

COMPARITIVE INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF MEDICAL FABRIC COATED WITH AZADIRACHTA INDICA AND ZIZIPHUS JUJUBE

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Abstract

Object: The primary object of this research work is to create the medical fabric to be utilized for hospital wear with antimicrobial properties through natural extracts of *azadirchta indica* barks and *ziziphus jujube* leaves.

Methods:

The dye extracts of *Azadirchta indica* barks was prepared through aqueous extraction method with the support of acetone and ethonal mixture of 25%(v/v) concentration . Similarly *Ziziphus jujube* leaves were dried and subjected to percolation process with 70% ethanol at room temperature for 24 h , at pH of 7. Three different dye concentration was prepared using the above extracts such as 10%,20%,30% for dyeing of cotton medical fabric with natural mordant (myrobalan)..

Results:

The color strength (K/S) of the all the dyed samples were good. More specifically the dye extracts of 20% concentration produces higher colour strength such as *Azadirchta indica* barks extracts yielded 9.74(K/S value) and *Ziziphus jujube* leaves extract yielded 11.9(K/S value). The washing, light and rubbing fastness properties of dyed fabrics were also good and 20% of dye concentration produces superior performance against all the fastness properties. The Antimicrobial property is one of the important characteristic of medical fabric ,which has been evaluated against two gram Negative bacteria and two gram positive bacteria such as *Pseudomonas aeruginosa*, *Klebsiella lebeliea*, *Staphylococcus aureus* and *Micrococcus mucilaginosus* microbes at a controlled environment and it zone inhabitation were analyzed

Conclusion:

It is inferred that all the samples showed better antimicrobial properties and 20% of dyed samples produces higher zone inhabitation than others. More than 20% of dye concentration increases the dye agglomeration and in turn reduces the dye affinity with the fabric, which may be the reason for minimizing the antimicrobial activity as well as dye ability of the fabric

1. Introduction

Natural dyes had been utilized as primary dye since from the ancient times. The invention of synthetic dyes reduces the application of natural dyes on textiles due to cheaper production cost, easy shade reproduce ability and lesser production time [1]. But recently days due to increased awareness on eco-friendly production methods and health concern,peoples are intended to go for natural dyed textile process and it is believed to be safe because of non-toxic, non-carcinogenic and biodegradable nature[2]. Further, natural dyes do not cause pollution and waste water problems. Long before mankind discovered the existence of microbes and its causes, the idea for healing through certain plants and currently we reconfirm these characterize.[3]. Since time immemorial, plants are used as a source of medicines. Traditional medicines were considered as the important art during ancient period [4].There are many plants are available as source of natural dyes which might be used in the textile industry. However, their common drawback is the lack of scientific information on the chemistry of extraction and dyeing [5].This present work with an attempt to study the effects of dye ability and antimicrobial effects of dyeing of cotton with aqueous extract of *Azadirchta indica* barks and *Ziziphus jujube* leaves. It is reported that Seeds , leaves barks of *Azadirchta indica* barks have been used to cure number of human ailments such as anti-inflammatory, anxiolytic, anti-androgenic, anti-stress, humoral and cell-mediated immune stimulant, anti-hyperglycemic, liver-stimulant, anti-viral and anti-malarial activities[6]. The aqueous

extract of *Azadirachta indica* barks and leaves had been utilized as a natural dye on textile materials [7]. The *Ziziphus jujuba* leaves of one of the traditionally used medicine to cure diarrhoea, syphilitic ulcers, asthma, stomatitis and gum bleeding and also used as poultice and astringent [8]. The leaves of *Z. jujuba* are reported to possess hypoglycaemic, antiulcer, immunomodulatory and Permeability enhancement activity [9]

2. Materials and methods

Plain woven Cotton fabric bleached with peroxide. The areal density of the fabric was 350 g/m² was sourced from KVIC shop, Gandhigram, Tamil Nadu. *AZADIRACHTA INDICA* barks AND *ZIZIPHUS JUJUBE* leaves were sourced nearby areas of Gandhigram university. These plant species were chosen based their potential antimicrobial activity over textiles. The collected *Azadirachta indica* barks and *ziziphus jujube* leaves from Gandhigram University washed under flowing water to remove dust particles and other soluble impurities and were allowed to dry at room temperature (24-25⁰ C) till the barks became completely dry, then were crushed into a fine powder. Further to that acetone, ethanol and sodium hydroxide were sourced from M/s GVR enterprises Madurai.

