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STUDY OF LEATHER DYING PROCESS USING CITRUS LIMON PEEL EXTRACT WITH COMMERCIALPROTEASE ENZYME AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Dyeing is an important process in the leather industry, which employs many synthetic colorants. The studies on process parameters of enzymatic treatment such as pH, temperature and duration on the exhaustion of the dye, levelness of dyeing, shade brightness, dye penetration and color intensity have been studied and the conditions are optimized. The change in shades due to enzymatic treatment has been quantified by reflectance measurements and compared with the visual assessment data. There is significant change in color due to enzymatic treatment as noticed by reflectance measurements and visual assessment data. The overall fastness of the leathers treated with enzyme is comparable with those obtained by control leathers. The current activity in the area of leather processing is shifting towards the design and utilization of cleaner and softer technology like enzymatically enhanced processes. The enzymes are successfully employed for the better quality leather production with less pollution impact and also for the treatment of waste discharged from the industry. It is very essential need to today polluted world. This process is mainly designed to save our environment. Antimicrobial activity is also done with the citrus limon extract for the three bacterial stains namely *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3940), *Salmonella typhi* (MTCC-734).

KEYWORDS: Dying, Protease, Citrus limon, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*.

INTRODUCTION

Leather and the leather industry have been around a long while. Leather was a very Important clothing material and its other uses were legion. Leather working is one of the oldest crafts known to humankind. The first leather objects were primarily Functional, but people soon learned the decorative potential of the material. The timeless appeal of leather lies in its luxurious texture, warm color, wholesome aroma and exceptional durability. The world's affair with fine leather continues to be influenced as much by its appeal as a status symbol as by its utilitarian value. The reputation of the leather industry across the centuries could be described as one of tolerated usefulness, with a wonderful end product. As the industry enters the 21st Century, it is now recognized as a major industry of great economic importance on an international scale.

The leather industry occupies a place of prominence in the Indian economy in view of its massive potential for employment, growth and exports. There has been an increasing emphasis on its planned development, aimed at optimum utilization of available raw materials for maximizing the returns, particularly from exports. Dyes are used for coloring the fabrics. Dyes are molecules which absorb and reflect light at specific wavelengths to give human eyes the sense of color. There are two major types of dyes - natural and synthetic dyes. The natural dyes are extracted from natural substances such as plants, animals, or minerals. Synthetic dyes are made in a laboratory. Chemicals are synthesized for making synthetic dyes. Some of the synthetic dyes contain metals too.

Naturaldyes

Direct Printing It is the most common approach to apply a color pattern onto a fabric. If done on colored fabric, it is known as over printing. The desired pattern is produced by pressing dye on the fabric in a paste form. To prepare the print paste, a thickening agent is added to a limited amount of water and dye is dissolved in it. Earlier starch was preferred as a thickening agent for printing. Nowadays gums or alginates derived from seaweed are preferred as they allow better penetration of color and are easier to wash out. Most pigment printing is done without thickeners because the mixing up of resins, solvents and water produces thickening anyway.

Aim and Objectives

Studies on the influence of commercial protease enzyme in natural leather dyeing process and antimicrobial activity of citrus limon peel extract.

- To prepare the dye from citrus limon peel.
- To collect commercial protease enzyme as a catalyst.
- To perform the dye on the natural leather.
- To study the anti microbial activity of citrus limon peel extract on *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3940), *Salmonella typhi* (MTCC-734).

