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Phytochemical Screening, Antioxidant and Antimicrobial Activity of Fabric Coated with *Catharanthus Roseus* Ethanolic Flowers Extract

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Abstract
The aim of the present study was to evaluate the free radical scavenging and antimicrobial activity of fabric coated of the *Catharanthus Roseus*. Ethanol flowers extract. Free radical scavenging was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Reducing power, Hydroxyl radical scavenging assay and antimicrobial activity of *Staphylococcus aureus, Escherichia coli* and standard drug of Streptomycin using disc diffusion method. This inhibition was observed with the individual extracts and when they were used in lower concentrations with ineffective antibiotics. The present investigation clearly indicates that the *Catharanthus Roseus* possesses antioxidant properties and serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Keywords
Catharanthus Roseus, Fabric coated, DPPH, *Staphylococcus aureus Escherichia coli*, Streptomycin, Antioxidant,

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INTRODUCTION
Natural fibres present many advantages like recyclable, biodegradable, plentiful, reveal fine mechanical properties, present improved working conditions and minimum abrasion to equipment in contrast to synthetic fibres, which produce drastic polluting environment. Cotton is a service to mankind since ages and its multipurpose functionality is unlimited as new experimentations are constantly being discovered (1). The most consumed natural fibre is cotton in the world. Cotton is a vegetable fibre which is in white or yellowish white obtained from plants (2). Cotton was used for clothing since long and its origin to be traced as 2300 B.C. Plant resources have a lead role in natural antimicrobial agents as they can be incorporated onto fabric for their antimicrobial potentiality. Many have worked on natural antimicrobial agents but only few have exploited combinational herbal extract coating on fabrics. Many Studies relate that the antimicrobial potential of plant extracts is due to their secondary metabolites. These metabolites are the reason for plants resisting the attack of microorganisms since immemorial (3).

Figure:1 *Catharanthus roseus*

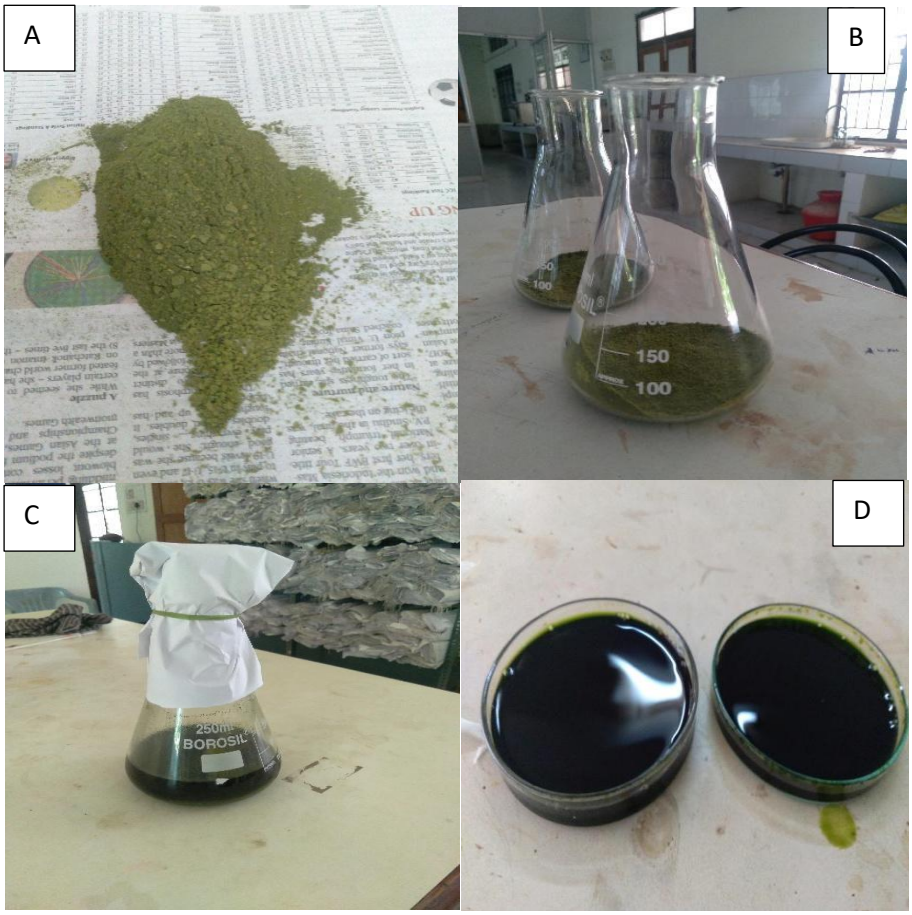

Figure 2A: powdered leaf of Catharanthus roseus
B. preparation of leaf powder for incubation
C. Incubation of extract
D. crude extract after filtration

Figure3 A and B: Before scouring and After scouring.