3. Dye extraction:

AZADIRACHTA INDICA barks AND *ZIZIPHUS JUJUBE* leaves were dried and made as power through grinding process was boiled with water mixed with sodium hydroxide.

3.1 *AZADIRACHTA INDICA* barks Extracts:

Few trails were conducted to optimize the best possible extraction process by varying the concentration. Different concentration of neem powder from (10,20,30) % (w/v) were dissolved in distilled water in separately and other co-solvents as water-acetone and water ethanol mixtures were tested at different concentrations ranged from (10-25) % (v/v), at (25-70) ⁰ C, for (15-120) min, at pH values of (3, 5, 7 and 9). This was carried out with the liquor ratio of 1:20. The extract which yields best colour strength was further utilized to optimize the dyeing condition

3.2 *ZIZIPHUS JUJUBE* leaves extracts:

The dried leaves of three different concentration such as 10%,20%,30%(w/v) subjected to percolation process separately with 70% ethanol at room temperature for 24 h , at pH of 7 . The Material liquor ratio of the process was 1:20[1].

4. Optimization of dyeing process variables

The dyeing parameters such as pH, Temperature of dye bath and duration of dyeing were optimized for both the dye extracted from *AZADIRACHTA INDICA* barks AND *ZIZIPHUS JUJUBE* leaves. Trails were conducted towards dyeing the cotton with the mentioned dyes separately using pH from 3 to 11 ,dyeing temperature 60⁰ C to 80⁰C and duration of dyeing from 1 to 2 hrs. The extracts *AZADIRACHTA INDICA* barks produces higher k/s value on cotton fabric with the pH of 9,dyeing temperature of 80⁰ C and the dyeing temperature was 90 min. Similarly The extracts *ZIZIPHUS JUJUBE* leaves produces higher k/s value on cotton fabric with the pH of 7,dyeing temperature of 90⁰ C and the dyeing temperature was 80 min. Further to this the researcher used these parameter for subsequent processing.

5. Dyeing

The cotton fabric samples were preprocessed with scouring and bleaching process .In dyeing three different concentration of dye extracts from *AZADIRACHTA INDICA* barks AND *ZIZIPHUS JUJUBE* leaves such as 10%,20%,30%(W/W) of the material. The dyeing process was carried out with the optimized process parameter.

5.1 Pre-mordanting

In this method the fabric was mordanted first and then dyed. The mordanting was done at the aqueous solution of with myrobalan mordant with the concentration of 4% for 40min with the temperature of 70⁰C with the MLR of 1:30. The pre-soaked fabric was dipped in the beaker and raised the temperature of solution up to boiling. Then the fabric was stirred continuously and heating carried out for one hour. The sample were allowed to cool at room temperature, remove from the bath, rinsed and dried in shade before dyeing.

5.2 Coloration

Dyeing process was executed with the laboratory model open beaker dyeing method. The instrument has the provision to control the dyeing temperature and pH. The samples were dyed with two class of dyes with three different concentration such as 10%, 20%, 30%(W/W). The fabric was entered into the dye bath at the temperature of 40°C and the temperature was gradually raised to the maximum temperature for the respective specimen. The MLR ratio was maintained in this process was 1:30 and the dyeing was continued till the maximum time duration was achieved. At the end of the dyeing ,the dyed samples were rinsed, soaped with non-ionic soap at the temperature of 60°C for 15 min, then rinsed with water and squeezed and dried .