RESULT AND DISCUSSION

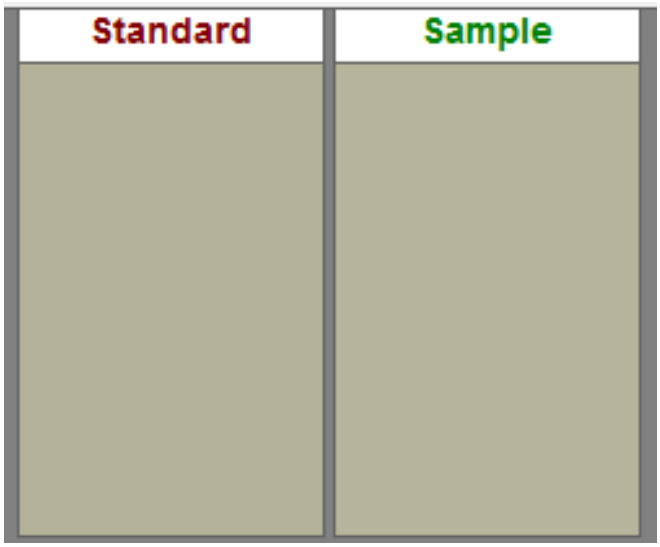
The dried powder sample of Citrus limon peel (CP), are not showed color formation immediately after the application of the sample in the aqueous solution. However PP showed a brown color after 24 hours immersed with aqueous solution.

Shows the extraction of CP

Sample Measurement – Reflectance

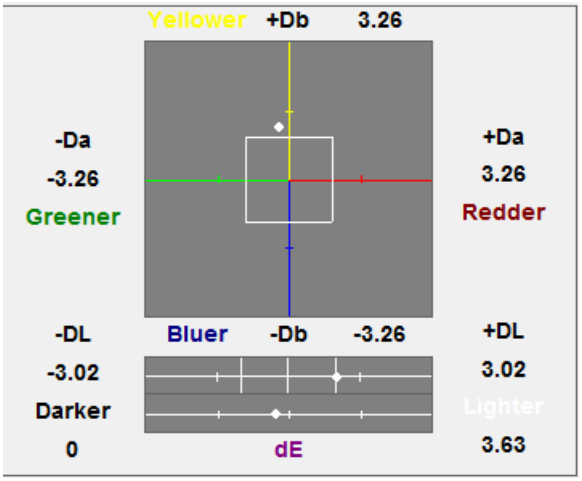
CIE Lab Values of the dyed leather sample

Enzyme	L*	a*	b*	C*	h*
Control (Dyeing with Citrus limon peel alone)	72.103	-3.462	11.064	11.593	107.404
Dyeing with Citrus limon Peel Protease enzyme	73.118	-3.689	12.324	12.864	106.694



Color difference value of the enzyme treated leather

DE^+	1.634	Overall Color Difference
DL^+	1.015	Lighter
Da^+	-0.227	Greener
Db^+	1.260	Yellower
DC^+	1.271	Brighter



DL – The leather has become lighter when compared to the control. This is because the enzyme treated leather has become more yellower and the yellowing of the leather shows that the enzyme treated leather is lighter when compared to the control.

Da* - In the enzyme assisted dyeing process there was no shift in hue. The negative value in 'a' shows that the color has become more greener by (0.227 units) when compared to the dyeing carried out without enzyme (Citrus limon peel dye alone).

Db* - Similarly, the color difference in terms of b value shows that the color has become more yellower for the enzyme assisted dyeing process when compared to the control leather.

DC – There is an increase in the intensity of color for the enzyme assisted leather dyeing process.

DE* - overall color difference of leather indicates perceptible color difference when the leather treated with the commercial protease enzyme.

Phytochemical screening of methanolic extract of dried fruits peel of Citrus limon (family-Rutaceae)

S. No.	Phytochemical test	Reagent used (test performed)	Observation	Result
1	Alkaloids test	Mayer's test Wagner's test Dragendroff's test	Frothing observed	+
2	Saponin test	Foam test	Turbidity obtained	+
3	Flavonoid test	Lead acetate test	Golden yellow colour observed	+
4	Carbohydrate test	Molisch's test Benedict's test Fehling's test	Yellow colour observed	+
5	Glycoside test	Modified Borntrager's	Brownish black ppt not observed	+
6	Steroid test	Salkowski's test	Ring not formed	+
7	Tannins test	Tannins test	Gelatin test	+
8	Proteins amino acids	Xanthoproteic test	Reddish black not seen	+

