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Phytochemical Profiling, Antimicrobial and Antioxidant Activities of Citrus Sinensis Peel Extract with its Application in Natural Dyeing of Cotton Fabrics.

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ABSTRACT
The present study investigated the optimum dyeing procedure of cotton fabrics using Citrus sinensis L. (orange) fruit peel extract as a natural dye source. The dye was extracted, lyophilized, and applied with different natural mordants (lemon, alum, and vinegar) under pre-, simultaneous, and post-mordanting techniques. The color strength (K/S values) of the dyed fabrics was determined using spectrophotometric analysis at 520 nm. Results demonstrated that lemon juice as a mordant, particularly in the post-mordanting technique, exhibited the highest color strength (K/S = 14.009), followed by pre-lemon (10.301), indicating its superior role in enhancing dye fixation. Phytochemical analysis of C. sinensis peel extracts confirmed the presence of bioactive compounds such as phenols, flavonoids, tannins, saponins, alkaloids, and coumarins, which not only contributed to color development but also imparted antimicrobial and antioxidant properties. Among different solvent extracts, ethyl acetate demonstrated the highest phenolic (1.103 ± 0.11 mg/g) and saponin (14.2 ± 0.28 mg/g) content, correlating with its strong antimicrobial activity against S. aureus (20.67 ± 2.08 mm) and K. pneumoniae (21.0 ± 1.51 mm). Antioxidant activity assessed by DPPH assay revealed significant radical scavenging effects, with IC_{50} values of 63.51 µg/mL (ethyl acetate), 60.66 µg/mL (hexane), and 70.66 µg/mL (methanol), comparable to the standard ascorbic acid (36.44 µg/mL). These findings establish C. sinensis peel extract as a sustainable, eco-friendly natural dye with added functional benefits, promoting its potential application in textile coloration, antimicrobial finishing, and value-added utilization of agro-waste.

Key words: Citrus sinensis, antimicrobial, phenol, saponin, natural mordants, DPPH assay.

I. INTRODUCTION:

Numerous scientific investigations have identified fruits and vegetables as significant sources of antimicrobial agents. However, limited attention has been directed toward the utilization of fruit by-products such as seeds and peels. In most cases, fruit skins are discarded as waste or used as livestock feed, despite being rich in bioactive compounds that are reported to exert beneficial effects on human health (Sachine et al., 2017; Zaki et al., 2022). The waste of fruit peels is a major concern for both processing industries and environmental groups alike. Packed with nutrients and phytochemicals, fruit peels present opportunities for use as medicines and nutritional supplements.As diabetes becomes more common, these options are being investigated as safe substitutes that have both antimicrobial and antidiabetic properties, while also acting as enhanced products for the food sector. (Apsara and Pushpalatha., 2002; Kumar et al., 2011).

Antioxidants are bioactive compounds that mitigate oxidative damage by neutralizing free radicals. Increasing attention is directed toward natural antioxidants, particularly plant-derived polyphenols, which exhibit strong radical scavenging activity and confer significant nutritional and physiological benefits (Priya et al., 2023). The extraction efficiency of phenolic compounds is strongly influenced by the properties of the solvents employed. Commonly used solvents

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include methanol, ethanol, propanol, acetone, and ethyl acetate (Spignoet al., 2007). Although phenolics dissolve better in highly polar solvents like methanol, the toxicity linked to it restricts its use. Therefore, ethanol, which is a solvent safe for food use, is chosen for the effective extraction of phenolic compounds from citrus peels.Antimicrobial agents that inhibit or destroy microorganisms, whereas disinfectants represent a class of antimicrobials specifically applied to nonliving surfaces. All citrus fruit products are known to potent antimicrobial agents against bacteria and fungus (Dayanand Dubey et al., 2011). Orange peel extract exhibits medicinal properties, including regulating cholesterol, aiding digestion, preventing respiratory disorders, and combating infections. Moreover the presence of high vitamin C content in orange peel helps to reduce aging spots, treat deficiencies, and protect against colds, flu, and scurvy (Shetty et al., 2016). The objective of the study was intended to investigate the antioxidant activity, and antibacterial activity of three different solvent extracts of C. sinensis L (Orange) fruit peels. In addition, total phenol content and total saponin content were also evaluated.

II. MATERIAL AND METHODS

2.1. Chemicals

The chemicals used in the study were purchased from Sigma Alrich and Merck. All the chemicals are analytical high grade with high purity.

2.2. Sample collection

The fresh and healthy C. sinensis L (orange) fruits were purchased from commercial market, Chennai, Tamil Nadu. The fresh fruit were peeled of and washed carefully under running tap water followed by sterile distilled water.

2.3. Extract of natural pigment from fruit peels of*C. sinensis*.

Colorants from C. sinensis fruit peels were extracted using Aqueous Two-Phase Extraction (ATPE). 0.1% ascorbic acid was added to prevent enzymatic browning. The extract was stored at 4 °C and used as needed. Poly Ethylene Glycol(PEG)6000 and ammonium sulfate were added to the crude extract to make 100% (w/w), mixed thoroughly, and allowed to separate for 4–5 h. The volumes of the top and bottom phases were recorded and analyzed. Experiments were conducted at 25 ± 1 °C with pH maintained at 5–5.5 (Stig E. Friberg 2007).