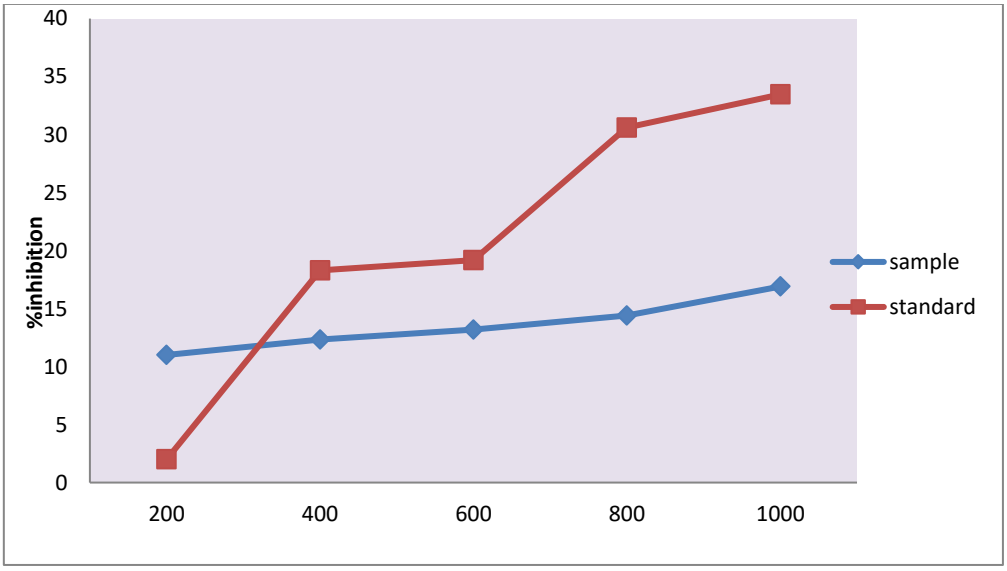

Figure 4 Results of graph of DPPH

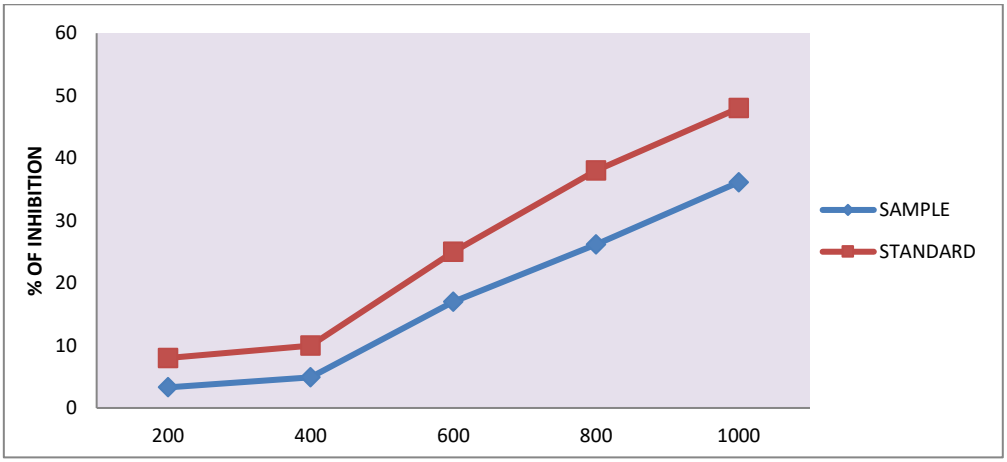

Figure 5 Results of graph of reducing power assay.

