substrate

Fig. 2

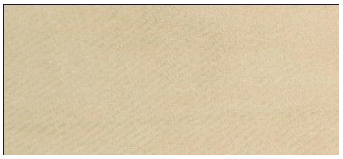


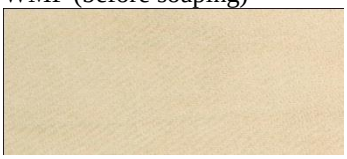
K/S of coloured substrates after colouring from crude extract



K/S of coloured substrates after colouring from methanol fraction

Fig. 3


WMF (before soaping)


WMF (after soaping)


NMF (before soaping)

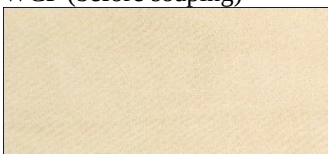

NMF (after soaping)

SMF (before soaping)

SMF (after soaping)

Fig. 4


WCF (before soaping)


WCF (after soaping)


NCF (before soaping)


NCF (after soaping)


SCF (before soaping)


SCF (after soaping)

Fig. 5

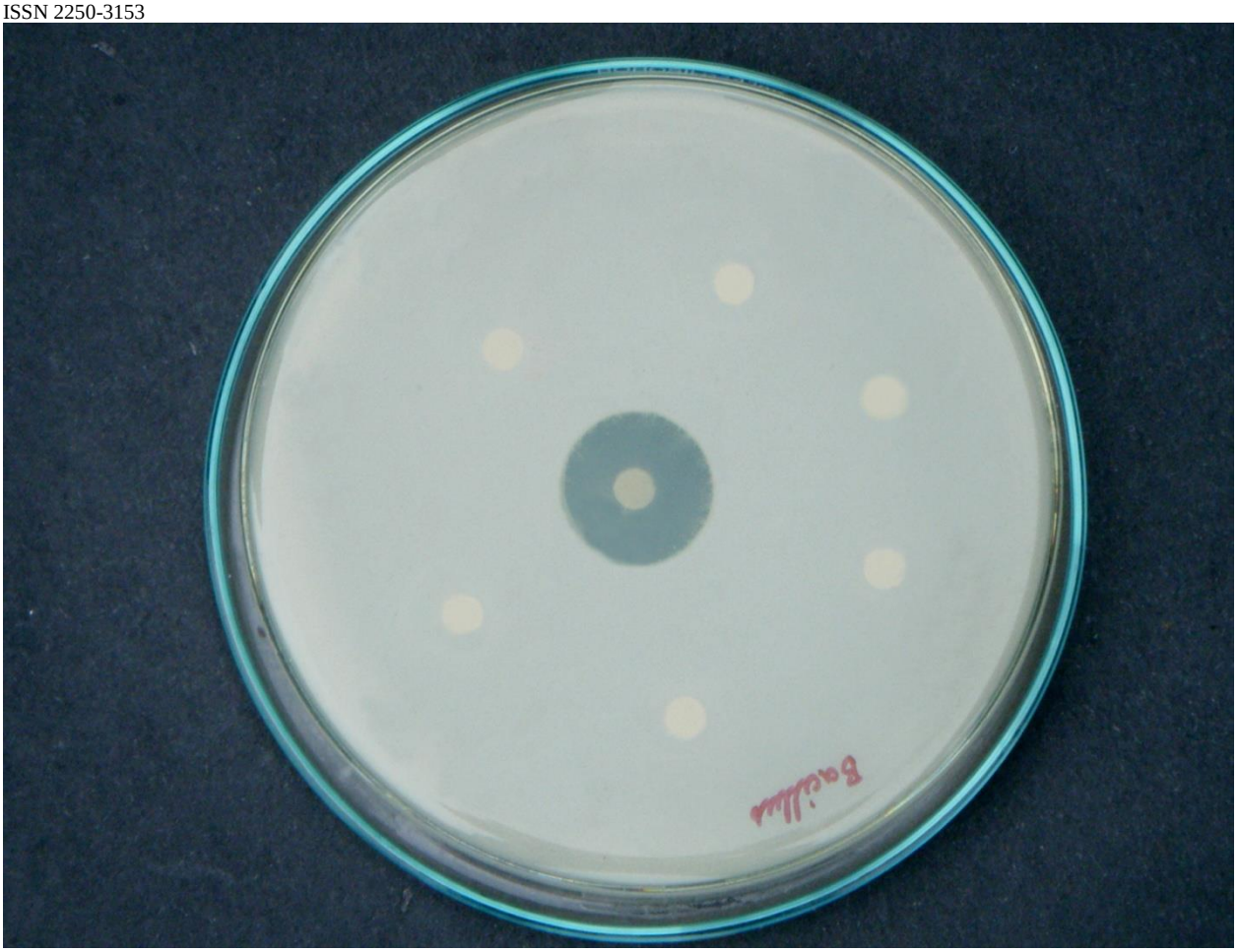


Fig. 6

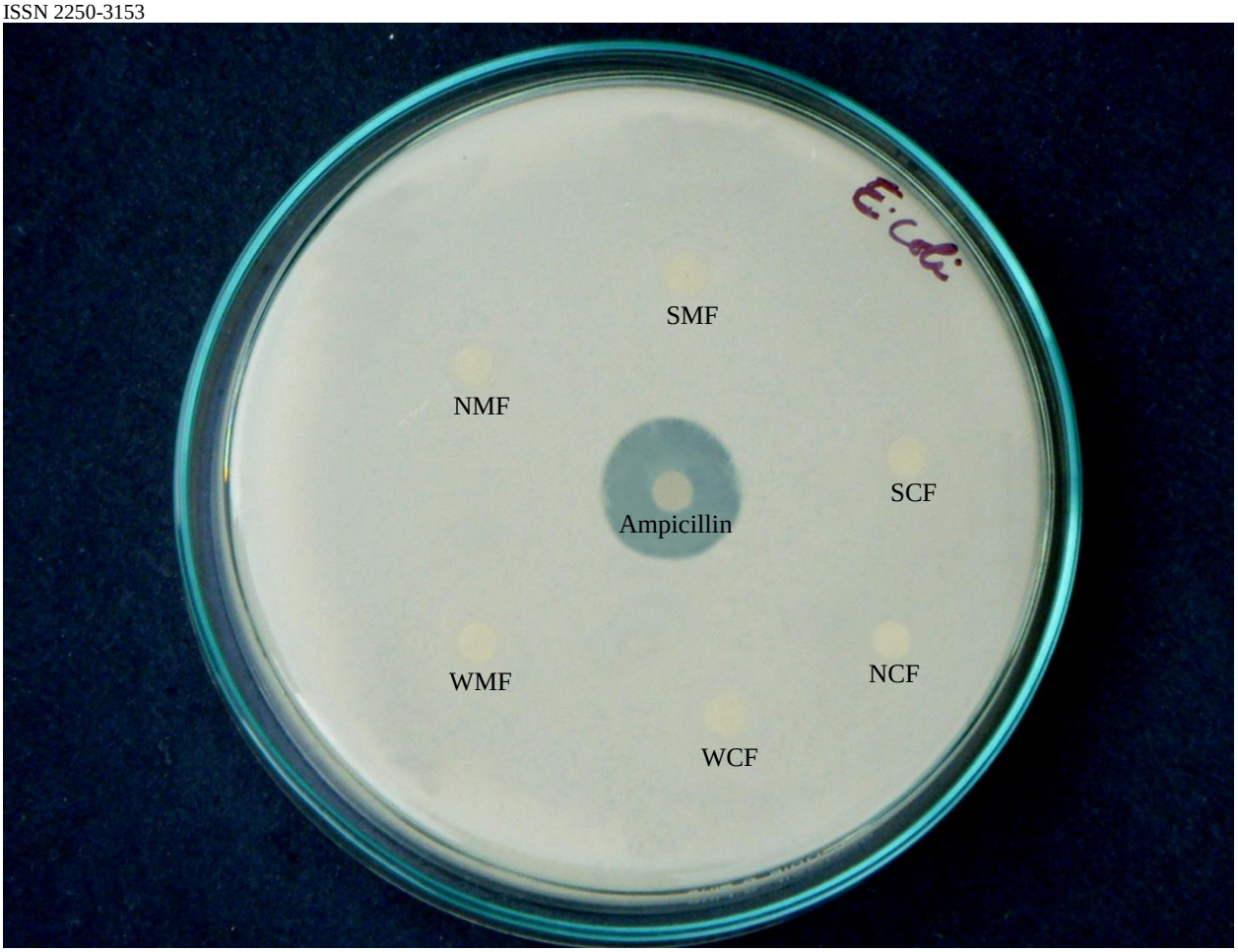


Fig. 7