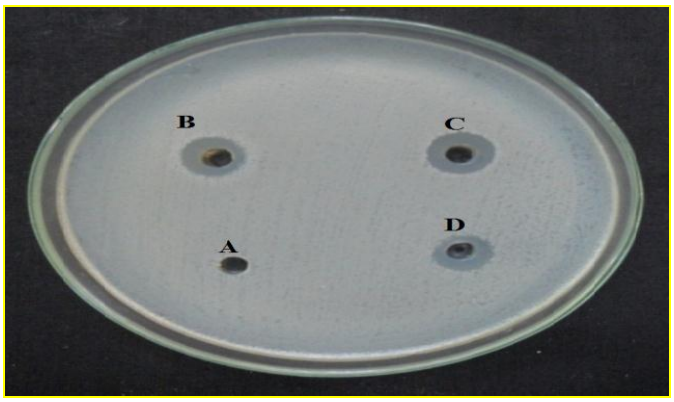
+sign indicates the presence and–sign indicates absence.

Antibacterial Activity

In this work the synthesis of citrus limon peel extract and the antibacterial activity against bacterial pathogens was evaluated.

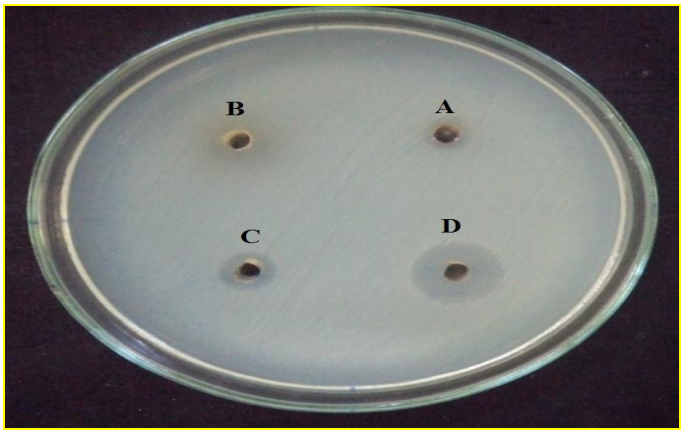
The aim of the present study was to evaluate the antibacterial activity of citrus lemon peel and its antibacterial activity. Three Bacterial pathogens *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* were used for experimental study (Table: 1; Figure: 7; Plates: 1-3). It was found that the minimum inhibitory concentration (MIC) of limon peel extract was 25µg in the three bacterial strains. Extracted citrus limon peel extract were considered for antibacterial activity against pathogenic microorganisms by using standard zone of inhibition. *Streptomycin, Gentamycin, Ampicillin, Erythromycin* of 10 mg/mL concentration were used as an antibacterial agents.

The Extracted citrus limon peel extract showed inhibition zone against all the test organisms. Maximum zone of inhibition was found due to the presence of *Bacillus subtilis, Salmonella typhi* and *Staphylococcus aureus* in all the tested bacterial organisms (Table:2; Plates: 4- 6).



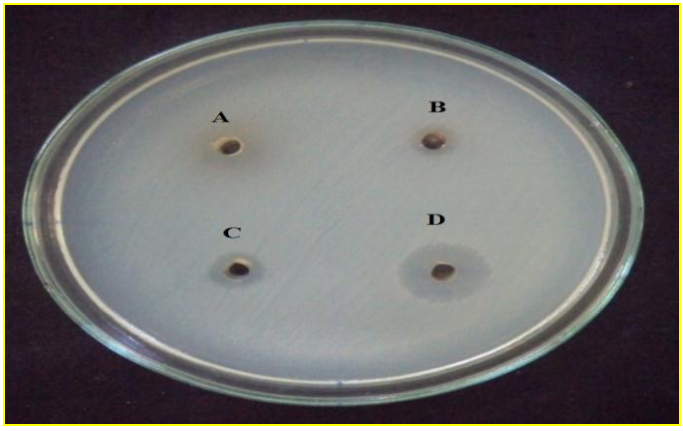
A- Crude extract, B-chloroform extract, C-methanol extract, D-reference drug.