2.3.1. Dyeing Procedure:

Scoured fabrics were mordanted using three techniques such as pre-mordanting, simultaneous mordanting, and post-mordanting (Ashis and Adwaita., 2011). Totally, nine experimental setups were performed based on an orthogonal array design. The C. sinensisdye was extracted from the fruit peel and was lyophilised and stored at 15^0C in a dry container. The powdered extract was then used to dye the scoured cotton fabric. Three types of mordants were used namely, lemon juice, alum and vinegar. All the mordanting techniques like pre, simultaneous and post mordanting techniques was used. The fabric was dried for week and then washed using specified quantity of detergent. The wash offs are collected and their absorbance and transmittance was read using UV spectrophotometer at 520nm. Then, based on these values, the K/S ratio was calculated to determine the most effective mordant and mordanting process using the well-known Kubelka–Munk theory.

2.4. Extract Preparation

The sample were shaded dried for 7-9 days and powdered using Pulvizier. The powdered samples were stored in air tight containers for further use. A conical flask containing 100 ml ofsolvents with different polarities (100% v/v ethanol, 100% v/v ethyl acetate, and 100% v/v hexane)was mixed with10 g of dried fruit peel powder and kept on a shaker for 24 hours at room temperature. The resulting material was filtered with muslin cloth and centrifuged at 10,000 rpm for 15 min (Remi C-854/6). The collected supernatant was concentrated with rotary vacuum evaporator, superfit, ROTAVAP, PBU-6. The concentrated crude extract was stored at 4^0 C for further use.

2.5. Prelimenary Phytochemical screening

The dried fruit peel extracts of C. sinensis was subjected to qualitative screening of phytochemical compounds viz., flavonoids, phenols, tannins, saponins, alkaloids, steroids, anthraquinons and cumarins followed by the method of Ushie et al.(2022).

2.5. Quantitative Phytochemical analysis.

2.5.1. Total Phenolic Content (TPC)

Total phenolics were measured by Folin-Ciocalteu method described by Rutuba et al. (2021). Phenolics were measured as gallic acid

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equivalents. One milliliter of fruit peel extracts was transferred to a test tube along with 1 mL of 95% ethanol, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent. The above mixture was kept for incubation at room temperature for 5 min, then 1 mL of 5% Na_2CO_3 was added and mixed well the solution was allowed for incubation in dark condition for 1 h. Then, the samples were vortexed and absorbance was measured at 725 nm at Elico double beam UV-VIS spectrophotometer (India).

2.5.2. Determination of saponins

Five gram of sample was extracted with 100 mL of 20% ethanol, heated at 55°C for 4 h with continuous stirring. The residue was re-extracted with another 100 mL of 20% ethanol. Combined extracts were concentrated to 40 mL at 90°C, transferred to a separating funnel, and extracted with 20 mL diethyl ether. The upper layer was discarded and the lower layer was further purified and extracted with 30 mL n-butanol. The resulting material was washed twice with 10 mL of 5% NaCl and evaporated to dryness. The residue was oven-dried to constant weight, and saponin content was expressed as a percentage (Obadoni et al., 2001)

$$\text{Total saponins (\% w/w)} = \frac{\text{Weight of saponin residue (g)}}{\text{Weight of plant material (g)}} \times 100$$

2.6.Antimicrobial activity

2.6.1. Antibacterial study:

The antibacterial activity of fruit peels extracts of C. sinensis was determined by disc diffusion method(Bharath et al., 2020). Four bacterial pathogens consisted of both gram positive and gram negative such as, Staphylococcus aureus (MTCC 3160), Klebsiella pneumoniae (MTCC 109), Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC 424) were used for the study. The sub-culture strains were inoculated into 50 mL of nutrient broth and incubated at 37°C for 24 hours. Mueller-Hinton agar plates were then spread with 0.2 mL of the overnight-grown bacterial inoculum. Sterile 5 mm discs were prepared from Whatman No.1 filter paper, loaded with the respective extracts, and allowed to dry at 37ºC for 20 minutes. The dried, sample-loaded discs were placed on the plates seeded with each test organism and incubated at room temperature for 24 hours. The experiment was performed in triplicate. The antibacterial activity of fruit peels of C. sinensis different solvent extracts was compared with streptomycin as a positive control and solvent as a negative control. The zone of inhibition was expressed as mm in diameter.

2.6.2. Antifungal activity

Two fungal pathogens (Aspergillus niger MTCC 872, Aspergillus flavus (MTCC 13062) were used as testing organisms to evaluate the antifungal activity of fruit peel extracts of C. sinensis according to the method of Nayan et al., (2011). Selected active culture was transferred from stock solution to glass tubes, possessing sabouraud dextrose broth and set up was kept on going at 48 hours at normal room temperature. The Fungal activities were examined using disc diffusion technique. Fluconazole served as a positive control. Antimicrobial activity was examined using the procedure explained above.