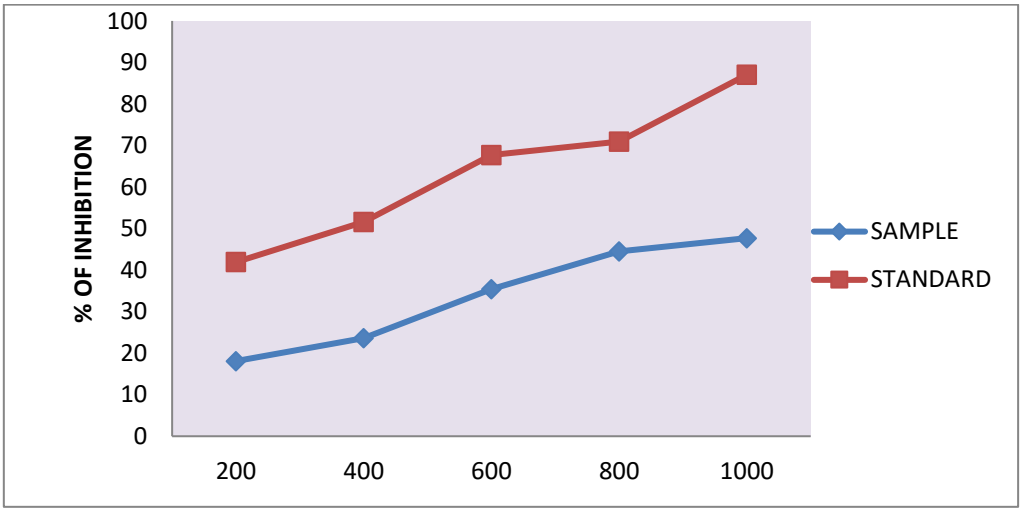

Figure 6 Results of graph of Hydroxy radical

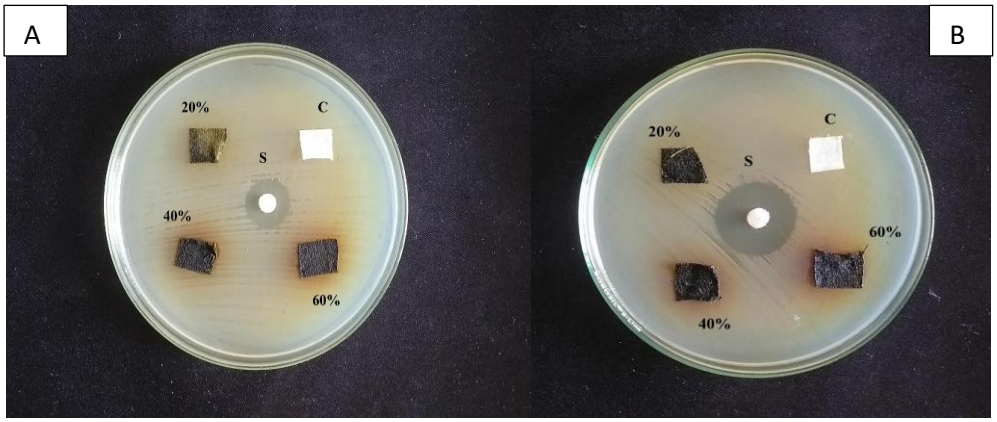

Figure 7 A,B: Antibacterial activity of Ecoli and S.A in coated fabric sample

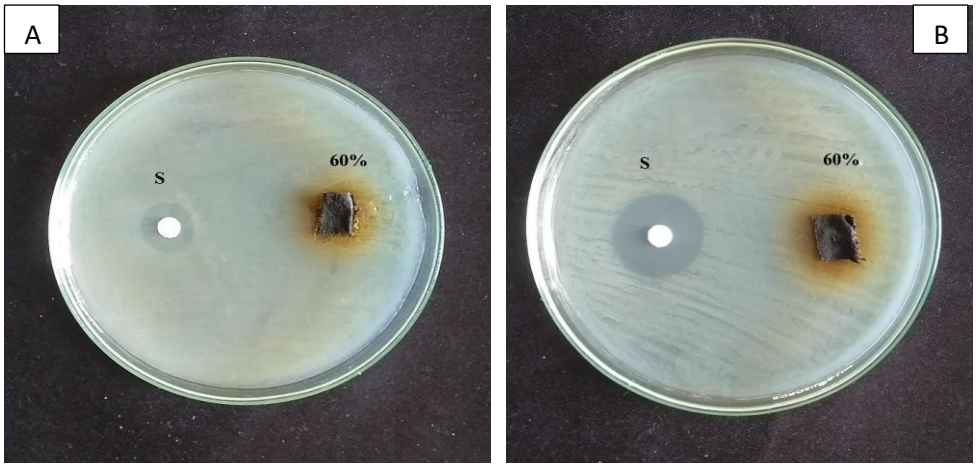

Figure 8 A, B: Antibacterial activity of highest concentration in *E.coli* and SA