Plate 1: Antibacterial activity of 1mM of citrus limon peel extract against *Bacillus subtilis* by agar well diffusion method.



A- Crude extract, B-chloroform extract, C-methanol extract, D-reference drug.

Plate 2: Antibacterial activity of 1mM of citrus limon peel extract against *Staphylococcus aureus* by agar well diffusion method.



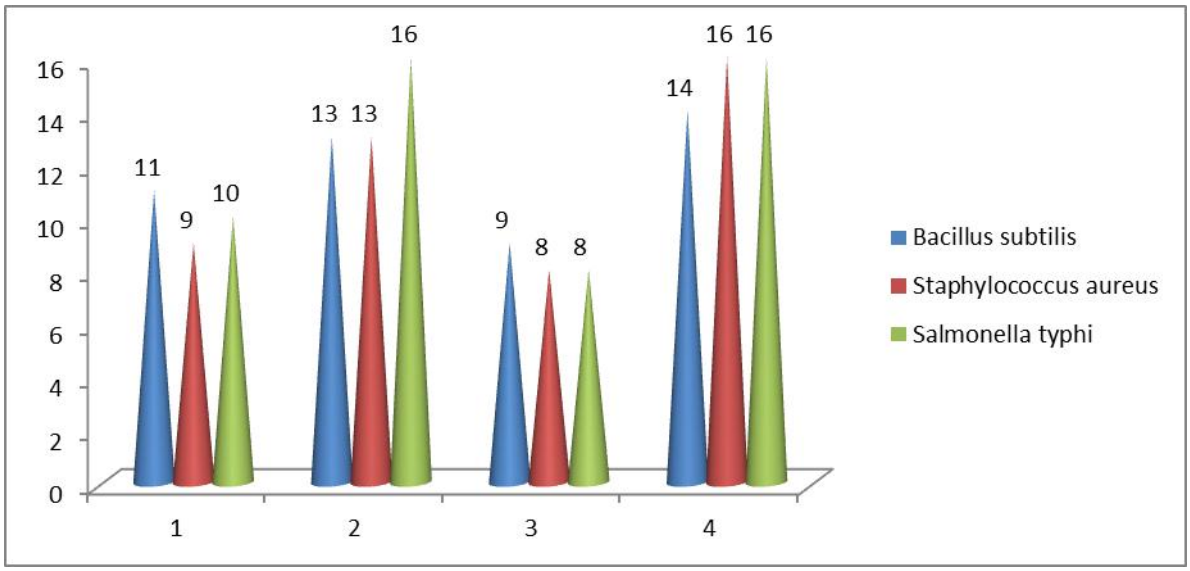
A- Crude extract, B-chloroform extract, C-methanol extract, D-reference drug.

Plate 3: Antibacterial activity of 1mM of citrus limon peel extract against *Salmonella typhi* by agar well diffusion method.

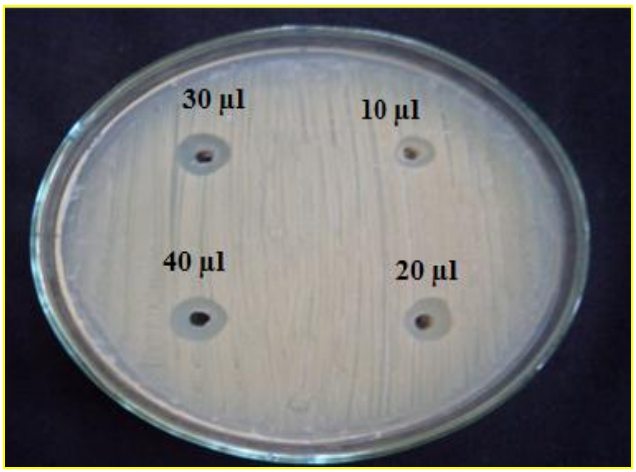
Table 1: Antibacterial activity of 1mM of citrus limon peel extract.