2.7.Antioxidant Activity

2.7.1. DPPH Radical Scavenging Assay

The Percentage of antioxidant activity of fruit peel extracts was assessed by DPPH free radical scavenging assay **(Tailor and Goyal., 2014)**. Briefly, a test tube contains different concentrations of sample (20, 40, 60, 80 and 100 µl/ml) were added with 3ml of ethanol and 0.3ml of 0.5 mM DPPH radical solution. The above experimental setup was allowed to stand for 30 min. and the absorbance was read at 517 nm using UV- Vis spectrophotometer and BHA was taken as standard. The scavenging activity of fruit peel extracts were expressed as percentage of inhibition by using the following formula.

$$\text{Inhibition (\%)} = \frac{\text{A Control - A Sample}}{\text{A Control}} \times 100$$

III. RESULT AND DISCUSSION

3.1. Optimum dyeing procedure using orange fruit peel extracts

The colour characteristics and K/S values (which reflects the color strength of dyed fabric) of the dyed fabrics are shown in Table 1.

The K/S values of dyed fabrics were enhanced through the application of natural mordants espicially, cotton fabrics exhibited significant improvement in K/S values when treated with lemon under post-mordanting (14.00), during alum simultaneous mordanting (4.258), and post vinegar in post-mordanting (2.687). These findings confirm the effectiveness of natural mordants in improving the color strength of cotton

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fabrics (plate 1). The result it is evident that for the orange skin extract, natural mordant (lemon) can be used for better color production. The earlier observations confirmed the finding that gum rosin generates a protective film on the fiber surface, thereby improving dye fixation and minimizing color loss. The fiber surfaces exhibited a granular morphology, which may be attributed to interactions between the gum rosin, pigments, fiber components, and natural mordants (Li, et al., 2014). In addition, the possible formation of coordinate bonds could facilitate stronger binding between fibers and pigments. Natural pigments exhibited selective staining efficiency on gram-positive and gram-negative bacteria, indicating their potential as eco-friendly alternatives to synthetic stains (Goldie & Rama., 2022). Orange is edible and the papery skin of orange is discarded as a waste but this papery skin contains pelargonidin (tetrahydroxy anthocyanidin) as coloring pigment in its structure. It was also reported by Tepparin et al., (2012) that tannins could improve the color yield and color fastness properties of the dyed fabrics. Antraquinone and flavonids helps in imparting the color to any substrate besides this the presence of these phytochemical provided antimicrobial and antioxidant properties (Yadav et al., 2013).

Table 1. K/S values and spectrometric data of dyed cotton fabrics with and without color fixation treatment

Method	A	T	R	K/S	Dyed cotton fabric
No Modrant	0.321	107.68	8.001	7.289	
Pre alum	0.163	69.66	30.17	0.807	
simultaneous alum	0.044	90.36	9.596	4.258	
post alum	0.295	183.72	84.01	2.015	
Pre vinegar	0.196	147.61	47.806	2.284	
Simultaneous vinegar	0.167	132.82	32.98	2.680	
Post vinegar	0.113	132.72	32.83	2.687	
Pre lemon	0.047	95.52	4.433	10.301	
Simultaneous lemon	0.173	107.52	7.537	7.671	
Post lemon	0.145	92.52	3.335	14.009	

A- percent absorbe,T-percent transmitte, R - reflectance of the dyed fabric, K - absorption coefficient, and S - scattering coefficient

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3.2. Phytochemical analysis

The phytochemical analysis of Citrus sinensis fruit peels extracts confirmed the presence of bioactive secondary metabolites (table 2). The result revealed that presence of phenols, steroids, saponins, coumarins, and flavonoids are present in all the three extracts. In addition, the methanol extract and hexane extract showed the presence of tannins, while ethyl acetate and hexane extracts showed the presence of alkaloids, respectively. Most of the flowering plants have an important physiological effect on mankind due to the presence of various alkaloid compounds such as morphine, quinine, ephedrine, nicotine and strychnine. Especially, morphine and codeine are narcotic analgesics as well as are anti-tissue agent (Sweta et al., 2021). Coumarin derivatives have higher anti-fungal activity against a range of soil borne plant pathogenic fungi and exhibit more stability. The terpenoids, alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases (Booker et al,. 2008).

3.2.1. Total Phenolic Content (TPC)

The total phenolic content of different solvent extract of C. sinensis fruit peels was observed by using Folin-Ciocalteu method. Phenolic content ranged from 0.83 ± 0.07 mg /g (hexane extract) to 1.10 ± 0.11 mg/g (ethyl acetate extract), with methanol extract showing an intermediate value (1.04 ± 0.05 mg/g). The relatively higher concentration of phenols in the ethyl acetate and methanol fractions indicates the ability of polar solvents to extract polyphenolic compounds more efficiently than non-polar solvents such as hexane (fig 1). Phenolic compounds are well known for their antioxidant potential and radical scavenging activity, which are attributed to their hydroxyl groups that can donate hydrogen atoms or electrons to neutralize free radicals (Shahidi et al., 2015; Simon et al., 2021). Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides (Okwu et al., 2001).

