Antibacterial Efficiency of Naturally Occurring Dyes and Mordants

TAIYABA NIMRA ANSARI (✉ taiyaba_ansari@rediffmail.com)

Government Holkar Science College <https://orcid.org/0000-0002-7718-5491>

Sanjeeda Iqbal

Government Holkar Science College

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Abstract

The popularity of natural dyes is increasing day by day due to their properties such as soothing colors, non carcinogenic, non hazardous and safe for environment. Some natural dyes also have been reported antibacterial properties. In present research work flowers of *Butea monosperma* and *Tagetes erecta* were used for dye extraction. Banana pseudostem sap and *Punica granatum* rind taken as biomordant. High performance thin layer chromatography was conducted for identification of components of *Butea monosperma*, *Tagetes erecta*, Banana (*Musa sp.*) pseudostem sap and *Punica granatum* rind. Dyeing of two types of cotton fabrics performed at pre-optimized conditions. The antibacterial activity of dyed fabrics was evaluated quantitatively and qualitatively. Research finding supported the usage of *Butea monosperma*, *Tagetes erecta*, Banana (*Musa sp.*) pseudostem sap and *Punica granatum* rind in textile dyeing process.

1 Introduction

Natural dyes are one of the most important natural products obtained from plants, animals, minerals, and micro-organisms. These dyes are used to color fabric, food, cosmetic products, and in decorative arts from the ancient period. After 1856 with the discovery of synthetic dyes, the popularity of natural dyes has diminished. Textile industries attracted to synthetic dyes which are cost-effective, fast in color and easy to apply on textiles. Wide application of synthetic dyes for long time resulted carcinogenic effects, mutagenic and non-biodegradable nature (Kiumarsi et al. 2017). Now a days awareness spreads for zero discharge emission, global warming, and ecosystem health for which green products are again in demand (Sagnella et al. 2014; Sangella et al. 2015). In this concern, people are also taking interest in hygiene and safe clothing. Cotton is one of the most common fabrics for apparels in all over the world. Cotton is produced naturally and a good substrate to grow on microorganism (Fu et al. 2011; Zhou and Kan 2015). As a hydrophilic nature, cotton easily provides suitable conditions, such as humidity, adequate temperature, and nutrition, for the growth of microorganisms, which generates an unpleasant odor, stains, and discoloration of the fabric. Therefore, it becomes necessary to apply an antibacterial finishing on cotton fabrics. The efficiency of any agent or substance which is used to mainly against bacteria for killing or inhibition is called antibacterial properties. Antibacterial finishes generally applied by using spun in additives, Padding, Spraying, Microencapsulation, Polymer modification etc. Many natural dyes have resistance towards bacterial colonies growth. Therefore, in this research work dyeing of cotton fabric is done with natural dyes and mordants to explore safe and hygienic clothing.

Flowers of *Butea monosperma* and Marigold were taken as a dye source. However, Pomegranate rind and Banana pseudostem sap is taken as mordant. Most of the natural dyes have very low affinity towards fabric, so a mordant (fixing agent) required to fix dyes on fabric. Chromium, copper, iron, tin and tannic acid etc. are metal salts used in synthetic dyeing extremely toxic either to human health, ecological health or both. Only a small amount of these metal salts get fixed on the fabric and the rest is discharged as effluent which leads to the contamination of land and water resources. Pomegranate rind and Banana

pseudostem sap are generally considered as waste. Here is an attempt to utilize the waste materials in dyeing process which are natural by products.

2 Objectives

- Evaluation of natural mordant qualities.
- Phyto-chemicals screening of dyes & mordants.
- Enumeration of antibacterial efficiency of dyed fabrics.

3 Results And Discussion

In present study, the dye stuff obtained from flowers of *Butea* and Marigold used for coloring of cotton fabrics at optimizing dyeing conditions with Banana pseudostem sap and Pomegranate rind as mordants. The detection of class of compounds in both dyes and mordants were done with the high performance thin layer chromatography (HPTLC). Inhibitory effects of natural dyed fabric were evaluated against gram positive and gram negative bacteria.