S No	Bacterial name	Zone of inhibition in mm			
		Crude limon peel extract	Extracted with 10% chloroform.	Extracted with 10% methanol	Reference Drug
1.	*Bacillus subtilis*	11±0.03	13±0.43	9±0.16	14±0.42[a]
2.	*Staphylococcus aureus*	9±0.36	13±0.56	8±0.24	16±0.49[b]
5.	*Salmonella typhi*	10±0.17	16±0.61	8±0.06	16±0.72[a]

Keys: crude: citrus limon peel extract; Reference drugs: a-Streptomycin, b-Gentamycin.

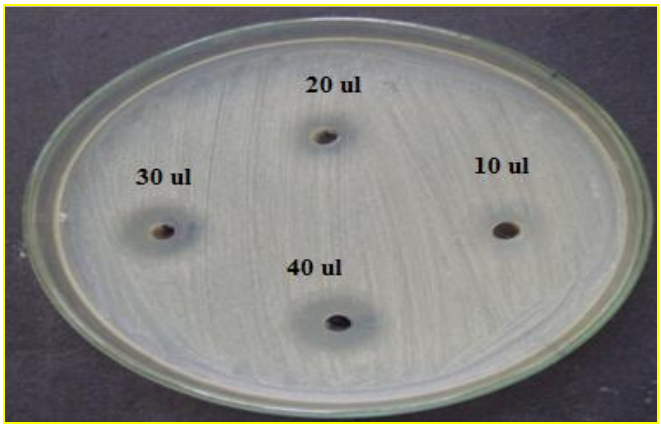


Graph 1: Antibacterial activity of Citrus limon peel extract.



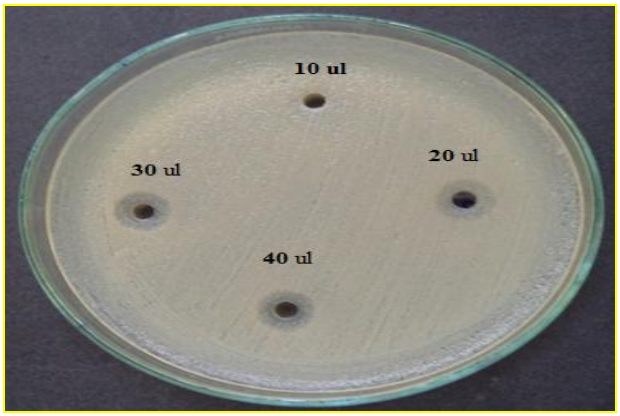
A- 10µl, B- 20µl, C- 30µl, D-40µl

Plate 4: Minimum inhibitory concentration (MIC) test for citrus limon peel extract against *Bacillus subtilis.*



A- 10µl, B- 20µl, C- 30µl, D-40µl

Plate 5: Minimum inhibitory concentration (MIC) test for citrus limon peel extract against *Staphylococcusaureus.*