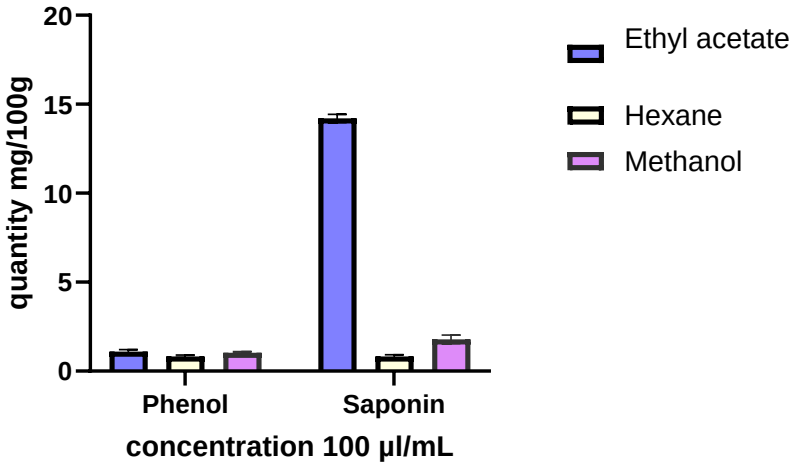

Fig: 1 - The quantitative phytochemical results of C.sinensis fruit peels using difference

3.2.2. Total Saponin Content (TSC)

Saponin content exhibited a more pronounced difference among the extracts. The ethyl acetate extract recorded the highest saponin concentration (14.20 ± 0.28 mg/g), whereas the hexane and methanol extracts contained significantly lower amounts (0.83 ± 0.09 mg/g and 1.79 ± 0.24 mg/g), respectively (fig 1).This indicates that saponins in C. sinensis peel are preferentially extracted by semi-polar solvents like ethyl acetate. Saponins are biologically important secondary metabolites associated with membrane-stabilizing, anti-inflammatory, and cholesterol-lowering activities (Francis et al., 2002). Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo et al., 2000).

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Table 2. Qualitative analysis of phytochemical in different solvent of C. sinensis.

Phytochemical screening	Solvents		
	Ethyl acetate	Hexane	Methanol
Flavonoids	+	-	+
Phenol	+	+	+
Tannins	-	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Steroids	+	+	+
Coumarin	+	+	+
Anthraquinones	-	-	-

+: Presence, -: Absence

3.3. Antimicrobial Activity

The antimicrobial activity of C. sinensis fruit peel extracts (ethyl acetate, hexane, and methanol) was evaluated against four bacterial pathogens (S. aureus, K. pneumoniae, E. coli, P. aeruginosa) and two fungal pathogens (A. niger,A. flavus) at varying concentrations (25, 50, 75, 100 µl/mL), as presented in tables 3 and plate 2.

3.3.1. Antibacterial activity

The ethyl acetate extract demonstrated significant antibacterial activity in a dose-dependent manner. The ethyl acetate extract possessed higher inhibition zone of 21.0 ± 1.51 mm at 100 µl/mL against K. pneumoniaewhich indicating a strong response compared with the control (23.60 ± 1.22 mm).

The hexane and methanol extracts exhibited relatively lower antibacterial activity compared with ethyl acetate. The study suggested that the bioactive constituents extracted by semi-polar solvents like ethyl acetate are responsible for significant antibacterial activity. The present findings was similar to previous report of Shalu Hasija et al., (2015) thatCitrus peel essential oils (orange, sweet lime, and lemon) obtained by hydro-distillation showed strong antimicrobial effects against E. coli, S. aureus, S. typhi, Shigella spp., and C. albicans. Their broad-spectrum activity highlights the potential of citrus peel oils as natural agents for food preservation and antimicrobial applications.

3.3.2. Antifungal activity

Interestingly, hexane extract exhibited stronger antifungal activity than antibacterial activity, particularly against A. flavus (18.6 ± 1.40 mm) and A. niger (17.2 ± 1.61 mm), it has highest inhibition zone when compared to ethyl acetate and methanol extract.

Overall, the ethyl acetate extract was the most effective against bacterial pathogens and hexane extract was potential against fungal pathogens.. The enhanced activity of ethyl acetate extract may be attributed to the presence of higher concentrations of phenolics and saponins, as confirmed in the phytochemical analysis (Table 1). These secondary metabolites are reported to exert antimicrobial effects by disrupting microbial membranes, binding to proteins, and interfering with metabolic enzymes (Cowan., 1999).

The moderate antifungal activity of hexane extract suggests that certain non-polar compounds, possibly terpenoids or essential oils, may contribute to fungal growth inhibition. These findings stress the importance of adopting effective post-harvest strategies to minimize fungal contamination and maintain fruit quality (Desalegn Amenu et al., 2024). The present study was correlated with previous finding of Sapna et al., (2016), C. sinensis peel extract has demonstrated in vitro antimicrobial activity against dental caries pathogens, supporting its potential use as a natural therapeutic agent. The ethyl acetate extract demonstrated the broadest and strongest antimicrobial spectrum, effectively inhibiting both Gram-positive and Gram-negative bacteria as well as fungal strains at higher concentrations.

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Table 3: Antimicrobial activity of C. sinensis fruit peel extract against different pathogens.