3.1 Bio-mordanting of fabrics

The natural substances used for fixation of dye are known as bio-mordants. Bio-mordanting was performed on two different types of cotton fabric. Banana pseudostem sap and Pomegranate rind extract were taken for the same. Orange and yellow dyes were obtained from the flowers of *Butea* and Marigold after extractions. For bio-mordanting of fabric, concentrations of mordants were selected through series of experimental trials. Constant dye concentration for dyeing was carried out for both fabrics. A range of orange and yellow shades have been developed on cotton through optimizing parameters and appropriate mordanting. Banana pseudostem sap has pale yellow in color which does not show any dominance on dyed fabrics whereas, Pomegranate rind extraction was dusty in color which showed a slight dusty effect on dyed fabric. However, the present investigation exhibited that both mordants have excellent efficiency to fix dye on fabric.

3.2 HPTLC analysis of dyes and mordants

Identification of major class of the compounds in the dyes and mordants were done with the help of HPTLC Chromatography. The details of detection of compounds are reported in Table 1.

HPTLC Chromatograms of *Butea monosperma*, Marigold, Pomegranate and Banana pseudostem sap are represented in Fig. 1.

Table 1
Rf of class of compounds

Class of compound	Name of the sample	Track no	No. of bands	Rf (Relative front)	Color
Alkaloids	<i>Butea</i>	2	1	0.42	Orange
	Marigold	4	0	-	-
	Pomegranate rind	6	0	-	-
	Banana pseudostem sap	8	1	0.42	Orange
Anthraquiones	<i>Butea</i>	2	2	0.6	Yellow
				0.69	Blue
	Marigold	4	1	0.82	Blue
	Pomegranate rind	0	0	0	
	Banana pseudostem sap	8	1	0.69	Blue
Bitter Principles	<i>Butea</i>	2	7	0.15	Brown yellow
				0.23	Yellow
				0.29	Orange
				0.49	Yellow
				0.54	Yellow
				0.81	Violet
				0.9	Violet
	Marigold	4	6	0.15	Brown
				0.45	Red Brown
				0.55	Red Brown
				0.68	Red
				0.82	Purple
				0.89	Purple
	Pomegranate rind	5	1	0.12	Brown
		6	2	0.56	Violet
				0.81	Purple

Class of compound	Name of the sample	Track no	No. of bands	Rf (Relative front)	Color
Essential oils	Banana pseudostem sap	8	2	0.14	Light yellow
				0.82	Violet
	<i>Butea</i>				
	Marigold	4	1	0.12	Blue
	Pomegranate rind	6	1	0.09	Blue
Flavonoids	Banana pseudostem sap				
	<i>Butea</i>	2	4	0.28	Aqua green
				0.32	light aqua green
				0.73	Blue
				0.78	Aqua green
	Marigold	4	1	0.93	Blue
	Pomegranate rind	0	0	0	
	Banana pseudostem sap	8	1	0.96	Blue
	<i>Butea</i>	2	4	0.27	Green
				0.67	Blue
				0.76	Blue
				0.97	Blue
		4	2	0.76	Blue
				0.86	Violet
		6	4	0.47	Blue
				0.59	Blue
				0.77	light blue
				0.85	Violet
	Banana pseudostem sap	8	1	0.12	Blue
Steroids	<i>Butea</i>	0	0	0	0

Class of compound	Name of the sample	Track no	No. of bands	Rf (Relative front)	Color
	Marigold	4	1	0.93	Purple
	Pomegranate rind	0	0	0	0
	Banana pseudostem sap	0	0	0	0
Sterols	<i>Butea</i>	2	2	0.64	light purple
				0.87	light purple
	Marigold	4	4	0.5	light purple
				0.65	Purple
				0.72	Purple
				0.83	Purple
	Pomegranate rind	6	2	0.63	Purple
				0.9	Purple
	Banana pseudostem sap	8	1	0.67	Purple
Triterpenoids	<i>Butea</i>	2	2	0.48	Violet
				0.87	Violet
	Marigold	4	3	0.66	Purple
				0.77	Purple
				0.87	Purple
				0.87	Purple
	Pomegranate rind	6	3	0.21	Violet
				0.49	Purple
				0.87	Violet
	Banana pseudostem sap	8	1	0.58	Violet

Through comparison of HPTLC chromatogram of the samples with chromatogram of standards of Alkaloids, Anthraquinones, Bitter Principles, Flavonoids, Saponins, Steroids, Sterols and Triterpenoids were detected. These compounds play an important role to provide special features to the dyes and mordants.

