

Extraction of Anti - Microbial Dye from the Flowers of *Delonix regia* for Sports Fabric

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Abstract: In a step towards ecofriendly practices in present study natural eco - friendly dye was extracted from petals of *Delonix regia* which performed well on cotton fabric with respect to its stability and quality leaving pleasant odor on fabric besides, on phytochemical analysis 70% ethanolic extract showed presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Amino acids and proteins, Saponins, Tannins and Terpenoids. On the other hand Fats and oils, Oxalates, Quinones, Sterols and Phlobatannins were reported to be absent and further the extract showed antimicrobial properties against two fungal (*Aspergillus niger*, *Candida albicans*.) and three bacterial (*E. coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*) species proving that the dye can be exclusively used to produce skin - friendly, antimicrobial sports clothing.

Keywords: *Delonix regia*, Gulmohar Antimicrobial, Dye, Sports fabric

Full forms: *Delonix regia* extract (sample D), alcoholic extract with alum mordant (ALAM), alcoholic extract with vinegar mordant (ALVN), alum mordant by heating at 75 °C (ALAM75°C), alcoholic extract without mordant by heating at 75 °C (AL75°C), alcoholic extract without mordant (AL), aqueous extract with alum mordant (AQAM), Aqueous extract with vinegar mordant (AQVN), aqueous extract with vinegar mordant by heating at 75 °C (AQVN75°C), aqueous extract without mordant by heating at 75 °C (AQ75 °C) aqueous extract without mordant (AQ).

1. Introduction

Textile industry has great potential of empowering society economically and socially, it has a huge base by providing livelihood to a large population, particularly in a developing country like India (Geetha, B & Sumathy, Judia.2013). At the same time these industries are forced to reduce their production and more over few startups have been permanently shut down due to backlash from various governmental and non - governmental organizations, environmentalists for a sound reason of them being a source of pollution, as they produce huge lot of toxins effluents in their time process as it includes various harmful chemical dyes and non - biodegradable, carcinogenic, toxic chemicals (M. M. Kamel et al, 2011)

Without an ounce of doubt we can say that there is an alternative for chemical dyes for instance there are various plants and there parts which can be used to extract natural non - toxic, biodegradable and non - carcinogenic dyes particularly from the bright colored petals for production of natural dyes, these can save the textile industries from the criticism (Naik Ramavathu et al., 2019). Natural dyes are safer for environment and also for the workers and artisans working in these industries. The fabrics produced using these natural dyes will be more acceptable in markets and do not pose a danger to the health of consumers Cristea G. Y., and Vilarem S. J 2003).

In India there are more than 500 varieties of plants documented that can yield natural dyes among them *Delonix regia* a species of flowering plants belonging to family *Fabaceae* and native to Madagascar. It is a huge tree, known for its scarlet orange to red colored flowers, the common

names include Gulmohar, Royal Poinciana etc. It is endemic and endangered in wild it is cultivated and naturalized in various tropical and sub - tropical regions of the world. In India it was introduced and naturalized.

The other major problem of textile industry is that the fabrics produced are generally not suitable for manufacturing sports clothing as majority of fabrics are prone to bacterial and fungal infections. Natural dyes extracted from plants can act as anti - microbial agents and this anti - microbial property can be well harnessed for production of anti - microbial dye for sportswear and inner wear (Kumaresan M, Palanisamy P N and Kumar P E, 2011).

The present study is focused on extracting natural anti - microbial dye from the petals of *Delonix regia* flowers so that it can be used by textile industries.

2. Materials and Methods

2.1 Collection of Materials

Fresh flowers of *Delonix regia* from Kolar region, Karnataka, India were collected, sealed packed in black polythene bags and in the laboratory of Dept. of Botany, BNU petals were separated from flowers washed thoroughly and stored for further use.

Dye Extraction: - Dye was extracted from fresh 500g of petals in 2000 ml of distilled water and 2000 ml of 60% ethanol each using soxhlet method.

Preparation of Mordants

Alum - 0.748g of alum and 0.187g of washing soda were mixed in 100ml of water and was stored for further use.

Vinegar - 50ml of 5% acetic acid is mixed with 100ml of water from that 25ml of it were taken and mixed with 100ml of distilled water (Geetha, B & Sumathy, Judia 2013)

2.2 Dyeing of fabrics

White cotton fabric cloth is used; the cloth was washed and dried before dyeing.