Pathogens	Type of extract	Zone of inhibition in (mm)				
		Control	25 (µl/mL)	50 (µl/mL)	75 (µl/mL)	100 (µl/mL)
S.aureus	Ethyl acetate	23.60±1.22	NA	10.67±1.53	16.60±1.51	20.67±2.08
	Methanol	21.03±1.65	NA	2.50±0.89	8.06±1.1	13.9±0.70
	Hexane	18.7±1.8	NA	2.5±0.89	7.15±1.33	12.5±0.81
K.pneumoniae	Ethyl acetate	24.3±1.40	NA	7.63±2.22	15.97±1.20	21.0±1.51
	Methanol	21.16±1.87	NA	3.96±0.70	7.6±1.17	13.2±0.74
	Hexane	18.2±1.2	NA	NA	3.23±1.51	11.3±1.1
E.Coli	Ethyl acetate	20.9±2.45	NA	7..03±1.55	8.33±1.62	14.83±1.69
	Methanol	16.96±18.3	NA	4.03±1.72	8.1±1.37	13.3±0.45
	Hexane	16.56±1.5	NA	NA	6.03±1.76	12.5±1.25
P. aeruginosa	Ethyl acetate	21.07±2.45	NA	9.1±2.13	16.07±1.94	18.93±2.29
	Methanol	21.53±1.80	NA	NA	7.5±0.82	12.5±0.75
	Hexane	16.76±1.5	NA	NA	7.4±2.95	13.2±0.74
A.niger	Ethyl acetate	20.90±1.71	1.77±1.25	5.47±1.70	13.60±1.91	16.70±1.39
	Methanol	14.13±041	NA	3.00±0.53	4.73±1.61	13.73±1.60
	Hexane	18.37±2.65	3.2±1.25	7.83±1.78	11.9±1.25	17.2±1.61
A. flavus	Ethyl acetate	21.93±1.43	1.87±0.32	4.87±2.31	10.37±2.22	14.63±1.83
	Methanol	20.1±1.08	1.77±0.38	1.93±0.49	7.53±0.97	16.0±1.25
	Hexane	20.23±0.49	3.3±1.15	9.13±1.20	13.07±1.95	18.6±1.40

NA – No Activity; All the Value are mean ±SD, SD- Standard Deviation

3.4. Antioxidant activity (DPPH assay)

The DPPH assay if frequently utilized for the assessment of radical scavenging activity due to its simplicity, reproducibility and reliability. This method is extensively employed to evaluate the free radical scavenging capacity or hydrogen-donating potential of bioactive compounds and to assess the antioxidant efficacy of plant extracts (Chanda et al., 2010; Nirmalraj et al., 2015). The antioxidant activity of different solvent extracts of C. sinensis fruit peels was evaluated using the DPPH radical scavenging assay, with ascorbic acid as the standard reference (Fig 2). All the extracts exhibited a concentration-dependent increase in scavenging activity, although their activity levels varied with the solvent used.

At the lowest concentration (20 µg/mL), the ethyl acetate, hexane, and methanol extracts showed inhibitory percentages of 14.92 ± 1.94%, 13.18 ± 2.87%, and 11.29 ± 2.43%, respectively, which were significantly lower than ascorbic acid (44.09–51.17%). However, with increasing concentrations, the inhibition improved notably. At 100 µg/mL, The ethyl acetate extract exhibited maximum inhibition of 82.30 ± 5.52%, followd by hexane extract 78.93 ± 3.95%, and methanol extract 71.29 ± 4.52%, respectively. However, all the extracts remained lower than the standard ascorbic acid (95.15± 2.68%). The IC_{50} values of hexane extract was (60.66 µg/mL) followed by ethyl acetate (63.51 µg/mL) and and ascorbic acid (36.44–68.25 µg/mL).

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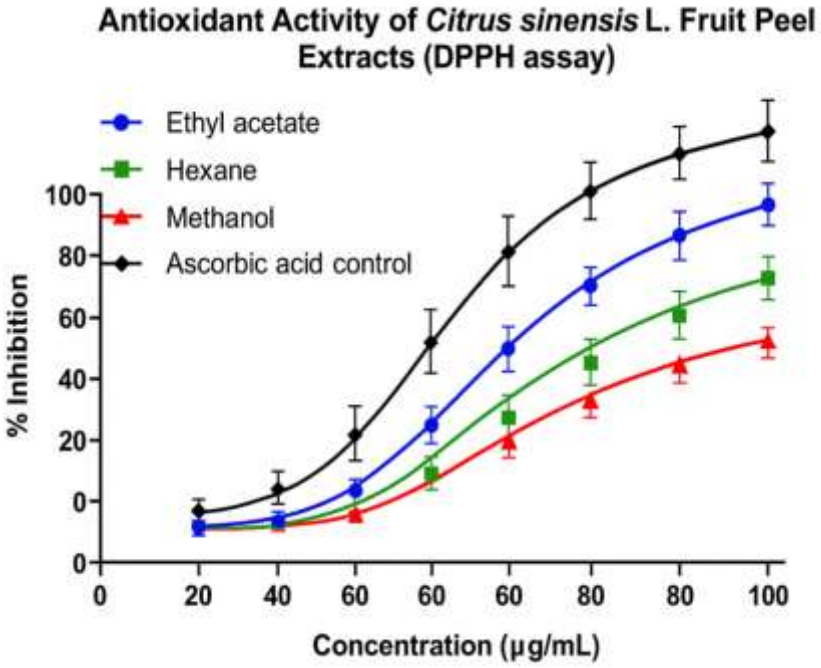

Fig 2. Antioxidant Activity of C. sinensis fruit peel different solvent extract

The observed differences in antioxidant activity may be attributed to the varying phytochemical compositions of the extracts. The higher activity in ethyl acetate and hexane fractions can be linked to the presence of phenolics and saponins, which were found in greater concentrations in these extracts. Phenolic compounds, in particular, are known to contribute significantly to antioxidant potential by donating hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative stress. (Shahidi and Ambigaipalan., 2015).