Alkaloids are the important secondary metabolites that were initially discovered and used as early as 4000 years ago and are well recognized for their rich therapeutic potential (Amirkia and Heinrich, 2014; Hussain and El-Anssary, 2018). Alkaloids have anti proliferative, antibacterial, antioxidant potential which

can be used for development of drugs (Qui et al, 2014). Alkaloids are an assembly of naturally occurring chemical composites which typically comprise basic nitrogen atoms. They may also contain some neutral or weakly acidic compound (Manske and Holmes, 2014). Alkaloids are low molecular weight structure and form approximately 20% of plant based secondary metabolites (Kaur and Arora, 2015).

Anthraquinones impart color to plants and have been widely used in natural dyes. They are also used as laxative and possess antifungal and antiviral activities. Anthraquinones as the name implies are photochemical compounds based on anthracene (three benzene rings joined together), Naturally occurring anthraquinones are a group of secondary metabolites structurally related to 1,10 dioxoanthracene and their glycosides.

The term bitter principle is usually used to indicate a group of natural products that have an extremely bitter taste and were traditionally used in liquid medicaments to stimulate appetite. Bitter principle are mainly of vegetative origin essentially comprise of C, H and O but rarely have or free from N.

Essential oils are aromatic, volatile liquid obtained from plant material through steam distillation and named after the plant from which they are derived. These fragrant substances are chemically pure compounds that are volatile under normal conditions (Rios, 2016). They also have medicinal application due to their therapeutic properties as well as agro alimentary uses because of their antimicrobial and antioxidant effect (Anand et al, 2019).

Saponins inhibit anti-inflammatory and immune boosting properties as well as antibacterial effects. In medicine they are used in vaccine formulation to regulate immune function. Additionally they act as antioxidants and scavenge oxidative stress. Saponins are naturally occurring bio-organic compounds having at least one glycoside linkage (C-O-sugar bond) at C-3 between a glycone and sugar chain.

A steroids is a biologically active organic compound with four rings arranged in a specific molecular configuration.

Sterols occur in the membranes of plants, animals, and microorganisms and are termed phytosterols, zoosterols, and mycosterols, respectively (Gordon, 2003). Sterols are monohydroxy alcohols with a four-ring core structure or steroid nucleus (Dupont, 2005).

Flavonoids are a class of phenolic compounds widely distributed in plants. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water (Trugo et al. 2003). They consist of two benzene rings connected by a short three carbon chain. One of the carbons in this chain is connected to a carbon in one of the benzene rings, either through an oxygen bridge or directly, which gives a third middle ring. They function as plant pigments and are responsible for the colors of many flowers and fruits. The biological activities of flavonoids have been demonstrated as anti-inflammatory, antihepatotoxic, antitumor, antimicrobial, antiviral, enzyme inhibitors, and antioxidant, and as having central vascular effects. Flavonoids also possess antibacterial, antiviral, and anti-inflammatory effects (Meserole, 2002).

Triterpenoids (such as lucidenic acids and ganoderic acids) have been reported to exhibit various biological activities, such as antibacterial, antiviral, antitumor, antiosteoclastic differentiation activity, anti-HIV-1, hepatoprotection, antioxidation, antihypertension, cholesterol reduction, and anti aggregation functions (Hsu and Yen, 2014). Triterpenoids metabolism plays an important role in medicinal plant drug discovery, development, and clinical uses. Triterpenoids are compounds with a carbon skeleton based on six isoprene units which are derived biosynthetically from the acyclic C 30 hydrocarbon, squalene. They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids (Ludwiczuk et al, 2017).

All above compounds have been regulated to the class of secondary plant products and great interest to the pharmaceutical industries. All compounds represent antibacterial properties that help to inhibit the bacterial colonies on fabrics too.

3.3 Assessment of antibacterial activity of dyed fabrics

Antibacterial efficiency of natural dye was evaluated against gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* using Agar Diffusion Method and Percentage Reduction Test. In *Butea* + Banana pseudostem sap dyed fabric 1 showed maximum zone of inhibition 11.00 ± 1.00 mm and 8.67 ± 1.53 mm with *E.coli* and *S.aureus* respectively. In fabric 2 Marigold + Banana pseudostem sap dye mordant combination showed maximum zone of inhibition 7.67 ± 2.08 mm and 10.33 ± 2.52 mm against *E.coli* and *S.aureus* respectively. However, maximum percent reduction in quantitative assessment of fabric 1 *Butea* + Banana pseudostem sap exhibited $53 \pm 4.58\%$ and $48.67 \pm 7.02 \%$ against *E.coli* and *S.aureus*. In fabric 2 *Butea* + Banana pseudostem sap showed $50.33 \pm 2.52 \%$ (*E.coli*) and Marigold + Banana pseudostem sap showed $54.33 \pm 2.52\%$ (*S.aureus*) bacterial resistance (Table 2&3).