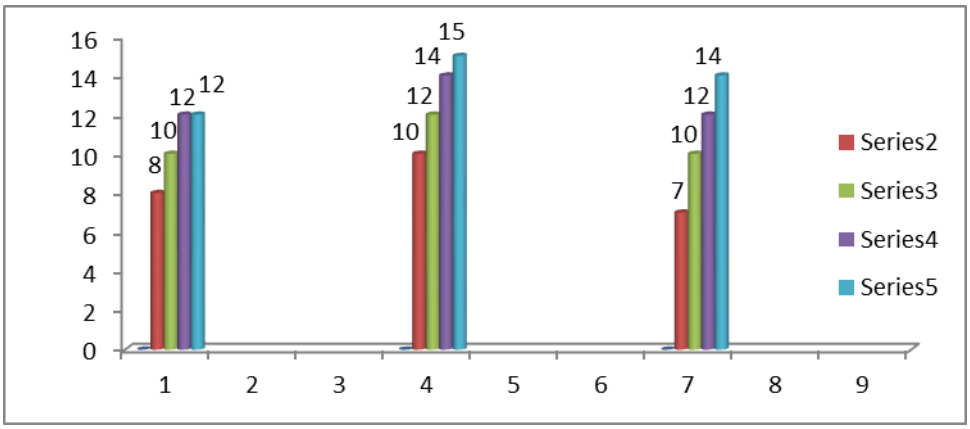


A-10µI, B- 20µI, C- 30µI, D-40µl

Plate 6: Minimum inhibitory concentration (MIC) test for citrus limon peel extract against *Salmonella typhi.*

Table 2: Determination of minimum inhibitory concentration (MIC) for Citrus limon peel extract.

S.No.	Bacterial name	Zone of inhibition (mm)			
		10 µl	20 µl	30 µl	40µl
1.	Bacillus subtilis	8±0.16	10±0.20	12±0.05	12±0.26
2.	*Staphylococcus aureus*	10±0.11	12±0.10	14±0.43	15±0.43
3.	*Salmonella typhi*	7±0.06	10±0.063	12±0.26	14±0.30



Graph 2: Determination of minimum inhibitory concentration (MIC) for Citrus limon peel extract.

It was found that the minimum inhibitory concentration (MIC) of Citrus limon peel ethanol extract was 25µg in the three bacterial strains. Syntheses of ethanolic extract of limon peel extracts were considered for antibacterial activity against pathogenic microorganisms by using standard zone of inhibition. *Streptomycin, Gentamycin, Ampicillin, Erythromycin* of 10 mg/mL concentration were used as an antibacterial agents.

The Citrus limon peel extract showed inhibition zone against all the test organisms. Maximum zone of inhibition was found due to the presence of *Bacillus subtilis, Salmonella typhi* and *Staphylococcus aureus* in all the tested bacterial organisms (Table:2; Figure: 8; Plates: 4- 6).

The mechanism of inhibitory action of citrus limon peel extract on microorganisms is not very well known. However, several mechanisms have been proposed to explain the inhibitory effect of citrus fruits on bacteria it is assumed that silver ion has high affinity towards sulfur and phosphorus molecules containing amino acids inside or outside of bacterial cell membrane protein are the key element of the antimicrobial effect (Rai and Bai, 2011) this in turn affects the osmotic stability leads to bactericidal activity.

CONCLUSION

The study concludes that the environment friendly approach for dyeing of leather with natural dye extracted from citrus limon Peel. The studies on enzyme on dyeing was also investigated and the following conclusions are made. Citrus limon Peel extract can effectively dye leather and it showed a yellowish green color. The current activity in the area of leather processing is shifting towards the design and utilization of cleaner and softer technology like enzymatically

enhanced processes. The enzymes are successfully employed for the better quality leather production with less pollution impact and also for the treatment of waste discharged from the industry. Enzyme assisted leather dyeing using the natural citrus Limon Peel extract showed a bright yellowish green color. The intensity of the color improved. The enzyme treated leather at the optimized conditions resulted in leathers with uniform dyeing, intense and bright shade.

The use of biochemistry by tanneries has increased in recent years. Enzymes can be applied during different steps of the leather production process. This study evaluated the performance of five commercial enzymes in soaking and unhairing by comparing the chemical and coenzymatic process. Antimicrobial activity is also done with the citrus limon extract for the three bacterial stains namely *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3940), *Salmonella typhi* (MTCC-734).

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