The moderate activity of methanol extract may be due to the extraction of other polar compounds that are less efficient radical scavengers, or possible antagonistic effects from certain constituents. Similar trends have been reported in citrus species, where ethyl acetate extracts consistently demonstrated stronger antioxidant potential compared with aqueous or polar methanolic extracts (Shafiya Rafiq et al., 2018). The therapeutic and protective role of phytophenols (plant-derived phenolic compounds) in human health. It explains that phytophenols are capable of preventing and managing diseasescaused by oxidative stress conditions in which excessive free radicals and reactive oxygen species (ROS) damage biological systems (Chikara et al., 2018) Specifically, these compounds help combat cancer (Huang et al., 2001), diabetes (Raimundo et al., 2020), neurodegenerative disorders (Perry et al., 2000), ageing-related degeneration, and cardiovascular dysfunctionsby neutralizing free radicals and quenching ROS (Cook and Sammon., 1996)(Młynarska et al., 2024).

IV. CONCLUSION

The study highlights the effectiveness of C. sinensis fruit peel extract as a natural dye for cotton fabrics. Among the tested mordants, lemon juice applied through post-mordanting exhibited the highest K/S value, making it the optimum mordanting technique for enhanced color strength. Phytochemical profiling confirmed the presence of secondary metabolites such as phenols, flavonoids, tannins, saponins, and alkaloids, which not only aid in dye fixation but also provide antioxidant and antimicrobial benefits. Ethyl acetate extracts, rich in phenols and saponins, exhibited superior antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungi, while also showing strong antioxidant potential in the DPPH assay. The valorization of orange peel waste into natural dye and bioactive extract supports the concept of sustainable textile processing, offering an eco-friendly alternative to synthetic dyes while addressing environmental pollution and adding functional value to fabrics.

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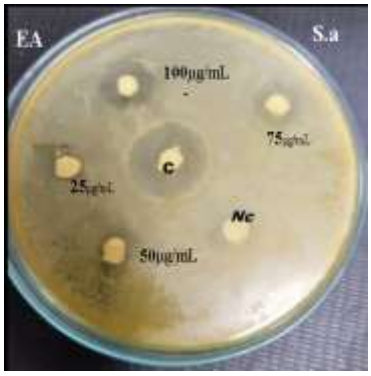

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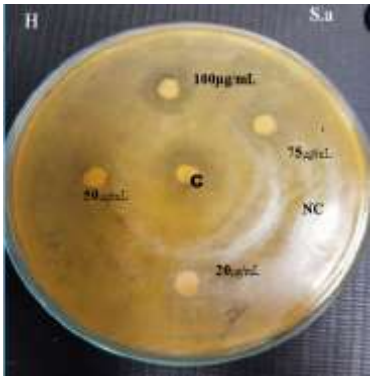

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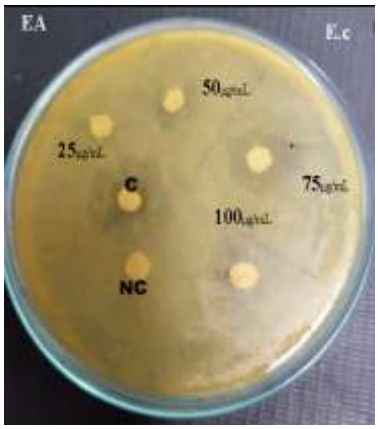

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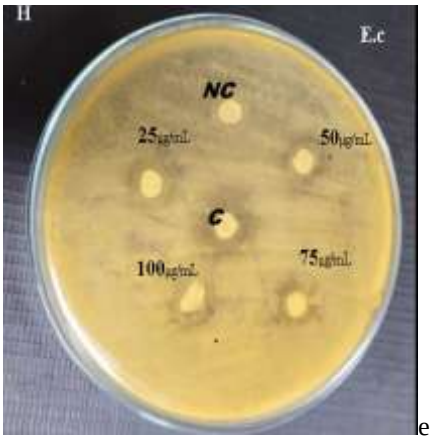

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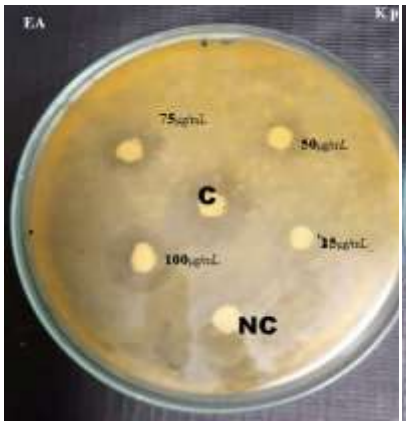

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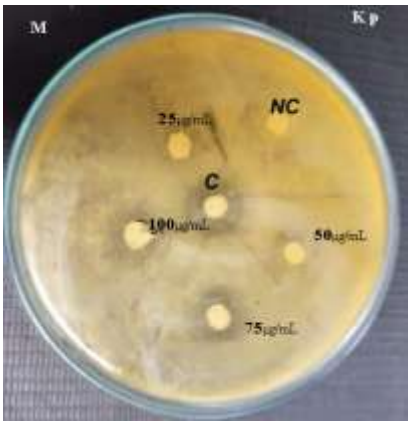