In general Bacteria can easily grow on any substrate and cotton fabric is also a good source of food and nutrition. Two representative microorganisms are specified for this testing, *S. aureus* and *E. coli*. The method is useful for testing against both Gram Positive (*S. aureus*) and Gram Negative (*E. coli*) bacteria. Mostly types of *E. coli* are non- dangerous and even help keep our digestive tract healthy. But some strains can cause diarrhea if we eat contaminated food or drink fouled water. While many of us associate *E. coli* with food poisoning, we can also get pneumonia and urinary tract infections from different types of the bacteria. In fact, 75–95% of urinary tract infections are caused by *E. coli*. Some versions of *E. coli* make us sick by making a toxin called Shiga. This toxin damages the lining of our intestine. One especially bad strain, O157:H7, can make us very sick. It causes abdominal cramps, vomiting, and bloody diarrhea. It is the leading cause of acute kidney failure in children. It can also cause life-threatening symptoms such as: Adult kidney failure, Fever, Bleeding etc. *S. aureus* has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections as abscesses (boils), furuncles, and cellulitis (Sukumaran and Senanayake, 2016). *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections.

Table 2
Quantitative antibacterial assessment of natural dyed fabric

Sample of dyed fabric	Bacterial resistance in percent (Cotton 1)		Bacterial resistance in percent (Cotton 2) Mean $\pm$ SD	
	Mean $\pm$ SD			
	E. coli	S.aureus	E. coli	S.aureus
<i>Butea</i> + Banana pseudostem sap	53 $\pm$ 4.58	48.67 $\pm$ 7.02	50.33 $\pm$ 2.52	41.00 $\pm$ 4.58
<i>Butea</i> + Pomegranate	39.33 $\pm$ 2.52	37.67 $\pm$ 3.21	39.67 $\pm$ 2.52	37.67 $\pm$ 2.52
Marigold + Banana pseudostem sap	50.00 $\pm$ 3.00	41.33 $\pm$ 2.31	48.17 $\pm$ 2.75	54.33 $\pm$ 2.52
Marigold + Pomegranate	32.33 $\pm$ 3.21	40.33 $\pm$ 3.51	39.67 $\pm$ 5.13	40.24 $\pm$ 3.92
Values are given in mean $\pm$ SD for three replicates.				

Table 3
Qualitative antibacterial assessment of natural dyed fabric

Sample of dyed fabric	Zone of inhibition in mm (Fabric1)		Zone of inhibition in mm (Fabric 2) Mean $\pm$ SD	
	Mean $\pm$ SD			
	E. coli	S.aureus	E. coli	S.aureus
<i>Butea</i> + Banana pseudostem sap	11.00 $\pm$ 1.00	8.67 $\pm$ 1.53	7.00 $\pm$ 1.00	3.33 $\pm$ 1.53
<i>Butea</i> + Pomegranate	3.67 $\pm$ 1.53	2.33 $\pm$ 1.53	2.33 $\pm$ 1.53	1.67 $\pm$ 0.58
Marigold + Banana pseudostem sap	8.00 $\pm$ 1.73	5.33 $\pm$ 1.53	7.67 $\pm$ 2.08	10.33 $\pm$ 2.52
Marigold + Pomegranate	1.33 $\pm$ 0.58	3.00 $\pm$ 1.00	3.33 $\pm$ 0.58	5.33 $\pm$ 1.53
Values are given in mean $\pm$ SD for three replicates				

Significant results obtained by qualitative and quantitative assessment of antibacterial properties of naturally dyed fabrics 1&2.

In case of quantitative antibacterial analysis percent reduction of both the bacterial strains calculated in combination of dye and mordant. The highest percent reduction of bacterial colonies found in fabric 1 with combination of *Butea* and Banana pseudostem sap for *E.coli* as well as *S. aureus*. Similar results obtained for fabric 2. Another good combination of Marigold and Banana pseudostem sap has given better results of resistance for both fabrics and both the strains of bacteria. However, all the results of naturally dyed fabrics shown significant resistance of bacterial growth.