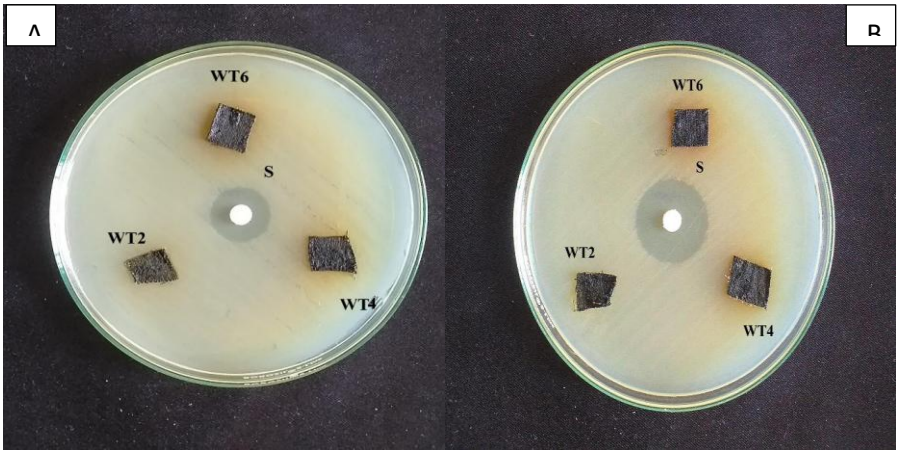

Figure 9A, B: Antibacterial activity of wash test samples in *E. coli* and SA.

Fig1. Shows the Periwinkle is the common name for a pair of perennial flowering shrubs belonging to the *Apocynaceae* family. It is cultivated as an ornamental plant almost throughout the tropical world. The herb has been used for centuries to treat a variety of ailments and was a favourite ingredient of magical charms in the Middle Ages. The present review evaluates the antibacterial activity, antihyperglycemic activity, antihypertensive activity, cytotoxic activity, antitumour activity, antidiabetic activity, diabetic wound healing activity and phytochemical constituents of *Catharanthus roseus*. The methanolic extracts of various parts of *Catharanthus roseus* was possessed high antioxidant activity due to the presence of flavonoids, coumarin, quinine and phenolic compounds. Herbal anticancer drug like *Catharanthus roseus* is wildly used because of their well-defined mechanism of action as anticancer drug. Proper chemical and biological investigations, understanding of the mechanisms of action, development of the structure activity relationship and high yield production by plant tissue culture of these herbal drugs promote their use against cancer as such or there semi synthetic analogues. In our present study was to evaluate the free radical scavenging and antimicrobial activity of fabric coated of the *Catharanthus Roseus* ethanol flowers extract.

MATERIALS AND METHODS

PLANT COLLECTION AND PLANT IDENTIFICATION

The plant material was collected locally from Dharapuram, Tiruppur, and District in the month of March 2022. The plant was identified from the Botanical survey of India, Coimbatore. The voucher number of specimen is **BSI/SRC/5/23/2021/Tech/306.**

PREPARATION OF SAMPLE EXTRACT

Collected leaves of *Catharanthus roseus* are shadow dried and powdered with the help of blender, from that 10mg of sample (*Catharanthus roseus* powder) is taken in conical flask and made up to 100ml with absolute ethanol. And kept for incubation for a period of 24hrs at room temperature.

FILTRATION AND INCUBATION

After the period of incubation for 24hrs the extract is filtered with the Whatman No. 1 filter paper to get the purest form of the extract. After the filtration process, the extract is transferred into petri plates and kept for incubation at 37°c in the oven for 20 mins, due to the high volatility of ethanol it will be easily evaporated by leaving the extracts alone in the petri plates. And the extracts left in the petri plates were collected and stored in an airtight container to avoid contamination and used for further studies Fig 2.

PHYTOCHEMICAL SCREENING OF THE PLANTS

The above said extract was carried out for phytochemical screening. The phytochemical screening of the plant extract was carried out by following the methods.

Test for alkaloids

Dragon droff's test: 1ml of the extract was treated with Dragon droff's reagent. An immediate orange red precipitate indicates the presence of alkaloids.

Test for flavonoids

Lead acetate test: 1ml of the extract was taken and few drops of lead acetate solution are added. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for phenols

Ferric chloride test: 1ml of the extract was treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for tannins

Ferric chloride test: 1ml of the extract was treated with 0.1% ferric chloride. Appearance of brownish dark blue colour indicates the presence of tannins.