6. Determination of absorption (%) and colour strength (K/S) values

The absorbance of the dye solution was recorded through spectrophotometer before and after dyeing of the fabric at particular wavelength. . The dye absorbance was calculated as given below .

$$\% \text{ dye absorption} = \frac{\text{OD of the dye liquor before dyeing} - \text{OD of the dye liquor after dyeing}}{\text{OD of the dye liquor before dyeing}} \times 100$$

Three to five reading were taken and it average data is recorded for analysis. Further to this *K/S* values of the dye samples were evaluated by light reflectance technique and the values were assessed using the below Kubel-Ka-Munk equation.

$$K/S = (1 - R)^2 / 2R$$

Where, R is the observed reflectance, K is the absorption co-efficient and S is the light scattering coefficient.

7. Antimicrobial assessment

The treated and untreated dyes samples were placed inside the bacteriostasis agar medium, which has been previously streaked with an inoculum of test organisms. After incubation, a streak of interrupted growth underneath and along the side of the test material indicates antimicrobial effectiveness of the fabric. The qualitative evaluation of antimicrobial test was carried out as per AATCC test method 147. The rectangular test specimen of size 25 X 50 mm was prepared for the evaluation. Sterilized nutrient agar at 47° C was dispensed in the petri dishes and allowed to gel firmly before inoculating. A loopful of the culture was transferred to the surface of the sterile agar plate by making five streaks approximately 60mm in length, spaced 10mm apart covering the central area of the petri dish. The specimen was then gently pressed transversely across the five inoculum streaks to ensure intimate contact with the agar surface. The plates were incubated at 37°C for 18-24 hours.

After the incubation period, the incubated plates were examined for interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of a zone of inhibition (ZOI) along a streak on either side of the test specimen was calculated using the following equation and the fabric samples were prepared with 22mm dia.

$$W = (T - D) / 2$$

Where

W: average width of clear zone of inhibition, mm

T: total diameter of test specimen and clear zone, mm

D: diameter of the test specimen, mm

8. Fastness determination

Colour fastness against washing was assessed as per the IS:764-1984 method using Sasmira launder-o-meter . The wash fastness rating was done with grey scale as per ISO-05-A02/03. Colour fastness to rubbing (dry and wet) was assessed as per IS:766-1984 method using Digital crock meter and grey scale reading followed as per the ISO-105-A03.colour fastness to light was measured as per IS:2454-1984 method. Shirley MBTF Micro fade-O-meter was utilized for measuring light fastness properties.

9. RESULTS AND DISCUSSIONS

9.1 Effect of Dye concentration on Colour strength(K/s)

To optimize the contraction of dye extracted from AZADIRACHTA INDICA barks(AZ) AND ZIZIPHUS JUJUBE (ZJ)leaves on cotton fabric was studied through measuring the colour strength of those samples with spectrophotometer. The below table 1. indicates the details. The Maximum colour yield (K/S value : 9.72)for the AZ class dye was achieved at 20% concentration , which was gradually declined by increasing the dye concentration. The K/S and colour co-ordinates such as L,a,b,c,h was reported in the table.1 .Similar to this the maximum colour yield for ZJ class of dye was (K/S value : 11.9) was achieved with 20% of dye concentration. Both the cases when dye concentration increases more than 20% reduces the color yield ,which was attributed that mostly the natural dyes are not the pure form of dye ,which contains large amount of foreign particles and this affects dye particle dispersion and minimized the dye exhaustion process.