As far as qualitative assessment is concerned the zone of inhibition exhibited by both fabric types with two bacterial strains and four combinations of dye and mordant. The biggest size of zone of inhibition observed in *Butea* + Banana pseudostem sap dyed fabric 1 for *E.coli* and Marigold + Banana pseudostem sap combination of fabric 2 for *S. aureus* respectively. These results shown excellent properties of naturally dyed fabrics for antibacterial effects.

Where synthetic dyes and chemical mordants are hazardous to human life and environment. The natural dyes and mordants give protection to users from harmful bacteria.

HPTLC analysis of dyes and mordants gives a class of phytochemicals present in all natural dyeing materials. Antimicrobial properties of Alkaloids, Anthraquinones, Essential oils, Saponins, Flavonoids and Triterpenoids studied by many researchers also present in dyes and mordants used in this investigation. All these six types of phytochemicals were analysed in *Butea* and Banana pseudostem sap by chromatogram studies. Marigold showed 5 such phytochemicals and Pomegranate rind analyzed presence of only 3 chemicals having antibacterial properties. Both quantitative and qualitative assessment of natural dyed fabric given outstanding results might be shown due to presence of these phytochemicals possessing natural antibacterial properties. Contribution of higher number of these phytochemicals present in dye and mordant shown the highest biocidal effects on fabrics as given in the Table 2&3. Therefore, Excellent results of both the assessments shown by combination of *Butea* + B.Sap and Marigold + B.Sap having maximum number of such phytochemicals in them, which are responsible for antibacterial effects naturally. Such type of natural dyeing given antibacterial finishing to fabric without any cost in ecofriendly manner.

Thus, naturally dyed fabrics are better choice to save health and wealth of both human beings and environment. Hence, popularization of natural dyes, mordants and their use in dyeing and printing must be accelerated to recover the nature.

4 Conclusions

The research work has investigated a new side in dyeing of cotton with natural dyes. Dyeability and fastness properties of dyed fabric were studied successfully. Phytochemical analysis is one of the best tools to know about the presence of compounds in plant material used. HPTLC give us a long list of phytochemicals present in dyes and mordants with antibacterial properties. Study of antimicrobial properties of any dyed sample is one of the very important parameter to know about the quality of dye and mordant that becomes more valuable criteria when we work with natural dyes and mordants. In present investigations the antimicrobial properties against *E.coli* and *S. aureus* revealed the significant results of biocidal qualities of natural dye and mordant in combination. As this research work was done with two representatives of gram positive and gram negative bacterial group. Thus, this also highlights the possibilities of antimicrobial activity of dyed fabric for many such broad spectrum of bacteria present in nature which may cause harmful allergies and infections to humans. Synthetic biocides have some side effects on human skin and cause water pollution as well. Therefore, the natural biocides are best

option to control microbial growth on fabric as well as pollution problems of nature. The fabric dyed with colorant from *Butea monosperma*, Marigold, Banana pseudostem sap and Pomegranate rind extracts displayed excellent antibacterial activity against both test organisms used. Application of natural dye and mordant, which contains antimicrobial properties, will also reduce the extra expenses of synthetic biocides used by textile industries. In present investigation the dyed fabrics were found to be hygienic, non-toxic, antibacterial and environment friendly.

5. Material And Methods

5.1 Selection and collection of Dye

Butea monosperma (Flame of forest) is a deciduous tree native to tropical and subtropical parts of Indian subcontinent. The flower are long, bright orange in color. Flowers were collected between March to April. Petals were separated from rest of the flowers and preserved. Marigold (*Tagetes erecta*) is an annual or perennial herbaceous plant. Flowers were plucked directly from plants. Petals were dried at room temperature and stored in airtight condition (Fig. 2).

5.2 Selection and collection of Mordants

1. Pomegranate (*Punica granatum*) is a deciduous shrub. Pomegranate fruit has anti-oxidant, anti-viral and anti-tumor properties. Therefore, it has been used in large quantities by people. A Pomegranate is a sweet, tart fruit with thick red rind. It holds hundreds of juicy seeds, while the skin is not edible and discarded which is used in present research work to find out its qualities.

2. Banana (*Musa sp.*) plant is larger herbaceous flowering plant. The Banana plant dies after fruiting. Another sucker replaces it and the whole plant body such as leaves and pseudostem thrown away as a waste. Pseudostem contains liquid material in leaf sheath. In this concern both waste materials i.e. pomegranate rind and Banana pseudostem sap taken as mordants (fixing agents) for dyeing (Fig. 3).