First the cloth was immersed in mordant, brought to boiling point, and then it was immersed in dye and left for one hour. Each Set comprising twenty (20) sample cloth were dyed in alcoholic extract with alum mordant (ALAM), alcoholic extract with vinegar mordant (ALVN), alcoholic extract with alum mordant by heating at 75 °C (ALAM75°C), alcoholic extract without mordant by heating at 75 °C (AL75°C), alcoholic extract without mordant (AL), aqueous extract with alum mordant (AQAM), Aqueous extract with vinegar mordant (AQVN), aqueous extract with vinegar mordant by heating at 75 °C (AQVN75°C), aqueous extract without mordant by heating at 75 °C (AQ75 °C) and finally aqueous extract without mordant (AQ) respectively. Later half of the set (10 samples) of dyed fabrics was dried in sun light and another half set (10samples) was dried in shade and observations were recorded (Naik Ramavathu et al., 2019).

2.3 Preliminary phytochemical analysis

Preliminary screening of phytochemicals in the extracts was performed for 14 compounds alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, cardiac glycosides etc. using alcoholic extract of petals of *Delonix regia* viz., The follows tests are performed. Wagner's test for alkaloids, Molisch's test for carbohydrates, Keller - killani test for cardiac glycosides, Alkaline reagent test for flavonoids, Ferric chloride test for Phenols, Preupitats test for Phlobatannins, Foams test for saponins, Liebermann Burchard test for steroids, Braymer's test for Tannins, Salkowski test for terpenoids, Tests for Quinones, Tests for Oxalates, Test for fats and fixed oils (Shaikh, Junaid & Patil, Matsyagandha, 2020).

2.4 Antimicrobial Analysis**Well diffusion method to check the Minimum Inhibition Concentration (MIC)**

The 60 % Ethanolic extract of *Delonix regia* petals (Sample D), was screened against organisms (*Aspergillus niger*, *Candida albicans*, *E. coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*)

Culture Preparation Culture Media Preparation for Fungi

Potato Dextrose Broth (PDB: Potato - 200g, Dextrose - 20g, Distilled water - 1000ml) 30ml was prepared in 2 Erlenmeyer flask respectively by boiling 6g of Potato in 30ml distilled water and filtered. 0.6g of Dextrose was added into the filtrate and the final volume was made upto 30ml with distilled water respectively. Autoclaved at 121°C for 15 mins. Later, *Aspergillus niger* (Isolated from natural source) and *Candida*

albicans (MTCC 3958) was inoculated and incubated at 25°C for 72h (Magaldi S et al., 2004).

Culture Media Preparation for Bacteria

Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000ml) 30ml was prepared in 3 Erlenmeyer flasks by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract 0.18g, Distilled water 30ml respectively and autoclaved at 121°C for 15 minutes. Later, *E. coli* (MTCC 433),

E. faecalis (ATCC 29212) and *P. aeruginosa* (MTCC 2453) was inoculated respectively in 30ml of sterilized LB broth flasks and incubated at 37° C for 24h (Valgas et al., 2004).

Bacterial Culture preparation

All the cultured organisms were centrifuged at 6000rpm for 10 minutes respectively, supernatant was discarded and the pellets were dissolved in 1% (w/v) Sodium chloride and adjusted to absorbance 1.0 at 600nm under UV spectrophotometer (Genesys 10S UV - VIS Spectrophotometer) (Valgas et al., 2004).

Sample preparation

100mg of sample D was dissolved in 1ml Dimethyl sulfoxide (DMSO). Different aliquots of the sample was prepared by pipetting 10µl (1mg), 20µl (2mg), 30µl (3mg) and 40µl (4mg) and the final volume was made upto 50µl by adding DMSO (Valgas et al., 2004).

Media preparation for MIC**For Fungal Plate - Potato Dextrose Agar**

(PDA: Potato - 200g, Dextrose - 20g, Agar - 20g, Distilled water - 1000ml) 100g of Potato was boiled in 300ml distilled water and filtered, final volume was made upto 500ml with distilled water. dextrose 10g, Agar 10g was added and autoclaved at 121°C for 15 mins (Valgas et al., 2004).

For Bacterial Plate - Luria Bertani (LB) agar media

(Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 20g, Distilled water 1000ml) 250ml was prepared in 3 Erlenmeyer flasks by adding Tryptone 2.5g, Sodium chloride 2.5g, Yeast extract 1.5g, Agar 5g, distilled water 250ml respectively and autoclaved at 121°C for 15 mins (Magaldi S et al., 2004).

Plating for MIC against organisms.