Table 1.Influence of dye bath concentration on colour strength of cotton fabric dyed with AZ and ZJ dye extracts

Dye Extract	Concentration	K/S	L	a	b	C	h
AZ	10%(W/W)	9.48	54.75`	5.41	16.40	17.27	71.34
	20% (W/W)	9.74	54.31	5.76	16.32	17.34	70.62
	30%(W/W)	9.68	54.63	6.04	16.72`	17.83	70.13
ZJ	10%(W/W)	8.71	36.6	4.85	86.45	16.53	74.56
	20% (W/W)	11.9	26.74	0.61	1.64	0.84	51.43
	30%(W/W)	9.25	53.82	4.03	19.77	20.18	73.43

9.2 Effect of Dye concentration on Colour fastness properties

The colour fastness towards light, wash and rubbing with respect to dye concentration on AZ and ZY dyes is presented in the table. The unmordant dyed cotton fabric by AZ and ZJ shows comparatively less fastness properties to washing, crocking and light. In the case of AZ dyes the fastness properties to washing, crocking and light are higher with 20% dye concentration further improvement in dye concentration, never improved the fastness properties. Similarly In the case of ZJ dyes the fastness properties to washing, crocking and light are higher with 20% dye concentration, further improvement in dye concentration never improved the any of the fastness properties.

Table 2. Colour fastness properties of dyed cotton mordanted (myrobalan) and unmordanted in different dye concentration

Dye Extract	Concentration	Wash		Rubbing fastness				Light fastness
		fastness		Dry		wet		
		CC	C S	CC	CS	CC	CS	
AZ	10%(W/W)Without mordant	3-4	2	3-4	3-4	2-3	2-3	2
	10%(W/W)	4	3	4	3-4	3	3	2
	20% (W/W)	4	3	4	3-4	3	3	3-4
	30%(W/W)	4	2	4	3-4	2	2-3	3
ZJ	10%(W/W)Without mordant	2-3	2	2-3	3-4	2	2-3	2
	10%(W/W)	3	2	3	3-4	2	2-3	2
	20% (W/W)	4	3	3	3-4	3	2-3	3-4
	30%(W/W)	3	2	3	3-4	3	2-3	3

9.4 Effect of Dye concentration on Antimicrobial activity

Antimicrobial activity of the natural extract of AZ and ZJ dyed fabric has been tested its antimicrobial activity through in vitro study against *Pseudomonas aeruginosa*, *Klebsiella lebelia*, *Staphylococcus aureus* and *Micrococcus mucilaginosus* microbes and its Zone of inhibition is shown in the table 3. The Dye AZ and ZJ has higher amount of antimicrobial activity than the control sample against all the selected pathogens. From the below table it is further inferred that 20% of dye concentration had higher antimicrobial activity in both the dye classes. Further increase of dye concentration never showed the positive effect on antimicrobial activity is concern. It is understood that the higher dye concentration increases the dye agglomeration and in turn reduces the dye affinity with the fabric, which may be the reason for minimizing the antimicrobial activity of the fabric as well. The below Figure 1 & 2 indicates the ZOI of Gram negative and Gram positive bacteria with different dye concentration.

Table 3. Influence of dye bath concentration on Zone of inhibition against Gram Negative and Gram positive bacteria

Dye	Name of the sample	Gram Negative		Gram Positive	
		<i>Pseudomonas aeruginosa</i>	<i>Klebsiella lebelia</i>	<i>Staphylococcus aureus</i>	<i>Micrococcus mucilaginosus</i>
AZ	10%(W/W)	20.5mm	21.5mm	21.5mm	21mm
	20% (W/W)	24.5mm	22.5mm	22.5mm	22mm
	30%(W/W)	23.5mm	22mm	20.5mm	22.5mm
ZJ	10%(W/W)	22mm	21mm	20.5mm	21mm
	20% (W/W)	23.5mm	20.5mm	21mm	22mm
	30%(W/W)	21mm	20mm	22mm	20.5mm