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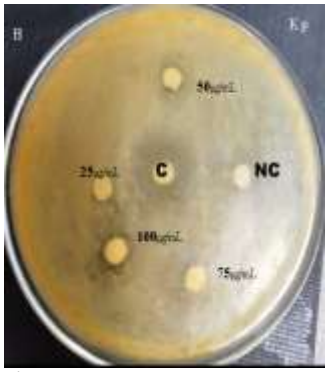

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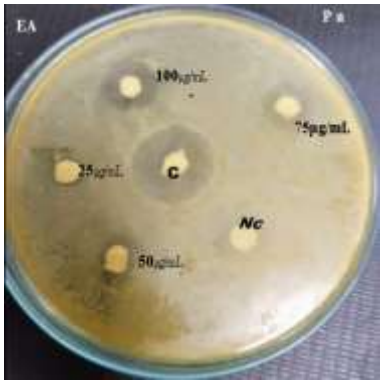

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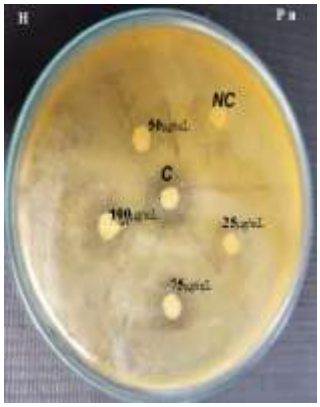

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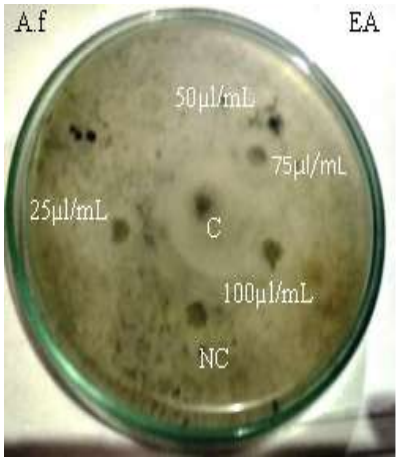

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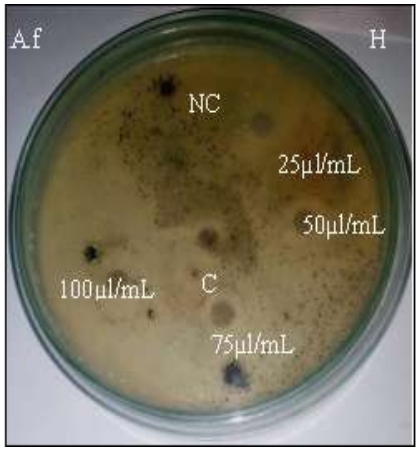

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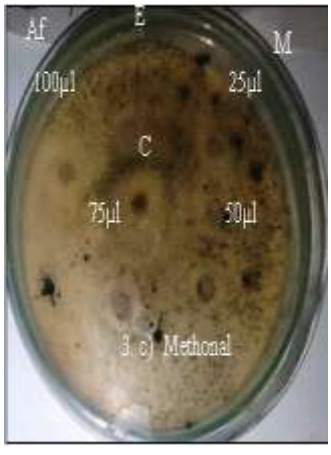

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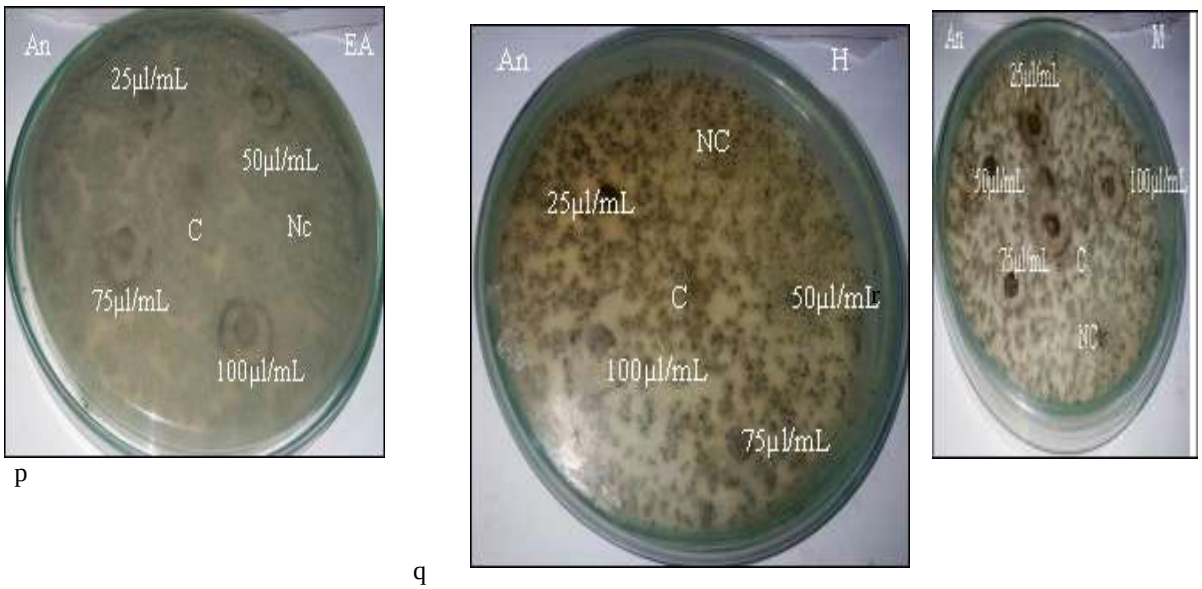