Test for proteins

Biurete test: 1 ml of the extract was treated with 1 ml of 40% sodium hydroxide solution and two drops of 1% copper sulphate reagent. Appearance of violet colour indicates the presence of proteins.

Test for amino acids

Ninhydrin test: 1ml of the extract was treated with 2 drops of freshly prepared 0.2% ninhydrin reagent, heat it. Development of violet colour indicates the presence of amino acid.

Test for carbohydrate

Molisch's test: 1 ml of the extract was treated with few drops of concentrated H_2SO_4, wait for 2 minutes and dilute it with 5ml distilled water. Appearance of red violet ring at the inter phase of two layers indicates the presence of carbohydrates.

Test for glycosides

Legal's Test: 1ml of the extract was treated with 3ml of reagent. The formation of pink to red colour which indicates the presence of glycosides.

Test for saponins

Foam Test: 1ml of the extract was treated with few drops of distilled water and shaken well. Appearance of foam while shaking indicates the presence of saponins.

DPPH radical scavenging activity

Various concentrations of *Catharanthus roseus* of the sample (4.0 mL) were mixed with 1.0 mL of ethanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517nm. Ascorbic acid was used as control (4). The percentage of DPPH decolorization of the sample was calculated according to the equation:

% decolorization = [1-(ABS sample/ABS control)] ×100

IC_{50} value (mg extract/mL) was the inhibitory concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used for comparison.

REDUCING POWER ASSAY

The reducing power was determined as described (5). Briefly, 0.13 mL of *Catharanthus roseus* extract of different concentration in phosphate buffer (0.2 M, pH 6.6) were mixed with 0.125 mL of potassium ferricyanide (1%, w/v) and incubated at 50^0C for 20 min. Afterwards, 0.125 mL of TCA (10%, w/v) were added to the mixture to terminate the reaction. Then, the solution was mixed with 1.5 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm.

HYDROXYL RADICAL ACTIVITY

The reaction mixture 3.0 mL contained 1.0 mL of 1.5mM $FeSO_4$, 0.7 mL of 6mM hydrogen peroxide, 0.3 mL of 20mM sodium salicylate, and varying concentrations of *Catharanthus roseus* sample. After incubation for 1 hour at 37°C, the absence of the hydroxylated salicylate complex was measured at 562 nm (6). The percentage scavenging effect was calculated as:

Scavenging activity = $[1-(A_1 - A_2)/A_0] \times 100\%$

Where A_0 was the absorbance of the control (without extract), A_1 was the absorbance in the presence of the extract, and A_2 was the absorbance without sodium salicylate.

TREATMENT OF FABRICS AND SCOURING

Scouring refers to the thorough washing (or) deep cleaning of an item. This is a good first step wherever you plan to dye fabric, especially with natural plant or food dyes.100% unfinished cotton fabric was taken for the dyeing process with the *Catharanthus roseus* extract.

SCOURING TECHNIQUES

Mix washing soda with water. Bring the water to boil and add the cotton fabric to it and leave for at least 2 to 4hrs with occasional stirring. After boiling, turn off the burner and allow the water to get cool, after that rinse the fabric with water to remove excess washing soda leftover in the fabric and allow it to dry.

DYEING PROCESS AND WASH DURABILITY TEST

There are several dyeing techniques that are used widely, but we are using a method, which is one of the common methods used for dyeing, especially for dyeing natural dyes. In this process, first the scoured fabric is taken and make into small pieces according to the needs and dip in the extract (*Catharanthus roseus*) and left for 24hrs undisturbed, so that the dye will get infused and tightly bound with fabric and get coated, after 24 hrs the fabric is taken out and kept in oven at 37°c for 10 mins to dry. Our sample is ready for the assay, we have taken three different concentrations of extract to study in-depth about the extract and the concentrations are 20%, 40%, 60% (ie. 20% of *Catharanthus roseus* extract in 80% of ethanol). The wash durability test is done to test the durability of the coated fabric against the detergents, which will be useful to know, how long the coated will extract stick with fabric, which makes us clear that for how many times we can re-use the coated fabric**.**

ANTIMICROBIAL SCREENING OF FABRIC COATED WITH PLANT EXTRACT Bacteria are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the

susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro bacterial growth Fig 3.