- Diameter of the control sample(bleached sample) was =16

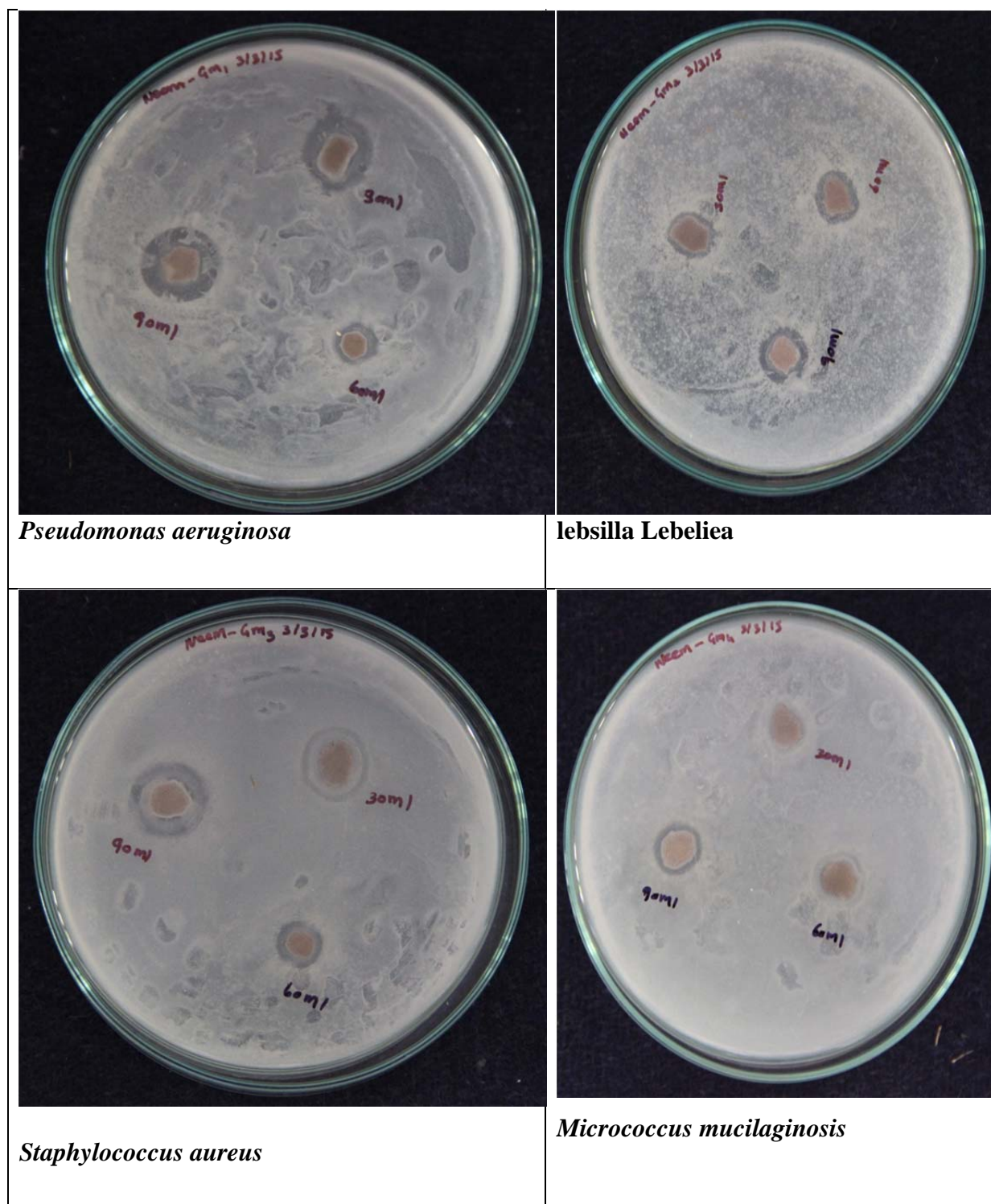


Figure 1: Zone of inhibition of cotton dyed AZ dye (Con. 10%,20%,30%) against various microbes.