PDA and LB agar Media plates were prepared. 200µl inoculum of (*Aspergillus niger*, *Candida albicans*, *E. coli*, *E. faecalis* and *P. aeruginosa*) was inoculated respectively by spread plate technique. Five wells measuring 0.6cm was made in each plate and 50µl of prepared sample containing 1mg, 2mg, 3mg and 4mg were loaded into the respective wells and 50µl of DMSO was loaded in the middle well as control blank.

The bacterial plates incubated at 37°C for 24h and Fungal plates incubated at 25°C for 72h. Later, zone of inhibition was recorded in mm (Millimeter) (Valgas et al., 2004).

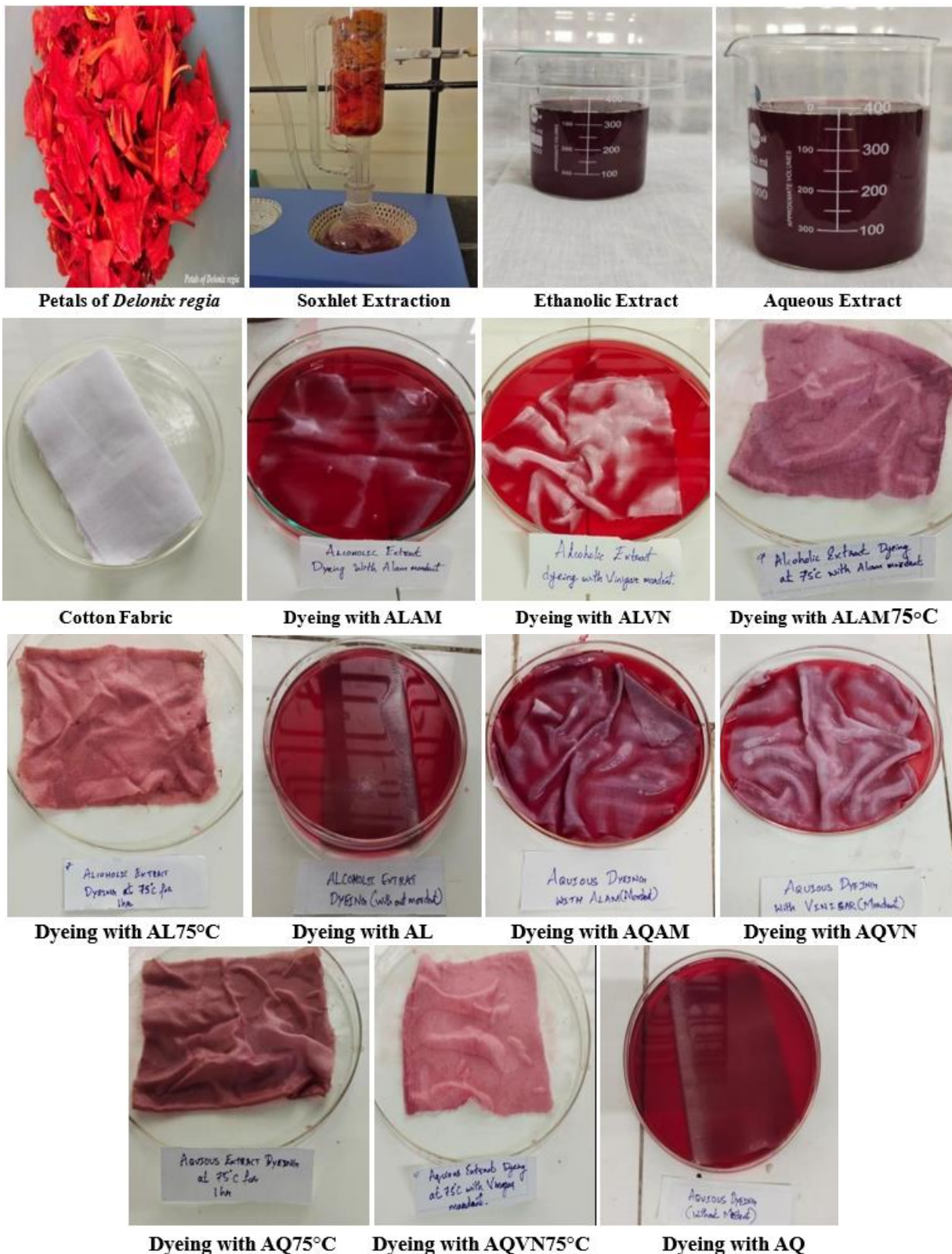
3. Results

Physical Characters of extracted dye

The aqueous extract was wine red in color, smelled like cooked raw green leaves, but the 60 % ethanolic extract had

little brownish tinge in red color and smelled more like alcohol.