5.3 Dyeing Process

5.3.1 Fabric selection: Two different type of cotton fabric taken i.e. handloom cotton (Khadi) as fabric 1 and power loom cotton as fabric 2 for study. Khadi was purchased from Khadi Bhandar (Indore Khadi Sangh) Rajwada Indore and fine cotton fabric procured from G. Sachanand kala mandir, Hukumchand marg, Indore.

5.3.2 Dye extraction (Win and Swe, 2008): Dried powders of dyes were taken for the preparation of aqueous extraction of dye. 15 Gram of dye powder (15% oven weight of fabric) weighed and poured in beaker and soaked in 100 ml water for 15 minutes at room temperature. Dye pot (beaker) kept on high temperature (95–100° C) till boiling then extracted for one hour at 60°C. The extraction liquor was then

filtered to obtain aqueous solution of dye. The temperature and time of extraction selected on the basis of reviewed literature and experimentation.

5.3.3 Biomordanting (Kumaresan, 2011): Banana pseudostem chopped in pieces and sap extracted with the help of grinder and stored in air tight bottles for further use. Dried Pomegranate rind grinded and soaked in water for 15 minutes then extraction process done for about an hour in water bath at 80°C. Extract was collected for mordanting.

5.3.4 Dyeing: Dyeing of fabric was performed under optimized conditions acquired through experiments. Fabric dyeing depends on concentration of mordants, pH, temperature and time. 15% concentration of dye was applied with both the mordants. However different concentration of mordants on cotton fabrics showed various color strength. Following concentration of mordants exhibited maximum color strength and taken for dyeing.

5.3.5 Sample selection for antibacterial activity study: On the basis of color strength of both fabrics in each dye and mordant combination 08 samples were selected to assess the antibacterial activity. Following concentration of mordant was selected for dyeing (Table 5).

Table 4
Selected concentration of mordant for each fabric.

Sample		Concentration of Mordant (gm/100ml)	
Dye	Mordant	Fabric 1	Fabric 2
<i>Butea</i>	Banana pseudostem sap	25	20
	Pomegranate	25	30
Marigold	Banana pseudostem sap	30	20
	Pomegranate	25	30

5.4.1 HPTLC analysis (Samanta and Konar, 2011): High performance thin layer chromatography was performed to identify the content of natural dyes obtained from *Butea monosperma*, *Tagetes erecta* and mordants such as dried powder of *Punica granatum* and *Musa paradisiaca* (banana) pseudostem sap.

HPTLC analysis was done at Anchrom Enterprises (I) Pvt. Limited by using HPTLC system (CAMAG, Switzerland). Predevelopment of stationary phase HPTLC silica gel 60F254 (Merck, Germany) was carried out by analytical grade methanol. HPTLC plate was kept in pre saturated twin trough chamber (10x20cm) for the development of samples. Following standardized developing solvent system (mobile phase) and derivatization were applied for the analysis.

Table 5
Mobile phase and derivative for class of compounds.

Class of compound	Mobile Phase	Derivative
Alkaloids	toluene: ethyl acetate: methanol: ammonia 25% (30:30:15:1) v/v/v/v	Dragendoff's solution
Anthraquinones	ethyl acetate: methanol: water (8:2:0.1) v/v/v	Alcoholic KOH
Bitter principle	ethyl acetate: methanol: water (8:2:0.1) v/v/v	vanillin sulphuric acid
Essential oil	Toluene: ethyl acetate (9.3: 0.7)	vanillin sulphuric acid
Saponins	Chloroform: acetic acid: methanol : water (6.4:3.2: 1.2: 0.8)	Anisaldehyde sulphuric acid
Steroids	n-butanol: methanol: water (3:1:1)	Anisaldehyde sulphuric acid
Sterols	Chloroform: ethyl acetate (4:6)	Anisaldehyde sulphuric acid
Triterpenoids	n-hexane : ethyl acetate (1:1)	Anisaldehyde sulphuric acid
Flavanoids	Ethyl acetate: formic acid : glacial acetic acid: water (10:0.5:0.5:1.3)	Natural product reagent

After development, scanning was executed in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV 366nm. The results were examined by using HPTLC Server LABSERVER, VERSION 2.5.18262.1 software .

5.4.2 Determination of R_f values

The identification of the separated compounds is usually based on their reaction with suitable location reagents and their position in the chromatogram.

The R_f value is constant for a given compound under standard condition.