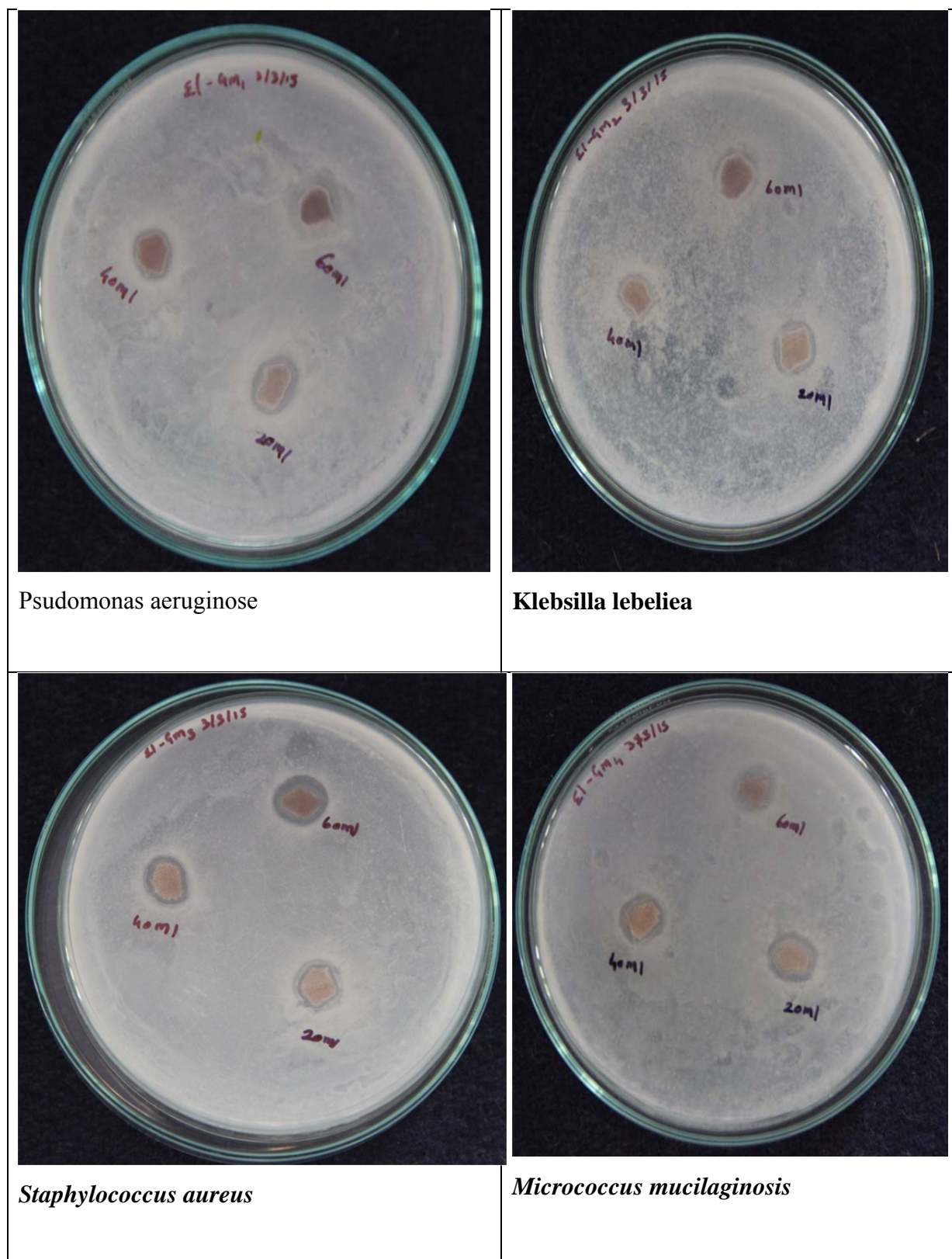
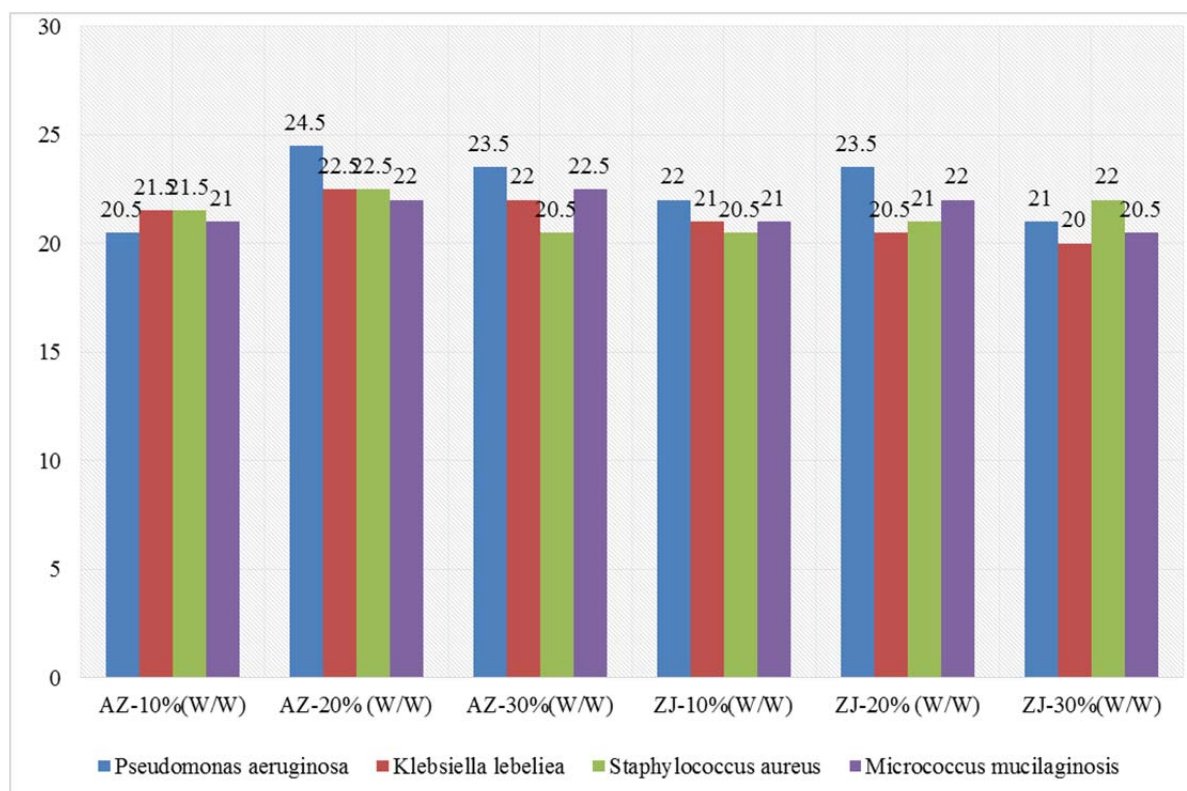


Figure 2: Zone of inhibition of cotton dyed ZJ dye (Con. 10%,20%,30%) against various microbes.



10. Conclusion:

Aqueous extracts of AZADIRACHTA INDICA barks(AZ) AND ZIZIPHUS JUJUBE (ZJ)leaves have been utilized to dye the cotton fabric with and without myrobalan mordant in three different dye concentration. The colour strength of dyed fabric was good and in comparison with all the three dye concentration. 20% concentration of dye extracts from both the dyes showed good colour strength. Similarly the colour fastness properties of dyed fabrics were analyzed against washing, crocking and light. All the dyed fabrics showed better colour fastness properties, but in comparison between the dyed samples, 20% dye concentrated fabric sample showed superior performance. Since the dye extracts are medical plants ,its antimicrobial test has been conducted through in vitro study of antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella lebeliea*, *Staphylococcus aureus* and *Micrococcus mucilaginosus* microbes and it Zone of inhibition(ZOI) was measured .All the dyed samples were shown better antimicrobial activity. It is also found that the 20% dye concentrated fabric sample produced highest antimicrobial activity.

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