Dyeing of Fabrics



Dyeing with AQ75°C Dyeing with AQVN75°C Dyeing with AQ

After Dying

Shade Dried and Washed



Shade drying



ALAM



ALVN



ALAM75°C



AL75°C



AQAM



AQVN



AQVN75°C



AQ



AQ75°C



AL

Sun dried and washed



Tabular Column 1

Sl. no	Shade dried	Color	Odor
1	ALAM	Light pink	Pleasant
2	ALVN	Pink	Pleasant
3	ALAM75°C	Light Brown	Pleasant
4	AL75°C	Dark pink	Pleasant
5	AL	Pink	Pleasant
6	AQAM	Bluish Green	Pleasant
7	AQVN	Light brown	pleasant
8	AQVN75°C	Dark pink	Pleasant
9	AQ75°C	Peach	Pleasant
10	AQ	Pink	Pleasant

Tabular Column 2

Sl. no	Sundried	Color	Odor
1	ALAM	Dark brown	Pleasant
2	ALVN	Brown	Pleasant
3	ALAM75°C	Brown with greenish tinge	Pleasant
4	AL75°C	Intense Dark brown	Pleasant soothing
5	AL	Brown	
6	AQAM	Greenish	Pleasant

7	AQVN	Light pink	pleasant
8	AQVN75°C	Dark brown	Pleasant
9	AQ75°C	Pale dark brown	Pleasant
10	AQ	Dark brown	Pleasant

Phytochemical analysis of ethanolic extract of petals of *Delonix regia*

Tabular column

Sl. no	Phytochemical name	Present/absent
1	Alkaloids	Present
2	Carbohydrates	Present
3	Cardiac glycosides	Present
4	Flavonoids	Present
5	Phenols	Present
6	Phlobatannins	Absent
7	Amino acids and proteins	Present
8	Saponins	Present
9	Sterols	Absent
10	Tannins	Present
11	Terpenoids	Present
12	Quinones	Absent
13	Oxalates	Absent
14	Fats and oils	Absent