Relative front (R_f) = Distance moved by the solute / Distance moved by the solvent

5.5 Antimicrobial Activity assessment of Dyed Fabric samples: Antimicrobial activity was evaluated by both qualitative and quantitative test methods.

5.5.1 Qualitative assessment by Agar Diffusion Method (SN 195920-1992)

This method was used to find out the antimicrobial efficacy of the treated fabrics qualitatively according to the Swiss standard- SN195920. For this test, bacteriostatic agar medium was used as a growth medium for evaluation. Non-sterile dyed samples of 1X1 cm and square in shape were taken as test

specimens. Undyed fabric samples used as control. The potato dextrose agar (PDA) was prepared and sterilized in an autoclave. In laminar air flow chamber, PDA added on a sterilized petri dish and allowed to harden for 30 minutes. The gram-negative *Escherichia coli* MTCC (Microbial Type Culture Collection and Gene Bank) 77 and gram-positive *Staphylococcus aureus* MTCC 96 culture (24 hours incubated) of the test organism were then evenly spread on separate agar plates using cotton swab. In this experiment, above two organisms were taken as representative bacteria for evaluation. The dyed and control fabric pieces (swatches) were placed on the solid agar inoculated with different bacterial cultures. The petri dish was then incubated at 37° C for 24 hours in incubator. At the end of the incubation time, the test dish was observed. The evaluation was made for the appearance of zone of inhibition around the fabric sample (swatch) measured in mm (milimeter).

5.5.2 Quantitative Assessment by Percentage Reduction Test (AATCC 100–2004)

AATCC Test Method 100–2004: Antibacterial finishes of textile material

Swatches in 4.8 ± 0.1 diameter were taken from the test fabric. The swatches stacked in 250ml wide mouth glass jar with screw cap. 1ml of *Staphylococcus aureus* (MTCC 96) broth poured on to the swatches. Two observations were taken for 0 contact time and 18–24 h at $37 \pm 2^\circ$. Then neutralizing solution was added and jar shake vigorously for one minute. Serial dilutions prepared for plating of nutrient agar. Report bacterial counts as the number of bacteria per sample observed in each petri plate. Percent reduction of bacteria calculated in all plates by following formula:

$$R = 100(B-A)/B$$

Where,

R= % reduction

A = the number of bacteria recovered from the inoculated dyed (treated) test specimen swatches in the jar incubated over the desired contact period

B = the number of bacteria recovered from the inoculated dyed (treated) test specimen swatches in the jar immediately after inoculation (at “0” contact time)

Abbreviations

HPTLC	High Performance Thin Layer Chromatography
E. coli	<i>Escherichia coli</i>
S. aureus	<i>Staphylococcus aureus</i>
SD	Standard deviation
B.Sap	Banana pseudostem sap
Rf	relative front / retardation factor
MTCC	Microbial Type Culture Collection and Gene Bank
AATCC	American Association of Textile Chemists and Colorists

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and approved the manuscript before submitting it to Bioresources and Bioprocessing.

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TNA performed the experiments. TNA and SI analyzed the data. TNA and SI wrote the manuscript. TNA and SI conceived the research. Both authors read and approved the final manuscript.

Authors' information (optional)

Department of Botany, Government Holkar Science College, Indore 452017, India

Taiyaba Nimra Ansari and Sanjeeda Iqbal

Corresponding Author

Taiyaba Nimra Ansari

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Figures

Figure 1

HPTLC chromatogram of *Butea monosperma*, Marigold, Pomegranate, Banana pseudostem under white light after derivatization with Dragendorff's reagent for Alkaloids (A), at visualizer R 366 after derivatization with alcoholic KOH for Anthraquinones (B), at white light after derivatization with vanillin sulphuric acid for Bitter principles (C), at white light after derivatization with vanillin sulphuric acid for Essential oil (D), at visualize R 366 after derivatization with ASR for Saponins (E), at R white after

derivatization with Anisaldehyde sulfuric acid for Steroids (F), at white light after derivatization with Anisaldehyde sulphuric acid for Sterols (G), at white light after derivatization with Anisaldehyde sulphuric acid for Triterpenoids (H), at R 366 after derivatization with NP (Natural product reagent) Flavanoids (I).

***Butea monosperma*(Flame of forest)**

***Tagetes erecta* (Marigold)**

Figure 2

Natural dyes

***Punica granatum*(Pomegranate)**

***Musa sp* (Banana pseudostem)**

Figure 3

Natural Mordant

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