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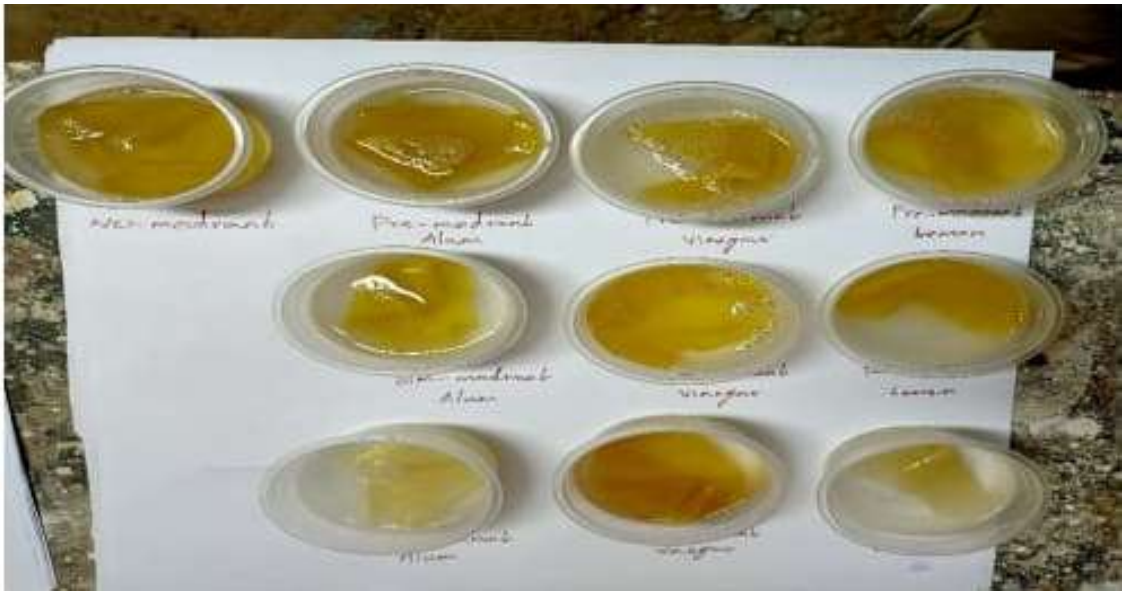
Plate 2. Plate showing aComparison of inhibitory activity of the 3 different extract of C. sinensis against the test organisms

(a-r) EA-Ethyl acetate, M-Methanol, H-Hexane, Sa-S.aureus, E c- E.Coli,K p-K.pneumoniae, P a-P. aeruginosa, Af- A. flavus, An-A.niger



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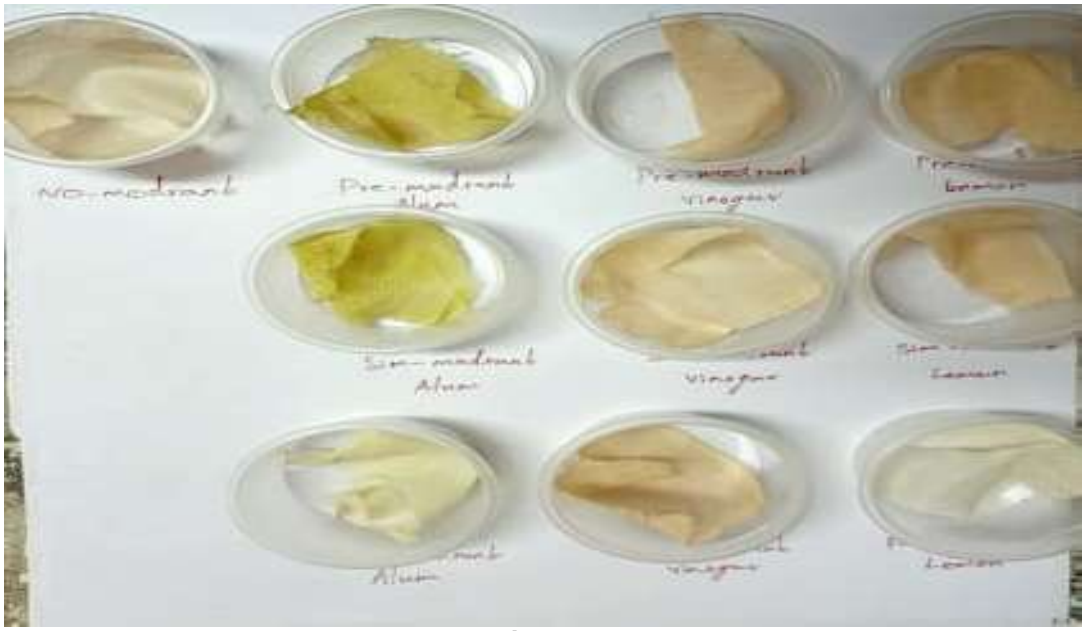

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Plate 1: The dyed cotton fabrics were treated with mordant solutions; a) Before dried, b) after dried