Anti microbial analysis

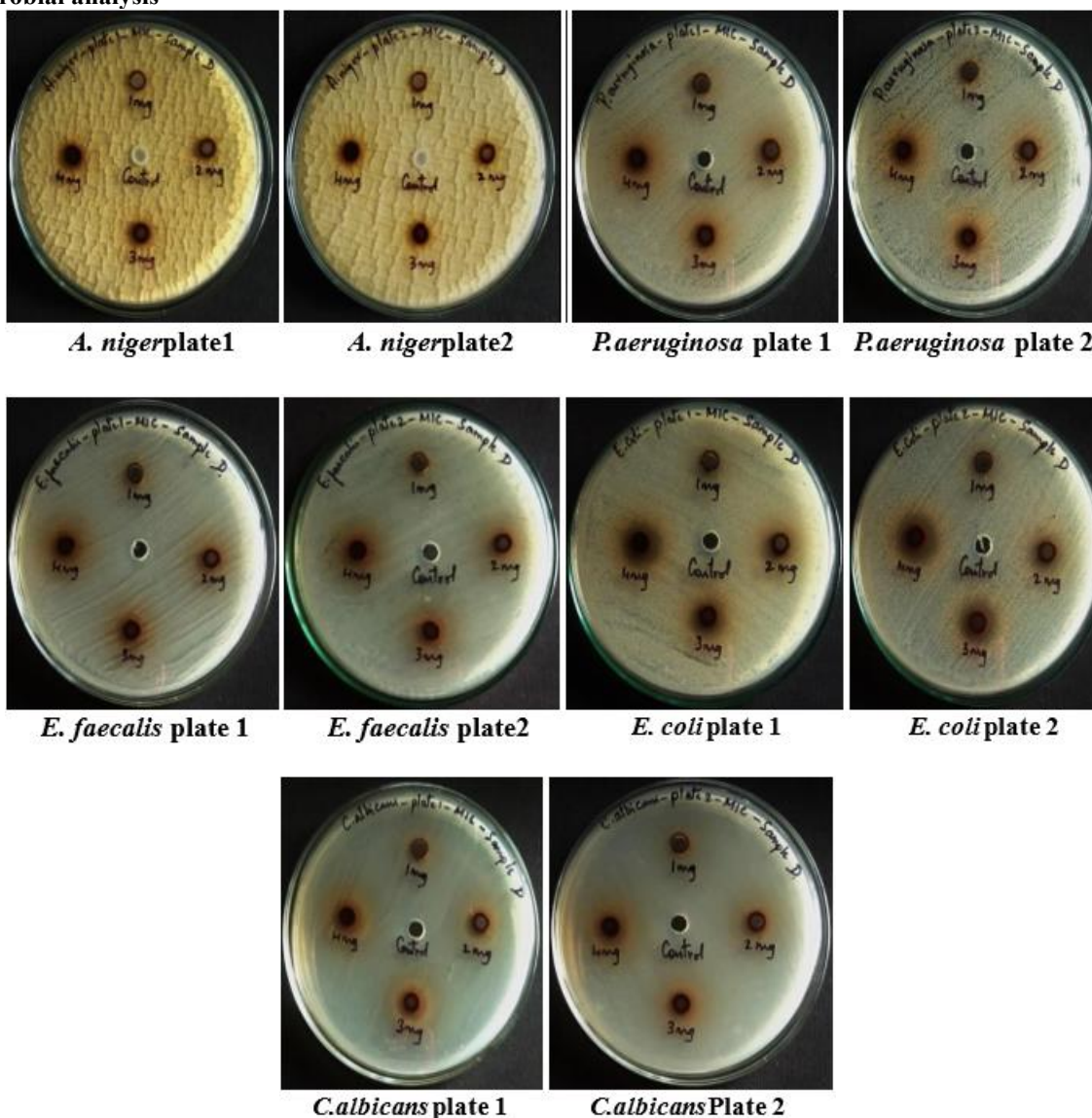


Table: MIC of ethanolic extract of petals of *Delonix regia* extract against Organisms

Organisms	Zone of inhibition (in mm) of Sample D. extract in µg.							
	1mg		2mg		3mg		4mg	
Plates	1	2	1	2	1	2	1	2
<i>A. niger</i>	10	10	12	12	13	12	14	14
<i>C. albicans</i>	10	10	12	11	13	12	15	15
<i>E. coli</i>	13	10	12	14	17	16	20	20
<i>E. faecalis</i>	10	10	12	12	13	13	14	14
<i>P. aeruginosa</i>	10	10	11	11	12	13	14	14

3. Discussion and Conclusion

Colouring agents are widely used for various purposes ranging from food to fabric manufacturing. From 1856 synthetic dyes were introduced and they became widely popular since they were cheap and easy to manufacture. The synthetic dyes are not eco - friendly and they are non - biodegradable, and most of them are carcinogenic and even few cause biomagnification, on the other hand, natural dyes are eco - friendly, bio - degradable and more acceptable in market (Naik Ramavathu et al, 2019).

In the present study, aqueous and alcoholic petals extract of *Delonix regia* were used for dyeing a cotton fabric. Two mordants namely **alum mordant and vinegar mordant** were used for proper adherence of the dye to fabric, dyeing was done in different combinations of alcoholic and aqueous extracts along with mordants. The aqueous extract was bright wine red in color and the 60% alcoholic extract was pale red with brownish tinge. One set (10 samples) was dried in shade and other set (10 samples) was dried in sun light which gave different shades of brown and pink. After drying the dye was stable on the fabric

with pleasant odor. Further Phytochemical analysis was done using 60% alcoholic extract which showed the **presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Amino acids and proteins, Saponins, Tannins and Terpenoids**. The chemicals and compounds such as **Fats and oils, Oxalates, Quinones, Sterols and Phlobatannins** were reported to be absent.

Apart from being natural and eco - friendly if a dye possess anti - microbial properties then it will be overwhelming and the dye can be exclusively used to manufacture anti - microbial clothing for sports and other purposes, hence, anti - microbial efficacy of the 60% alcoholic extract was analysed on two fungal samples (*A. niger*, and *C. albicans*) and three bacterial samples (*E. coli*, *E. faecalis* and *P. aeruginosa*). The results show that 3mg concentration is best showing inhibition zone of **13mm** for *A. niger*, **13mm** for *C. albicans*, **17mm** for *E. coli*, **13mm** for *E. faecalis* and **13mm** for *P. aeruginosa*.

The 60% alcoholic and aqueous extracts from petals of *delonix regia* can be used as an excellent anti - microbial dye for exclusively dyeing sports clothing.

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