AGAR DISC DIFFUSION METHOD

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

ANTIBACTERIAL ACTIVITY

Antibacterial activity of sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton agar medium was weighed as 3.8 gm and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured into sterile petri plates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Discs were prepared with 20 µl of samples (WT, UT, and C sample) and positive control 20 µg of Streptomycin (1mg/ml) was placed on MHA plates. These plates were incubated for 24 hrs at 37°C. Then the microbial growth was examined

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING

Phytochemicals are chemicals of plant origin; they are produced by plants through primary or secondary metabolism. They are naturally occurring bioactive compounds and play important role in plant growth as well as defence mechanism. Preliminary phytochemical screening was done, and the result is shown in Table 1. Based on the phytochemical analysis of plant extract. *Catharanthus roseus* showed the presence of flavonoid, alkaloid, tannins, protein, phenol, glycoside, carbohydrate, and absence of Saponins, Amino acid.

Table:1 Results of phytochemicals screening

SL. No.	TEST	ETHANOL
1	Alkaloid	+
2	Flavonoid	+
3	Phenols	+
4	protein	+
5	Amino acid	-
6	Carbohydrate	+
7	Tannins	+
8	Saponins	-
9	Glycoside	+

+ presence - absence

FREE RADICAL SCAVENGING ACTIVITY

Free radicals can generate oxidative stress and produce biological disorders in plants when exposed to contamination sites**.** When plants are grown in healthy condition, they are naturally occurring antioxidants and are good free radical scavengers, they were investigated for their free radical scavenging potential. The water extract which showed higher positive to phytochemicals is used for DPPH, Hydroxyl radical and reducing power assay.

1, 1-DIPHENYL-2-PICRYLHYDRAZYL (DPPH)

DPPH is a dark coloured crystalline which is composed of stable free radical molecules which is insoluble in water. Mainly used for testing the antioxidant activity and appeared as violet solution in ethanol. Antioxidant molecules cause reduction of DPPH solution result into colourless solution (7). This method is easy and applies to measure the all over antioxidant capacity and free radical scavenging activity of plant extract (8). Fig 4 Shows the graph represented the results of DPPH radical scavenging activity of *Catharanthus* ***roseus*** flower extract, and standard as ascorbic acid. The potential of L-ascorbic acid and sample to scavenge DPPH radical is directly proportional to the concentrations.

HYDROXYL RADICAL AND REDUCING POWER ASSAY

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects. Hydrogen peroxide scavenging activity of the extract is presented graph shown in Fig 5. Hydroxyl radical scavenging activity ***Catharanthus*** **roseus** flower extract, and standard as ascorbic acid. Fig 6 shows

the potential of standard and sample to scavenge Hydroxyl radical is directly proportional to the concentrations.

ANTIMICROBIAL ACTIVITY.

The study of antimicrobial activity on the ***Catharanthus roseus*** extract coated cotton fabric is shown in the Fig 7, Table 2. Antimicrobial activity of coated fabric of different concentrations (20, 40, 60 in **Staphylococcus aureus and E. coli**. **Streptomycin** is taken as the standard is about the highest performing concentration coated fabric is taken for the experiment to study its antimicrobial activity Fig 8, Table 3. The wash Durability test is done to know about the durability of the coated fabric against the detergents. The result of wash durability test is shown in the Fig 9, Table 4 shows the Against *Staphylococcus aureus* and *E. coli.*

Table 2 Antibacterial activity of *E. coli* and S.A in coated fabric sample

Microorganisms/Sample Concentration (µl)	Zone of Inhibition in mm	
	C-Sample (60%)	Streptomycin (20 µg)
Escherichia coli	14	13
Staphylococcus aureus	19	21

Table 3 Antibacterial activity of highest concentration in *E. coli* and SA

Microorganisms/Sample Concentration (µl)	Zone of Inhibition in mm			
	WT2	WT4	WT6	Streptomycin (20 µl)
Escherichia coli	13	10	5	15
Staphylococcus aureus	18	14	6	21

Table 4 Antibacterial activity of wash test samples in *E. coli* and SA

Microorganisms/Sample Concentration (µl)	Zone of Inhibition in mm				
	Control	20%	40%	60%	Streptomycin (20 µl)
UT					
Escherichia coli	0	9	10	13	15
Staphylococcus aureus	0	8	11	18	21

CONCLUSION

The present study shows that the selected herbal extracts have excellent control on the antioxidant activity and microbial strains such as *Staphylococcus aureus* and *E. coli.* The reason for the activity of extracts against these harmful pathogens may be due to the presence of secondary metabolites. Further, extensive research on characterization of the herbal compounds and methods to adhere these compounds on the surface of the fibre might be useful to develop potential antimicrobial finished natural textiles on commercial scale for the benefit of the society.

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